Toxicological Profile for Nitrobenzene

Draft for Public Comment

April 2022

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

CS274127-A

DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

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 and EPA.

Each profile includes the following:

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

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

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

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

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

Written comments may also be sent to:

Agency for Toxic Substances and Disease Registry
Office of Innovation and Analytics
Toxicology Section
1600 Clifton Road, N.E.
Mail Stop S102-1
Atlanta, Georgia 30329-4027

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

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

Patrick N. Breysse, Ph.D., CIH
Director, National Center for Environmental Health
and
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

Christopher M. Reh, PhD
Associate Director
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

VERSION HISTORY

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

CONTRIBUTORS & REVIEWERS

CHEMICAL MANAGER TEAM

Malcolm Williams, DVM, PhD (Lead)
Jennifer Przybyla, MS, MS, PhD

ATSDR, Office of Innovation and Analytics, Toxicology Section, Atlanta, GA

Lauren Brown, MS, DABT
Meghan Lynch, DSc
Kerry Diskin, DSc
Rekha Balachandran, PhD
Hannah Derrick, BS
Abt Associates, Inc., Rockville, MD

Chimmeddulam Dalaijamts, PhD
Texas A&M University, College Station, TX (subcontractor to Abt Associates)

REVIEWERS

Interagency Minimal Risk Level Workgroup:

Includes ATSDR; National Center for Environmental Health (NCEH); National Institute for Occupational Health and Safety (NIOSH); U.S. Environmental Protection Agency (EPA); National Toxicology Program (NTP).

Additional reviews for science and/or policy:

ATSDR, Office of Community Health and Hazard Assessment; ATSDR, Office of Capacity Development and Applied Prevention Science; ATSDR, Office of Science; NCEH, Division of Laboratory Science; NCEH, Division of Environmental Health Science and Practice; EPA.

PEER REVIEWERS

1. Mary Beth Genter, Ph.D., Professor, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio
2. Stephen M. Roberts, Ph.D., Director, Center for Environmental & Human Toxicology, Professor, College of Veterinary Medicine, College of Medicine, and College of Public Health and Health Professions, University of Florida, Gainesville, Florida
3. Andrew Gow, Professor, Ernest Mario School of Pharmacy, Rutgers University, New Brunswick, New Jersey

These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(i)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

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.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Nitrobenzene ($C_6H_5NO_2$; CAS 98-95-3) is a synthetic chemical mainly used to produce aniline, quinolone, 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 dye (Dai et al. 2010a; Dasgupta et al. 2018). Nitrobenzene does not naturally occur.

While most nitrobenzene is retained in closed loop systems, data collected for the Toxics Release Inventory (TRI) suggests that 64,4532 pounds of nitrobenzene were released to the environment from industrial activities in 2017 (TRI17 2019). Human exposure to nitrobenzene results from releases to air and wastewater from industrial sources. Nitrobenzene may also be an air pollutant in ambient air, especially in urban areas. The general public may also be exposed to nitrobenzene in drinking water. The most likely routes of human exposure to nitrobenzene are through the skin and through inhalation (IRIS 2009). 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). Information on nitrobenzene levels in soil and sediment is limited in the available literature, but it has been detected in soil at the former site of a dye manufacturer and is assumed to be present in soil at hazardous waste sites due to its presence in the air above some sites (Harkov et al. 1985; LaRegina et al. 1986; Nelson and Hites 1980).

Biomonitoring can be done for nitrobenzene in urine; however, this will only reflect recent exposures. The presence of p-nitrophenol and p-aminophenol are two metabolites of nitrobenzene which may also be used in urine. However, these metabolites are not specific to nitrobenzene and therefore this complicates using these metabolites as indicators of nitrobenzene exposure specifically (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. However, there are many case studies in humans, most of which are a result of intentional ingestion. The results of these case studies clearly indicate that the most common adverse outcome associated with nitrobenzene exposure in humans is methemoglobinemia. This is supported by data in experimental animal studies which observed increased methemoglobin (metHb) levels after inhalation,

oral and dermal exposure. In addition to the hematological effects observed with nitrobenzene exposure, adverse respiratory, hepatic, renal and reproductive effects are also outcomes which have been observed, regardless of exposure route. Additionally, inhalation studies have demonstrated inflammatory effects in the nasal passages. As illustrated in Figure 1-1 the most sensitive effect in lab animals after inhalation exposure include effects on the hematologic and respiratory system. One study also found low dose effects on the endocrine system, specifically on the adrenal gland.

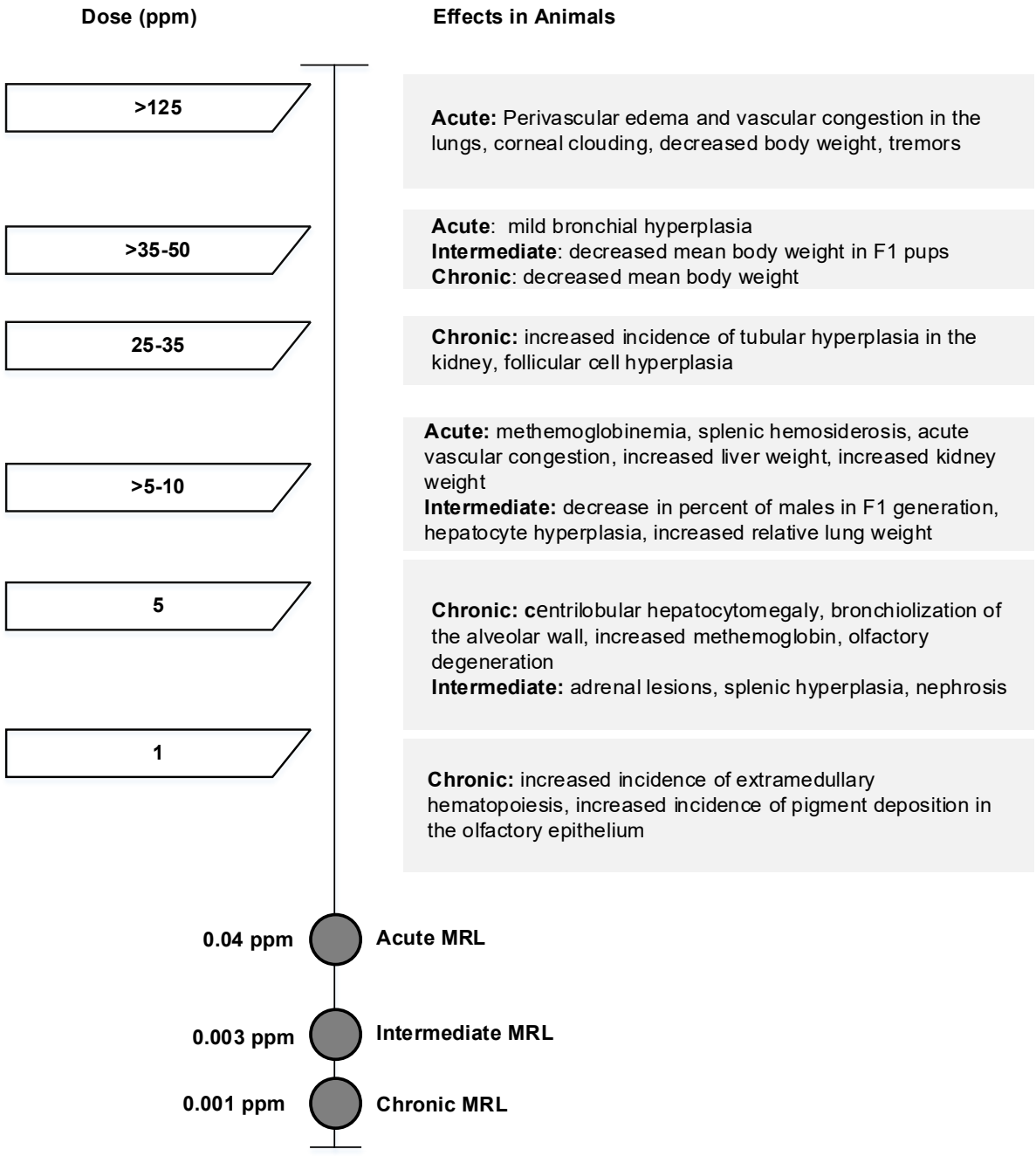
Figure 1-2 demonstrates that the hematological system is also a sensitive target after oral exposure as are the hepatic and renal systems. Figure 1-1 and Figure 1-2 demonstrate that the minimal risk levels (MRLs are established below any doses at which effects have been demonstrated.

- Respiratory: Results from inhalation studies indicate effects of nitrobenzene exposure on the nasal passages. Specifically, in chronic rodent studies B6C3F1 mice had significantly increased degeneration of the olfactory epithelium and F344 rats had a significant increase in pigment deposition in the olfactory epithelium (Cattley et al. 1994). In the same study, a significant increase in bronchiolization of the alveoli was observed in mice (Cattley et al. 1994). In addition, acute (≤14 days) and intermediate (15-364 days) dermal exposure studies have demonstrated lung congestion after nitrobenzene exposure in F344 rats (NTP 1982).
- Hematological: In general, when considering the toxicological effects of nitrobenzene exposure via ingestion, inhalation or dermal routes, methemoglobinemia is the most commonly observed systemic adverse health outcome. Specifically, in all human case studies evaluated, with both inhalation and ingestion exposure to nitrobenzene, methemoglobinemia was the main adverse effect reported in humans (e.g., 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 an increase in metHb 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; CIIT 1993; Hamm Jr. et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b). A variety of other adverse effects in the hematologic system such as extramedullary hematopoiesis, alterations in hemoglobin levels, changes in bone marrow in response to the anemia and congestion of the spleen 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; Hamm Jr. et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b)
- Hepatic: There is limited evidence that the liver is a target for nitrobenzene toxicity in humans. This evidence comes from two human case studies (Gupta et al. 2012; Ikeda and Kita 1964). However, there are several experimental animal studies that reported adverse liver effects

(Cattley et al. 1994; Hamm Jr. et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b). In the case studies there were disruptions in the liver as evidenced by an increase in the retention of bromosulphthalein (BSP), a dye used in liver function test, and an increase in icterus index (i.e., jaundice) and indirect bilirubin levels in liver function tests (Ikeda and Kita 1964) and pathological observations of hepatic centrilobular necrosis (Gupta et al. 2012). 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; Hamm Jr. et al. 1984; Medinsky and Irons 1985; NTP 1983a).

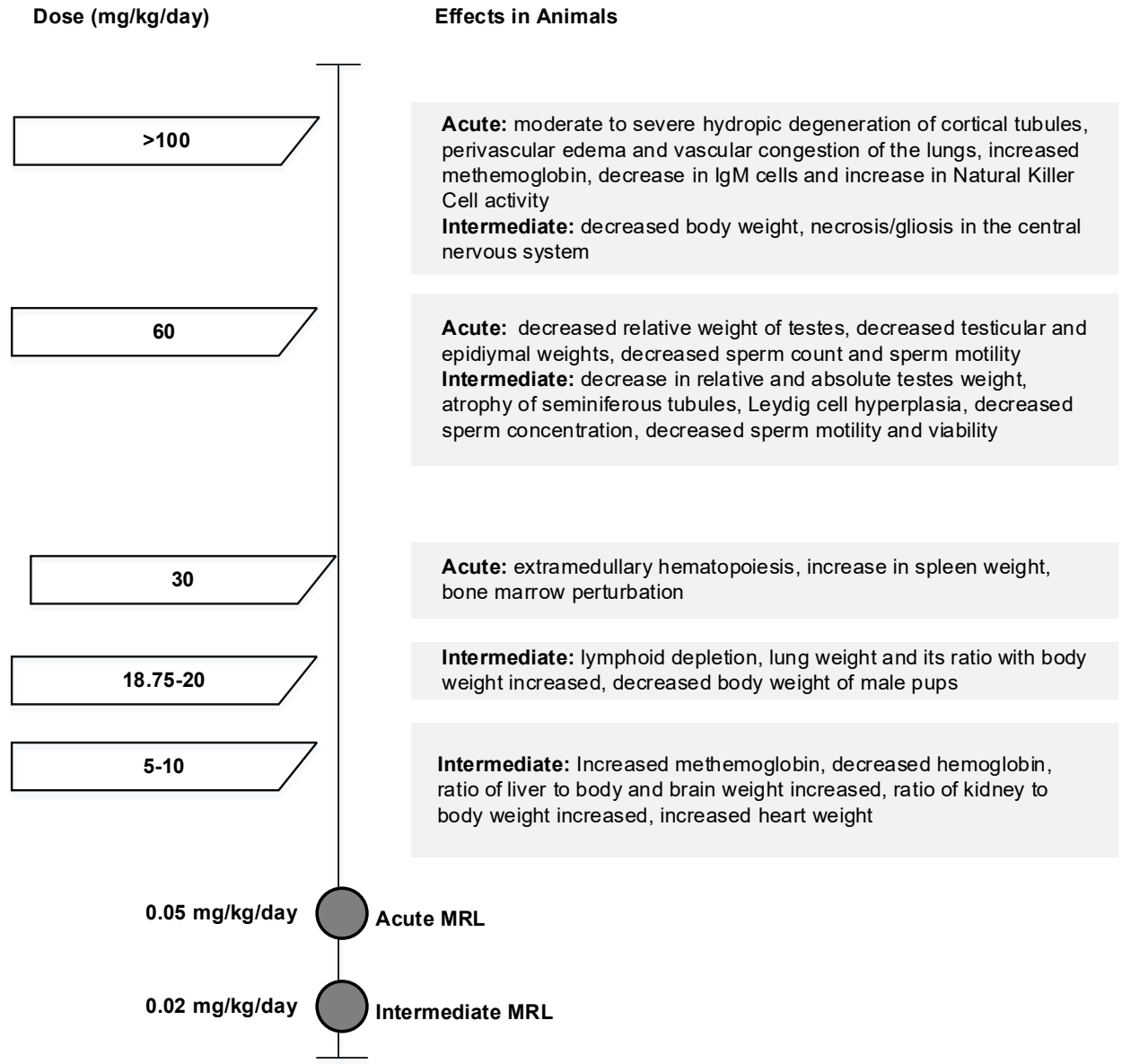
- Renal: The kidney also appears to be a target for nitrobenzene toxicity. However, the evidence for this in humans is limited. Specifically, in one case study, renal tubular necrosis was seen following the death of the subject who ingested nitrobenzene (Gupta et al. 2012). Several experimental animal studies have demonstrated increases in kidney weight and degenerative changes in the cortical tubules (Medinsky and Irons 1985; NTP 1982, 1983a).
- Reproductive: 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; Allenby et al. 1991; Linder et al. 1992). Common effects seen after exposure to nitrobenzene include atrophy of the seminiferous tubules, hypospermatogensis, Sertoli cell hyperplasia and dysspermiogensis. 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; Dodd et al. 1987; Hamm Jr. et al. 1984; Kawaguchi et al. 2004; Kawashima et al. 1995; Linder et al. 1992; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b). The effects on the male reproductive system have also been observed to decrease fertility indices in intermediate inhalation studies of Sprague-Dawley rats (Dodd et al. 1987).

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



MRL = Minimal Risk Level

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



MRL = Minimal Risk Level

1.3 MINIMAL RISK LEVELS (MRLs)

The inhalation database was considered adequate for deriving an MRL for all durations of exposure for nitrobenzene. As presented in Figure 1-1 the available inhalation data indicate the respiratory system and hematological systems are the most sensitive, especially when considering chronic exposure. The oral database was also considered adequate for deriving MRLs for acute and intermediate exposure durations. Given there were no studies located that evaluated oral exposure to nitrobenzene for more than 1 year, the database to derive a chronic MRL for the oral exposure route was deemed inadequate. The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

As illustrated in Figure 1-3, hematological, hepatic, renal, respiratory and endocrine effects appear to be the most sensitive targets of nitrobenzene inhalation. Hematological, hepatic, and cardiovascular effects appear to be the most sensitive targets of ingested nitrobenzene (Figure 1-4). The lowest-observed-adverse effect levels (LOAELs) in Figures 1-3 and 1-4 reflect actual doses (levels of exposure) employed in animal studies.

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, and cardiovascular endpoints are the most sensitive targets of nitrobenzene oral exposure.
Numbers in circles are the lowest LOAELs among health effects in animals.

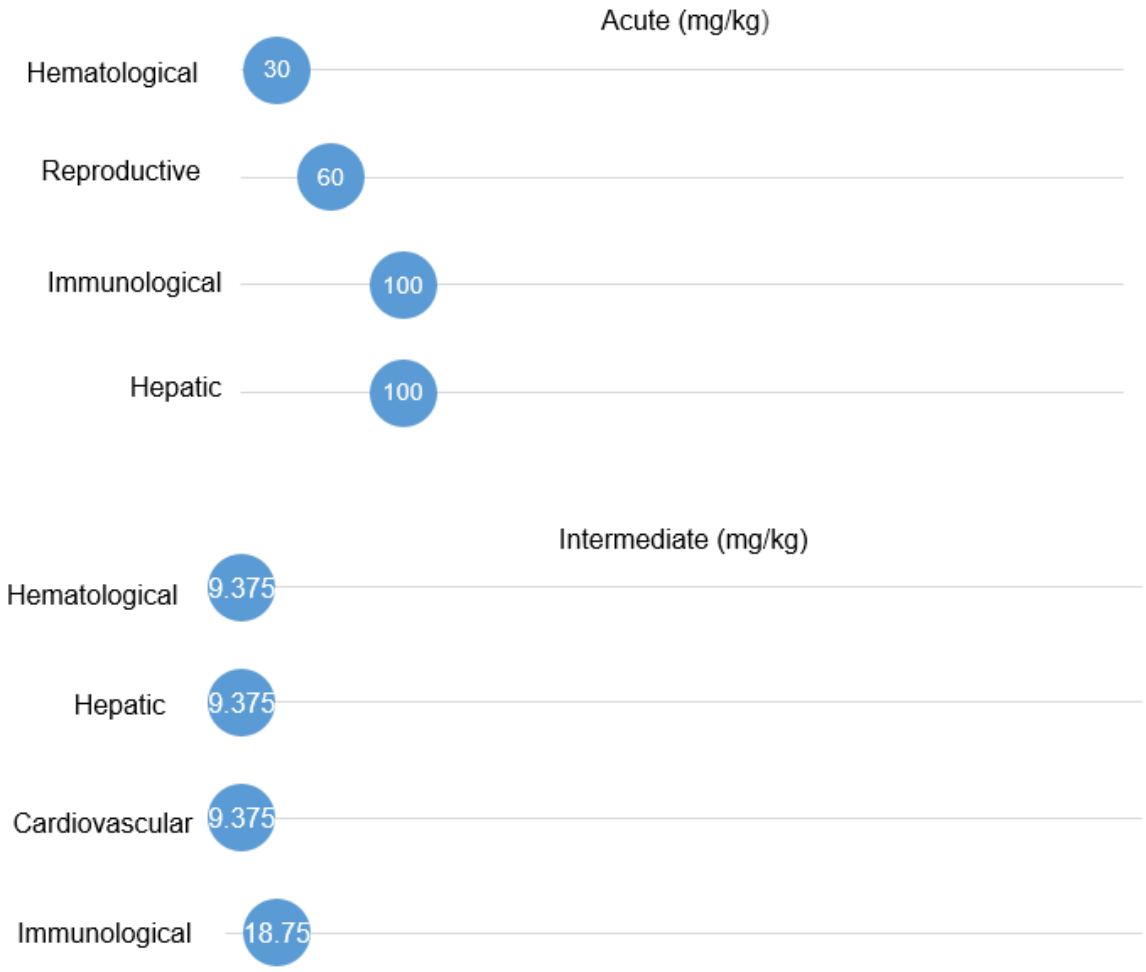


Table 1-1. Provisional Minimal Risk Levels for Nitrobenzene

Exposure Duration	Provisional MRL	Critical Effect	Point of Departure/Human Equivalent Concentration	Uncertainty & Modifying Factor	Reference
Inhalation Exposure (ppm)					
Acute	0.04	Increased methemoglobin	$BMCL_{1SD}$: 16 ($BMCL_{HEC}$: 4)	UF: 30 MF: 3	Medinsky and Irons 1985
Intermediate	0.003	Hemolytic anemia and increased methemoglobin	LOAEL: 5 ($LOAEL_{HEC}$: 0.89)	300	Hamm Jr. et al 1984
Chronic	0.001	degeneration of the olfactory epithelium and bronchiolization of the alveoli	$BMCL_{10}$:0.93 ($BMCL_{HEC}$: 0.04)	30	Cattley et al 1994
Oral Exposure (mg nitrobenzene/kg/day)					
Acute	0.05	Dose-dependent changes in the bone marrow including increases granulocyte-monocyte progenitor cells and, DNA synthesis	$BMDL_{1SD}$: 4.7	100	Burns et al. 1994
Intermediate	0.02	Increased methemoglobin	$BMDL_{1SD}$:1.8	100	NTP 1983a
Chronic	Insufficient data for MRL derivation				

[a]See Appendix A for additional information.

1 SD = 1 standard deviation; BMCL = benchmark concentration limit; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; $LOAEL_{ADJ}$ = LOAEL adjusted to continuous exposure; MF = modifying factor; MRL = minimal risk level; UF = uncertainty factor

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 D). 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 comes from experimental animal studies. Even though there were more than 30 human studies the majority of these were case studies. The most examined endpoints were hematological, reproductive, hepatic and renal.

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

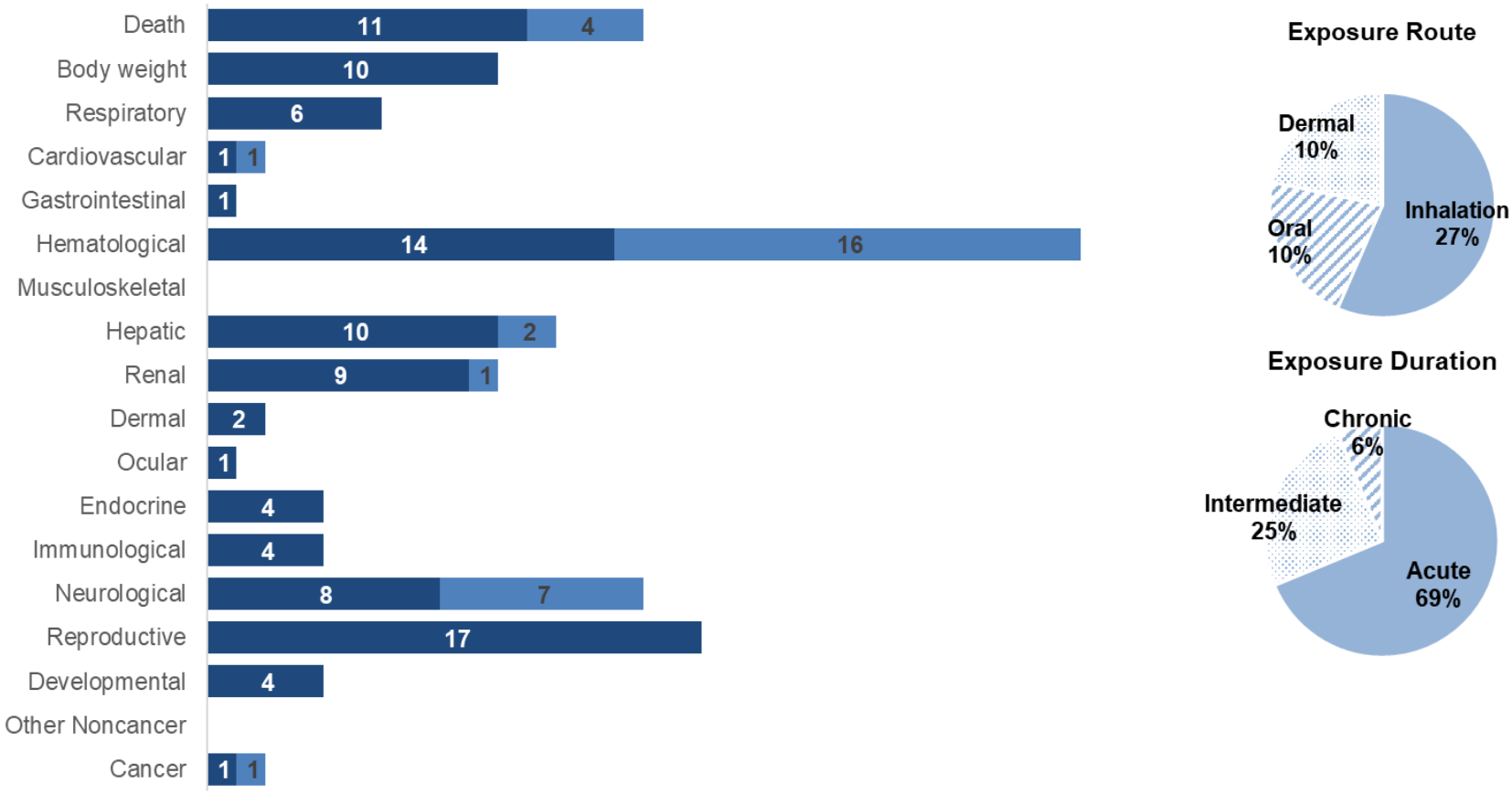
- Respiratory: Nitrobenzene exposure has been associated with a variety of adverse respiratory outcomes. Specifically, in the lungs of exposed experimental animals, congestion was observed as was bronchiolization of the alveoli. The nasal epithelial tissue was also adversely affected after nitrobenzene exposure as evidenced by degeneration of the olfactory epithelium and pigmentation deposition in the olfactory submucosa.
- Hematological: The most indicated outcome associated with nitrobenzene exposure, via any route of exposure, is methemoglobinemia. Specifically, in all human case studies evaluated with nitrobenzene exposure, methemoglobinemia was the main adverse effect observed. Additionally, experimental animal studies have demonstrated an increase in methemoglobin (metHb) levels in mice and rats of both sexes exposed through any exposure route with acute, intermediate and chronic exposure. There are a variety of other adverse effects observed in the hematologic system such as extramedullary hematopoiesis, alterations in hemoglobin levels, and congestion of the spleen.
- Hepatic: The liver is a target for nitrobenzene toxicity as evidenced by a case study and experimental animal studies which observed centrilobular necrosis. In one case study occupational exposure to nitrobenzene was associated with retention of BSP and increases in

icterus index and indirect bilirubin level. Degenerative changes in hepatocytes, hepatocytomegaly, and increased liver weights have also been observed in animal studies.

- Renal: The kidney also appears to be a target for nitrobenzene. In one case study renal tubular necrosis was seen following the death of the subject. In addition, experimental animal studies have demonstrated increases in kidney weight and degenerative changes in the cortical tubules.
- Reproductive: Nitrobenzene is a known male reproductive toxicant. Common effects seen after exposure to nitrobenzene include atrophy of the seminiferous tubules, hypospermatogensis, Sertoli cell hyperplasia and dysspermiogensis. The effects on the male reproductive system have also been observed along with decreased fertility indices.

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

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



*Includes studies discussed in Chapter 2 including those finding no effect. Most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
ACUTE EXPOSURE									
1	RAT (Fischer-344) 10M, 10F	14 days, 5 days/week, 6 hours/day	0, 9.1, 35.8, 124.5	BC, GN, HE, HP, LE, OW	Death	124.5			
					Resp	124.5			
					Hemato		9.1	124.5	SLOAEL: methemoglobin level at 13.4% for females and 11.7% for males at acute sacrifice, effects did not persist after 14 day recovery period LOAEL: concentration dependent increase in the number of hemosiderin-laden macrophages infiltrating the red pulp in the spleen, increased extramedullary hematopoiesis and acute sinusoidal congestion in the spleen at acute sacrifice. In females, methemoglobin increased from 3.6% to 4.8%
					Hepatic	9.1 F	35.8 F		Significant 27% increase in relative liver weight at acute sacrifice
					Hepatic		9.1 M		Significant 13% increase in relative liver weigh at acute sacrifice
					Renal		9.1		15% increase for males and 6% increase for females in relative kidney weight at acute sacrifice
					Neuro	124.5			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Repro	35.8 M		124.5 M	Increase in the number of multinucleated giant cells, Sertoli cell hyperplasia and dysspermiogenesis, lumen of the ductus epididymis contained reduced mature sperm.
Medinsky and Irons 1985									
2	RAT (Sprague-Dawley) 26F	10 days, gestational days 6-15, 6 hours/day	0, 1, 9.8, 39.4	CS, BW, OW, GN	Hemato	1 F	9.8 F		40% increase in absolute and relative spleen weight
					Develop	9.8		39.4	41% increase in the percent of litters that had animals with holes in the parietal skull plate; 32.5% increase in the percent of liters that had ecchymosis on the trunk
					Repro	9.8M		39.4M	44% decrease in testes weight 3 days and 14 days post exposure; development of testicular lesions which consist of an increase in multinucleated giant cells, Sertoli cell hyperplasia and severe dysspermiogensis were observed
Tyl et al. 1987									
3	RAT (Sprague-Dawley) 10M/10F	14 days, 5 days/week, 6 hours/day	0, 9.1, 35.8, 124.5	BC, BI, GN, HE, HP, LE, OW	Death			124.5	5 male and 3 female Sprague-Dawley rats were found dead after 4 days of exposure
					Resp		124.5		Perivascular edema and vascular congestion

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Hemato		9.1 F		Methemoglobin increased to 6.3% from 4.8% in controls; spleen weight increased 44% at acute sacrifice
					Hemato	9.1 M	34.8 M [b]		Methemoglobin levels increased from 6.9% to 8.7%. 74% increase in relative spleen weight in males; ($BMCL_{1SD}$=16 ppm)
					Hepatic	9.1	34.8		Mild degree of hepatocyte necrosis
					Renal		124.5		Moderate to severe hydropic degeneration of cortical tubular cells
					Neuro			124.5	Perivascular hemorrhage in cerebellar peduncle accompanied by varying degrees of edema and malacia
					Repro	35.8 M		124.5 M	Dysspermiogenesis of moderate severity
Medinsky and Irons 1985									
4	MOUSE (B6C3F1) 10M, 10F	14 days, 5 days/week, 6 hours/day	0, 9.1, 35.8, 124.5	BC, BI, GN, HE, HP, LE, OW	Death			124.5	Early morbidity noted for male and female mice, not otherwise described
					Resp	9.1	35.8		Mild bronchial hyperplasia not otherwise described observed 3 days after exposure ended
					Hemato	9.1 F	35.8 F		Extramedullary hematopoiesis and acute vascular congestion
					Hemato	35.8 M	124.8 M		Extramedullary hematopoiesis and acute vascular congestion
					Hepatic		35.8		Nonnecrotic hepatocyte degenerative changes observed at acute sacrifice

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Hepatic			124.5 M	4/10 exhibit centrilobular necrosis superimposed on a severe centrilobular hydropic degeneration (10/10)
					Renal	9.1			
					Renal		35.8 M		Minimal to moderate multifocal degenerative changes in the renal tubular epithelium
					Immuno	9.1		35.8	Lymphoid aplasia
					Neuro	35.8		124.5	Cerebellar lesions (perivascular hemorrhage)
Medinsky and Irons 1985									
5	RABBIT (New Zealand) 88F	13 days, 6 hours / day	0, 9.9,41, 104	DX, GN, HE, CS, HP, LE	Death			104 F	One killed in moribund condition 2 days post mating
					Bd wt	104 F			
					Hemato	104 F			
					Hepatic	9.9 F	41 F		Mean absolute liver weight increased 9.6% mean relative liver weight increased 11.5%
					Repro	104 F			
Biodynamics 1984									
6	RABBIT (New Zealand) 48F	6 hr/day; 12 days	10, 40, 81	BC, BW, OW, LE,	Death	40 F			
					Bd wt	81 F			
					Hemato	81 F			
					Hepatic	81 F			
					Renal	81 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
Biodynamics 1983									
INTERMEDIATE EXPOSURE									
7	RAT (Sprague-Dawley) 120M 120F	10 weeks (2 generations), 5 days/week, 6 hours/day	0, 1, 10, 40	BW, LE, RX, GN, OW	Repro	10		40	Decreased fertility rates, decreased parturition and gestation indices, atrophy of seminiferous tubules, spermatocyte degeneration and reduced testicular and epididymal weights
					Develop	10	40		12% decrease in mean body weight of F1 pups
Dodd et al. 1987									
8	RAT (Fischer-344) 10M 10F	90 days, 5 days / week, 6 hours / day	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Resp	15.8	48.7		Minimal to slight hyperplasia of the bronchial epithelium
					Hemato		5 F		Splenic hyperplasia accompanied by decreased RBCs, decreased hemoglobin, decreased hematocrit, increased RBC width, increased platelet volume, increased platelet width
					Hemato		5 M [c]		Significantly increased methemoglobin, increased from 1.2% in controls to 3.0% accompanied by other hematologic changes that were consistent with hemolytic anemia.
					Hepatic			48.7	Necrosis

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Renal	15.8 F		48.7 F	Nephrosis consisting of accumulation of hyaline or eosinophilic droplets in the cytoplasm of proximal tubular epithelial cells, moderate severity
					Renal		5 M	48.7 M	LOAEL: nephrosis consisting of accumulation of hyaline or eosinophilic droplets in the cytoplasm of proximal tubular epithelial cells, mild severity SLOAEL: nephrosis consisting of accumulation of hyaline or eosinophilic droplets in the cytoplasm of proximal tubular epithelial cells, moderate severity
					Endocr	15.8	48.7		Increased basophilia of the medullary cells in the adrenal glands
					Repro	15.8 M		48.7 M	Severe degeneration of tubular epithelial cells, absence of mature sperm
Hamm Jr. 1984									
9	RAT (Sprague-Dawley) 10M 10F	90 days, 5 days / week, 6 hours / day	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Hemato		48.7		Splenic lesions and increased methemoglobin (M: 0.6% to 10.5%; F: 2.1% to 9.6%)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Renal	5	15.8	48.7	SLOAEL: Toxic nephrosis that was moderate to severe in 9 of 10 males and minimal in 5 of 10 females exposed to 50 ppm LOAEL: Accumulation of lipid in proximal convoluted tubule in 4/10 female mice and 1 male mouse
					Repro	5 M	15.8 M	48.7 M	SLOAEL: Bilateral testicular atrophy LOAEL: Slight reduction in mature sperm in 2 animals
Hamm Jr. 1984									
10	MOUSE (B6C3F1) 10M 10F	90 days, 5 days / week, 6 hours / day	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Hemato	15.8	48.7		Methemoglobinemia significantly increased from 0.7%in controls to 5.8% in males and 1.3% in controls to 5.1% in females; extramedullary hematopoiesis
					Hepatic		48.7 F		7/9 females exhibited centrilobular hyperplasia and hypertrophy (less severe when compared to the male mice)
					Hepatic	5 M	15.8 M	48.7 M	SLOAEL: Hepatocyte hyperplasia primarily in the centrilobular region in 9/9 mice LOAEL: Hepatocyte hyperplasia primarily in the centrilobular region in 4/9 mice

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Endocr		5 F		Adrenal lesions that consisted of prominent cellular vacuolization that was restricted to the zona reticularis contiguous with the medulla
					Endocr	48.7 M			
					Repro	48.7			
Hamm Jr. 1984									
CHRONIC EXPOSURE									
11	RAT (Fischer-344) 280B	505 days over 2 years, 5 days / week, 6 hours / day	0, 1, 5, 24.8	HE, HP, CS	Death	24.8			
					Hemato	5 F	24.8 F		Increased pigmentation in the spleen, methemoglobin at 5%
					Hemato		1 M		90% incidence of extramedullary hematopoiesis of the spleen versus 77% in control, 91% incidence of pigmentation of the spleen versus 80% in control
					Hepatic	5 F	24.8 F		Increase in eosinophilic foci (9/66 in control; 16/70 in 25 ppm); increase in spongiosis hepatitis (0/70 in control; 6/70 in 25 ppm)
					Hepatic	1 M	5 M		Incidence of eosinophilic foci increased from 26/69 in control to 44/70; incidence of centrilobular hepatocytomegaly increased from 0/69 in controls to 8/70.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Renal	24.8 F			
					Renal	5 M	24.8 M		16% increase in incidence of tubular hyperplasia of the kidney
					Endocr	24.8			follicular cell hyperplasia of the thyroid
					Repro	24.8 M			
					Cancer			24.8 F	20% increase in the incidence of endometrial stromal polyps
					Cancer			24.8 M	20% increase in incidence of hepatocellular adenoma, 22% increase in incidence of hepatocellular adenoma or carcinoma, 7% increase in incidence of tubular adenoma of the kidney, 9% increase in incidence of tubular adenoma or carcinoma of the kidney
Cattley et al. 1994									
12	RAT (Sprague-Dawley) 70M/70F	505 days over 2 years, 5 days / week, 6 hours / day	0, 1, 5, 24.8	HE, HP, CS	Death	24.8 M			
					Resp	5 M	24.8 M		Increased incidence of inflammation, suppurative exudate and squamous epithelial hyperplasia – not otherwise described
					Hemato		1 M		Methemoglobin levels at 4.08% at 15 months sacrifice, rats at 24 months did not have increased methemoglobin at this dose level

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Hepatic	1 M	5 M		15% increase in the incidence of centrilobular hepatocytomegaly; 19% increase in multinucleated hepatocytes
					Renal	24.8 M			
					Endocr	24.8 M			
					Repro	5 M		24.8 M	43% increase in incidence of bilateral atrophy of the testes, 41% increase in incidence of bilateral hypospermia of the epididymis
					Cancer			24.8 M	9% increase in incidence of hepatocellular adenoma, 11% increase in incidence of hepatocellular adenoma or carcinoma
Cattley et al. 1994									
13	MOUSE (B6C3F1) 70M/70F	505 days over 2 years, 5 days / week, 6 hours / day	0, 5, 24.8, 49.1	BI, BW, CS, GN, HE, HP, LE, OW	Death	49.1			
					Bd wt	49.1			
					Resp	1	5 [d]		87% increase in incidence in males and 92% increase in incidence in females of bronchiolization of the alveoli, females also had 32% increase in incidence of degeneration and loss of the olfactory epithelium ($BMCL_{10}$= 0.93 ppm). 19% increase in incidence of pulmonary adenoma

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Hemato		5 F		7% decrease in red blood cell count, 8% decrease in hematocrit, and 8% decrease in hemoglobin
					Hemato		5 M		11% increase in incidence of bone marrow hypercellularity in femur
					Hepatic	24.8 F	49.1 F		11% increase in incidence of centrilobular hepatocytomegaly
					Hepatic		5 M		22% increase in incidence of centrilobular hepatocytomegaly, 19% increase in incidence of multinucleated hepatocytes
					Renal	49.1 F			
					Renal	24.8 M	49.1 M		15% increase in incidence of kidney cysts
					Endocr	5 M	24.8 M		9% increase in incidence of follicular cell hyperplasia in the thyroid
					Immuno	24.8 F	49.1 F		involution of the thymus increased by 22% 11% increase in incidence of mononuclear cell infiltrate in the pancreas
					Immuno	49.1 M			
					Repro			49.1 M	13% increase in incidence of epididymis hypospermia
					Cancer			49.1 F	8% increase in incidence of adenocarcinoma of the mammary gland
Cattley et al. 1994									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	NOAEL Endpoint (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects

[a] The number corresponds to the entries in Figure 2-2
[b] The data on methemoglobin changes was used in deriving an MRL of 0.04 ppm based on a $BMCL_{1SD}$ of 16 ppm, extrapolated to continuous exposure and converted to a $BMCL_{HEC}$ of 4 ppm. The $BMCL_{HEC}$ was then divided by a composite uncertainty factor and modifying factor of 90 (10 for human variability 3 for animal to human after dosimetric adjustment, 3 for a modifying factor to account for humans increased susceptibility to methemoglobin increases compared to rodents)
[c] The data on adverse hematologic effects was used in deriving an MRL of 0.003 ppm based on a LOAEL of 5 ppm, extrapolated 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 after dosimetric adjustment, 10 for use of a LOAEL)
[d] The data on increased incidence of degradation of the olfactory epithelium was used in deriving an MRL of 0.001 ppm based on a $BMCL_{10}$ of 0.93 ppm extrapolated to continuous exposure and converted to a $BMCL_{HEC}$ of 0.04 ppm. The $BMCL_{HEC}$ was then divided by an uncertainty factor of 30 (10 for human variability 3 for animal to human after dosimetric adjustment)

BI = Biochemical Changes; BC = blood chemistry; Bd wt or BW = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; G = gavage; Gastro = gastrointestinal; GGT = gamma-glutamyl transferase; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE= lethality; LOAEL = lowest-observed-adverse-effect level; LD_{50} = lethal dose, 50% kill; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SLOAEL = serious LOAELUR = urinalysis

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

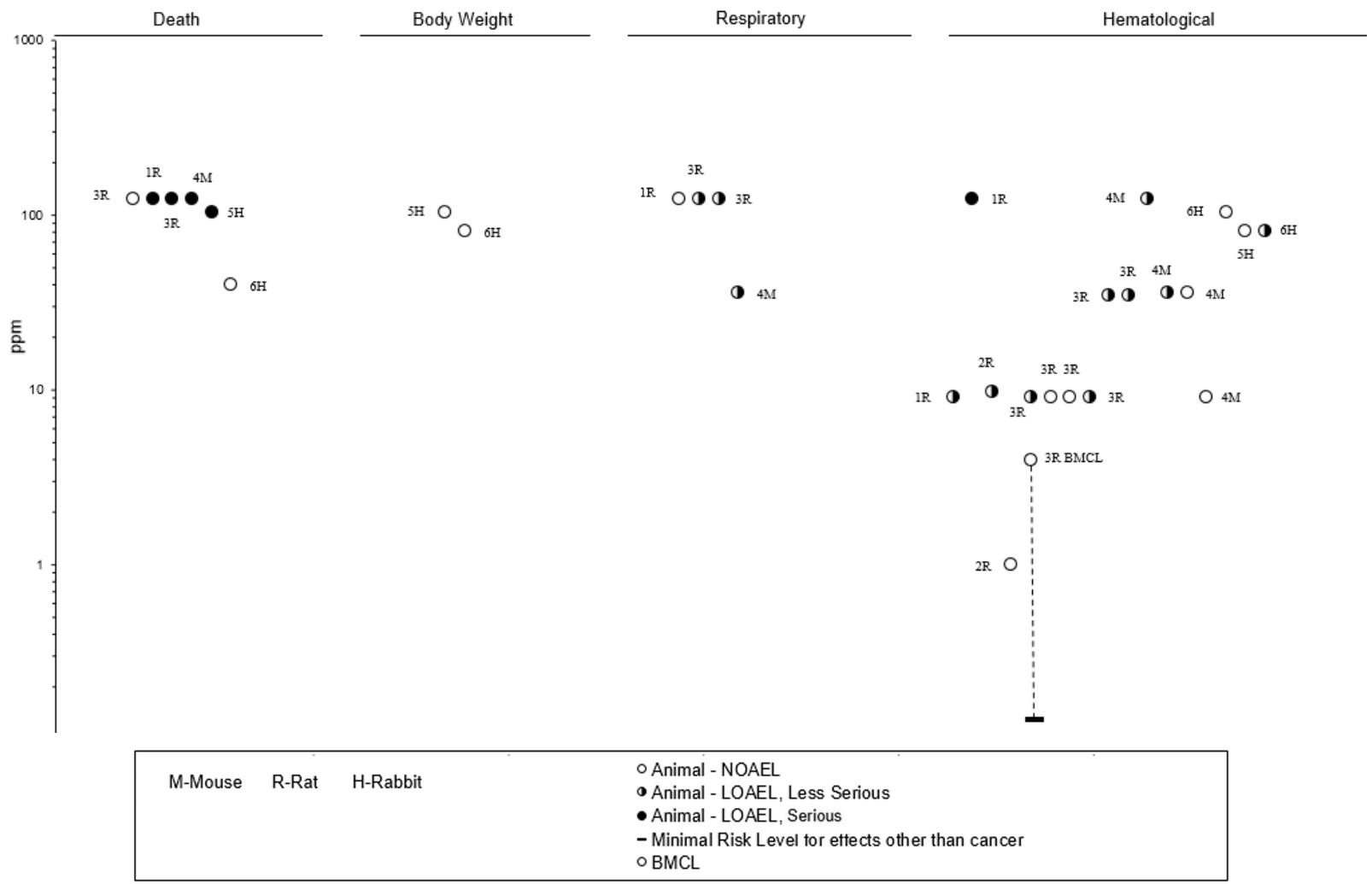


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Acute (≤ 14 days)

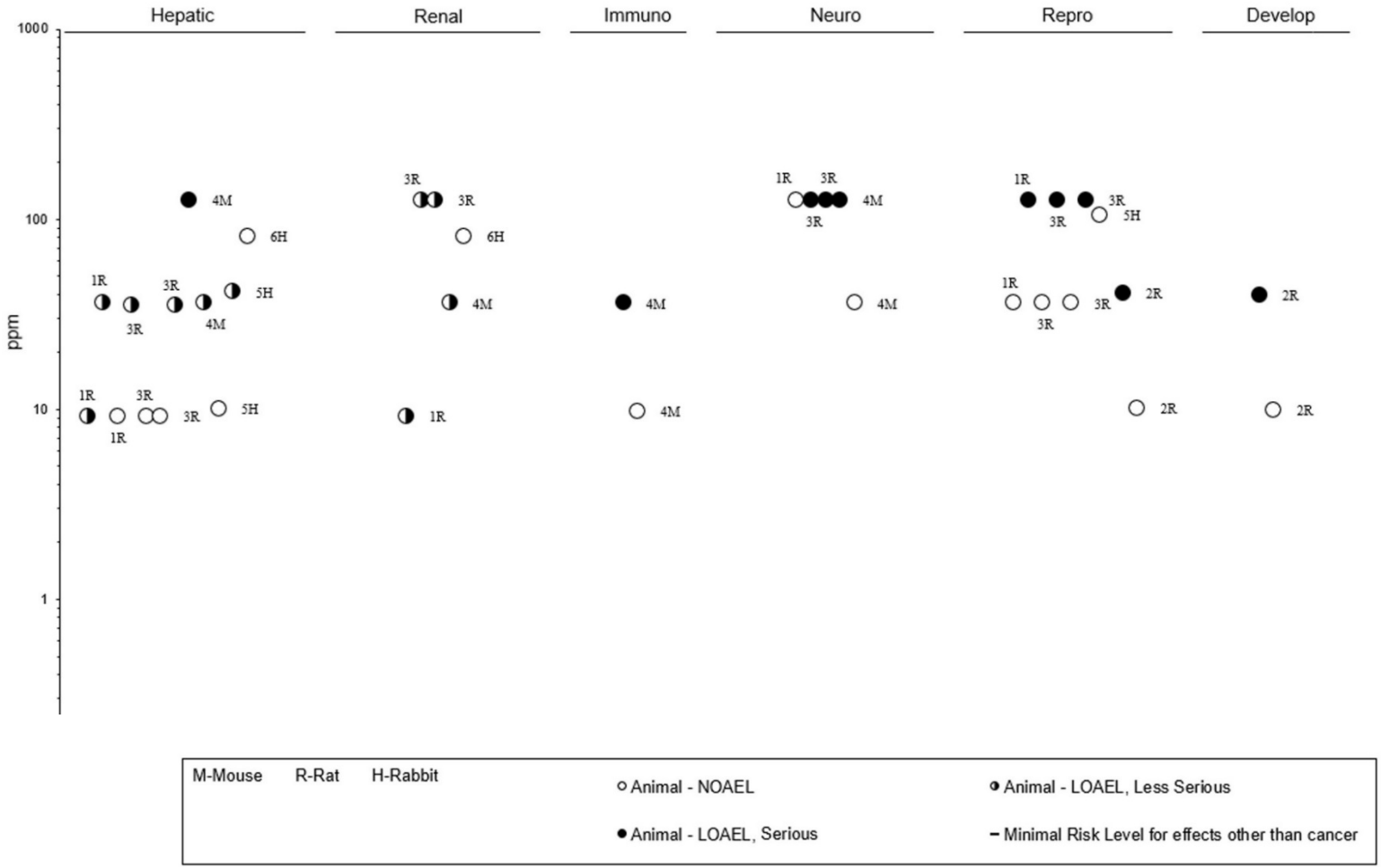


Figure 2-2. Levels of Significant Exposure to Nitrobenzene - Inhalation
Intermediate (15-364 days)

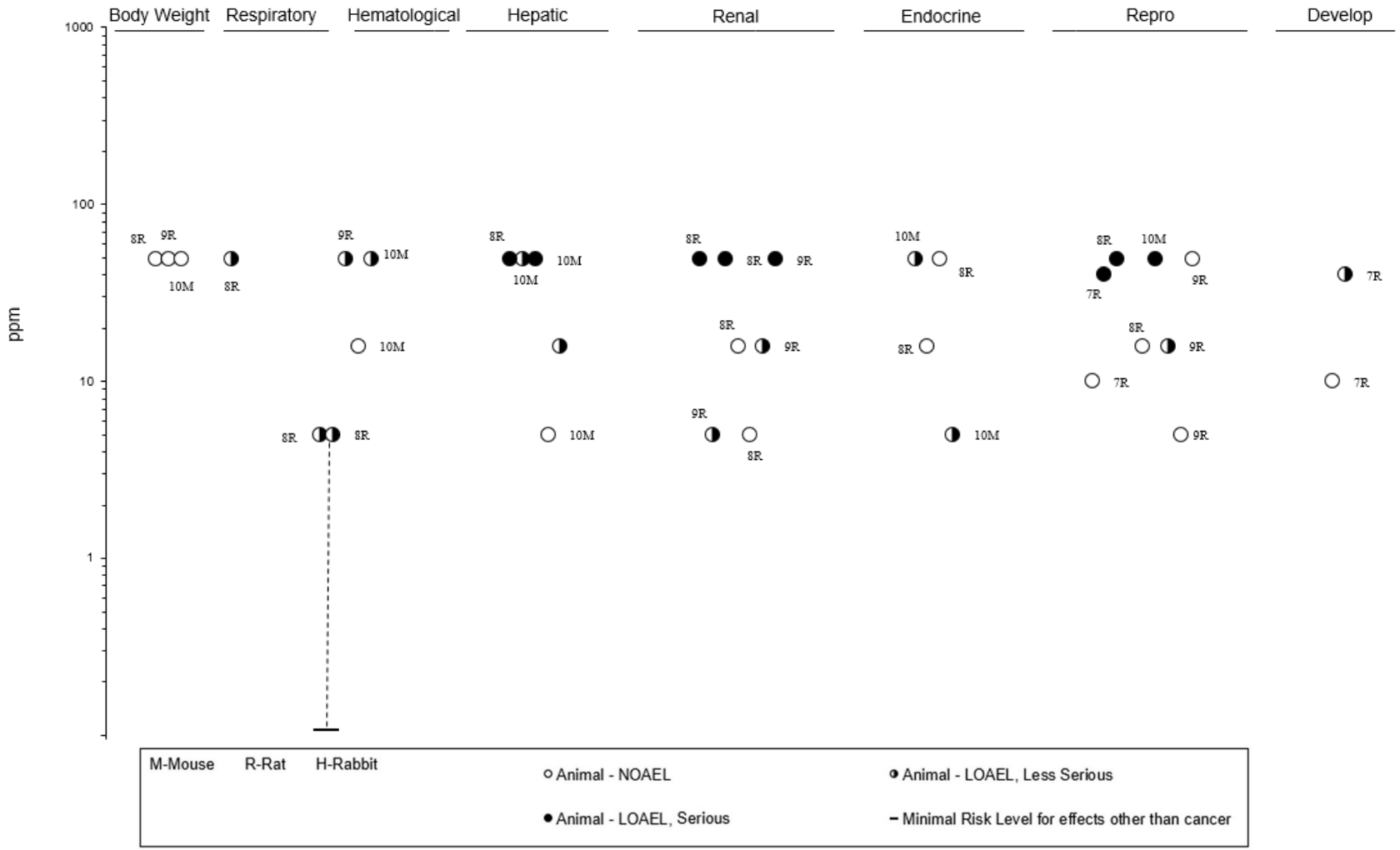


Figure 2-2. Levels of Significant Exposure to Nitrobenzene - Inhalation
Chronic (≥365 days)

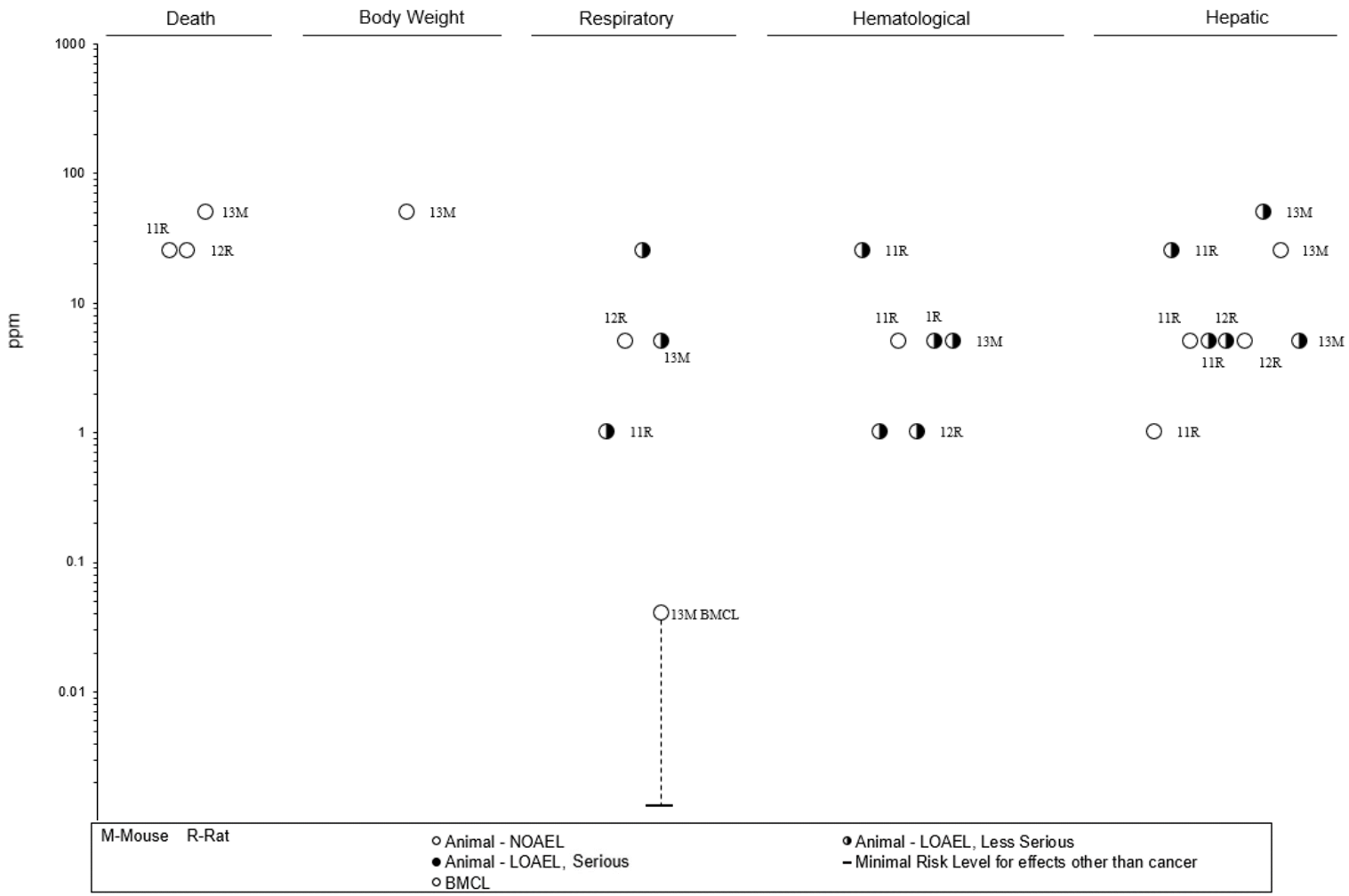


Figure 2-2. Levels of Significant Exposure to Nitrobenzene - Inhalation
Chronic (≥365 days)

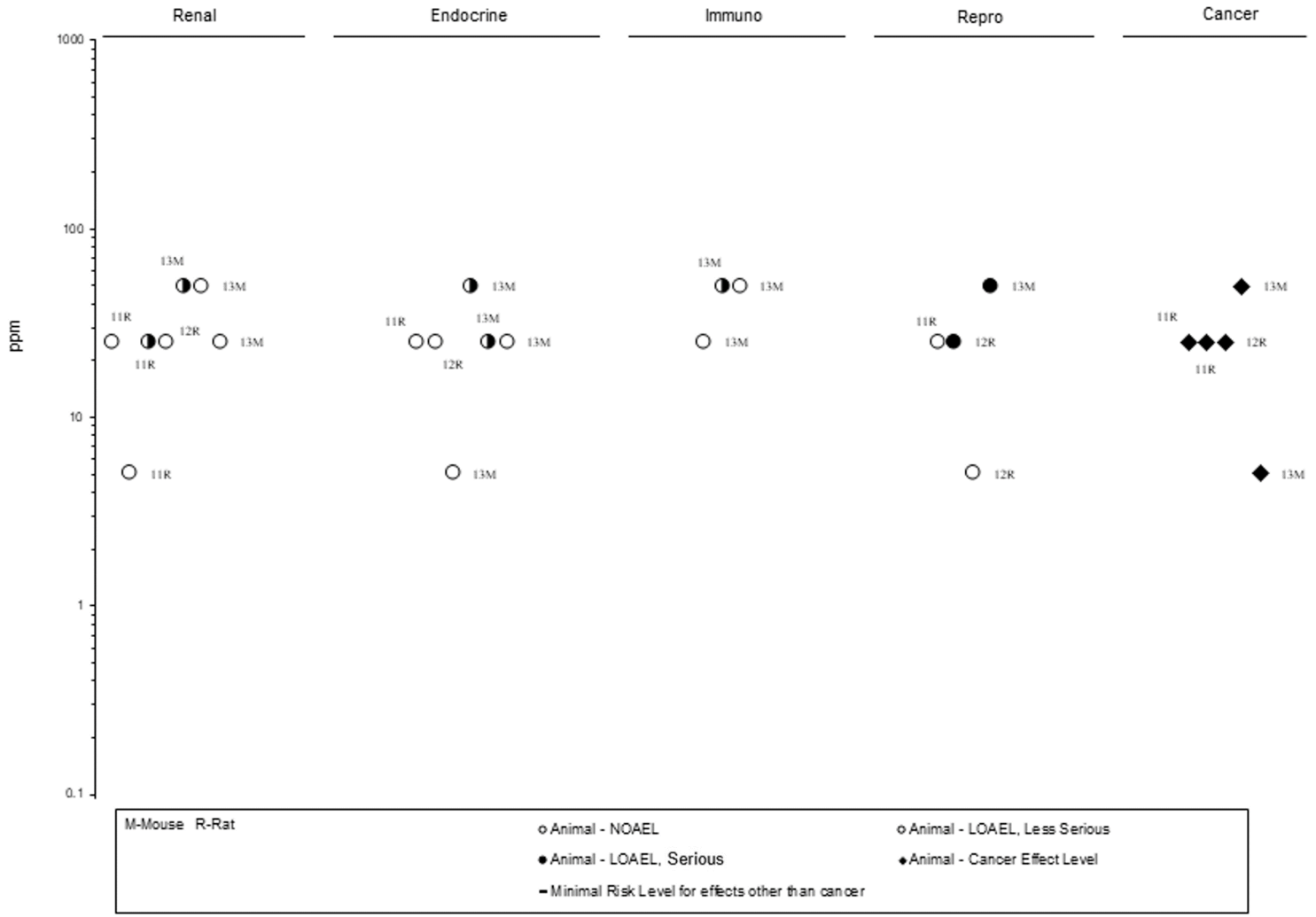


Table 2-2. Levels of Significant Exposure to Nitrobenzene – Oral

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)	Effects
ACUTE EXPOSURE									
1	RAT (Fischer-344) 50M	Once; 3 groups with varying pectin in diet (0, 5% and 8.4%) (GO)	0, 50, 100, 150, 200, 400, 600	HE	Hemato	150 M		200 M	NOAEL: All groups regardless of diet SLOAEL: In groups with 5% and 8.5% pectin: 30-40% methemoglobinemia
Goldstein et al. 1984									
2	RAT (Fischer-344) 10M	Once (GO)	550 mg/kg	HP	Neuro			550 M	Hemorrhages in the brain stem and cerebellum, bilateral symmetric degeneration in the cerebellum and cerebellar peduncles
Morgan et al. 1985									
3	RAT (Fischer-344) 6M	Single dose (GO)	300 mg/kg	RX	Repro			300 M	Cessation of sperm output 32 days after exposure. On days 76-100 after treatment rate of sperm production increased to 78% of controls.
Levin et al. 1988									
4	RAT (Fischer-344) 45 M	Single dose (GO)	300 mg/kg	HP	Repro			300 M	Degeneration of the seminiferous epithelium within 3 days of treatment
Levin et al. 1988									
5	RAT (Sprague-Dawley) 15M	Once / day for 1 -2 weeks (GO)	60 mg/kg	OW	Repro		60 M		Relative weight of testes decreased to approximately 50% of controls with 2 weeks of dosing.

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
Iida et al. 1997									
6	RAT (Sprague-Dawley) 16M	Once/day for 3 days (G)	0, 60	HP, RX, OW, BW	Bd wt	60 M			
					Repro	60 M			
Kawaguchi et al. 2004									
7	RAT (Sprague-Dawley) 40M	Once / day; exposed for 70 days observed at 21 days, 28 days, 42, days, 56 days, or 70 days (GO) (GO)	60 mg/kg	RX, OW	Repro			60 M	Significantly decreased testicular and epididymal weights, sperm count decreased to 34% of controls, decreased sperm motility, all after 14 days. No impacts after only 7 days.
Kawashima et al. 1995									

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
8	RAT (Sprague-Dawley) 12M	Once (GO)	0, 300	OW, RX, HP	Repro			300 M	Two-days post-treatment: degenerating and missing pachytene spermatocytes, immature germ cells and debris in epididymis; 14 days post-treatment: maturation depletion of spermatids, some multinucleated giant cells, testicular debris throughout epididymis, significantly decreased weight of testes and epididymis (magnitude not specified), decreased cauda and caput counts, abnormal caput and cauda sperm morphology
Linder et al. 1992									
9	RAT (Wistar) 36M	Once (GO)	0, 300	OW, RX, BI	Repro		300 M		13 and 23% decrease in testicular weight compared to control 1 and 3 days post-treatment, respectively; decrease in S-methionine incorporation in later stages of spermatogenic cycle
McLaren et al. 1993									

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
10	RAT (Sprague-Dawley) 21M	Once (GO)	0, 250	HP	Repro			250 M	Degeneration of late pachytene spermatocytes and evidence of cell apoptosis 24 hours after dosing, spermatid degeneration and formation of multinucleated giant cells derived from spermatids after 2 and 3 days post-treatment
Shinoda et al. 1998									
11	MOUSE C57 and CBA NS M	Once (GO)	0, 100 to 800 mg/kg	HP	Repro		400 M		Increased apoptosis index of primary and secondary spermatocytes
Richburg and Nañez 2003									
12	MOUSE B6C3F1 8F	once/day (GO)	30, 100, 300	BC, BI, BW, CS, FI, GN, HE, HP, IX, LE, OW	Death			300 F	8.5% of mice died between 1st and 14th day of exposure
					Bd wt	100 F	300 F		12% increase in body weight
					Resp	300 F			
					Hemato		30 F [b]		Dose-dependent increase in number of cells in the bone marrow, DNA synthesis, and the number of granulocyte-monocyte progenitor cells per femur ($BMDL_{1SD}$= 4.7 mg nitrobenzene /kg/day)
					Hepatic	30 F	100 F		12% increase in relative liver weight

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
					Renal	100 F	300 F		10% increase in kidney weight
					Immuno	30 F	100 F		50% decrease in IgM AFC in the spleen cells; increase in Natural Killer Cell activity at 30:1 and 100:1 effector: target ratios. 26% increase in relative thymus weight that was not apparent at 300 mg/kg/day
					Neuro		300 F		10% decrease in relative brain weight
Burns et al. 1994									
INTERMEDIATE EXPOSURE									
13	RAT (Sprague-Dawley) 10M	Once / day for 3 wks (GO)	60 mg/kg	OW, HP	Repro			60 M	Relative weight of testes decreased by more than 50% of controls; atrophy of the epididymis
Iida et al. 1997									

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
14	RAT (Sprague-Dawley) 16M	Once / day for 18 days (G)	60 mg/kg	HP, RX, OW, BW	Bd wt	60 M			
					Repro			60 M	Testes weight approximately 40% of control, epididymis weight decreased to approximately 11% of control. All rats exhibited atrophy of seminiferous tubules and decreased sperm concentration in the cauda. 3 out of 8experienced decreased sperm concentrations in the caput/corpus. 4 and 7 out of 8 rats had cell debris in the caput/corpus and cauda, respectively.
Kawaguchi et al. 2004									
15	RAT (Sprague-Dawley) 100M	Once / day; 21 days, 28 days, 42, days, 56 days, or 70 days (GO)	60 mg/kg	RX, OW	Repro		60 M		Significantly decreased testicular and epididymal weights, Sperm count decreased to 10% of controls, decreased sperm motility, decreased sperm viability, increased abnormal sperm rate, all starting at 21 days.
Kawashima et al. 1995									

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
16	RAT (Sprague-Dawley) 40M 40F	Once / day, 54 days (GO)	0, 20, 60, 100	BW, HE, GN, HP, LE, RX, DX, BC, OW	Death			100	Decrease in number of pups alive on day 0 after gestation by 80% compared to controls. 2/10 male rats. 7/10 during gestation period. 2/10 during gestation lactation period.
					Bd wt		100 M		Approximately 16% decrease in body weight
					Hemato		20 M		Approximately 13% decrease in red blood cells; approximately 11% decrease in hemoglobin, methemoglobin levels rose to 3.64 percent, compared to 0.70% in controls, increase of hematopoiesis in bone marrow, increased absolute and relative spleen weight, increase in extramedullary hematopoiesis in spleen and brown pigmentation in the spleen

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
					Hepatic		20 M		Increased absolute (approximately 20%) and relative liver weight (approximately 18%), centrilobular swelling of hepatocytes, brown pigmentation in Kupffer cells, extramedullary hematopoiesis
					Renal		20 M		Brown pigmentation in proximal tubules
					Neuro			100 M	Necrosis / gliosis in central nervous system
					Repro			60 M	Approximately 60 % decrease in absolute and relative testes weight, approximately 20% decrease in absolute epididymis weight and approximately 18% decrease in relative epididymides weight, atrophy of seminiferous tubules, Leydig cell hyperplasia, loss of intraluminal sperm or cell debris in epididymis
					Develop		60		Decrease in bodyweight of male and female pups alive on day 0 and day 4 post-gestation

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
					Develop		20 M		6.25% decrease in bodyweight on day 4 of lactation
Mitsumori et al. 1994									
17	RAT (Fischer-344) 10 M/10F	once/day (GO), 90 days	9.375, 18.75, 37.5, 75, 150	BW, CS, FI, GN, HE, HP, LE, NX, OW	Death	75		150	Nine male and three females died prior to study completion
					Resp	9.375 F	18.75 F		Lung weight ratios with body weight and brain weight were significantly increased
					Cardio		9.375 F		Heart weight and its ration to final body weight significantly increased
					Hemato		9.375 [c]	75	SLOAEL: Cyanosis LOAEL: Increased methemoglobin (2.4-fold of controls) and absolute reticulocyte (16% of controls), and decreased hemoglobin (3% of control) ($BMDL_{1SD}$ =1.8 mg nitrobenzene/kg/day)
					Hepatic		9.375 F		Liver weight, ratio of liver to body, and ratio of liver to brain weight significantly increased

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
					Renal		9.375 M		Ratio of kidney with final body weight were consistently increased and significant.
					Neuro			75 F	Ataxia
					Repro			75 M	SLOAEL: 9/10 atrophy of the testes, 10/10 hypospermatogenesis.
NTP 1983a									
18	MOUSE (B6C3F1) 10	once/day (GO), 90 days	18.75, 37.5, 75, 150, 300	BW, CS, FI, GN, HE, HP, LE, NX, OW	Death	150 M		300 M	3 mice died prior to study completion between weeks 4 and 5
					Hemato		18.75		Increase in methemoglobin in males 2-fold and in females 27% of controls
					Hepatic		18.75 F		8% increase in absolute liver weight
					Hepatic	75 M	150 M		15% increase in relative liver weight
					Renal	37.5 M	75 M		Right kidney weight and its ratio with brain weight was significantly increased by 12%.
					Immuno			300 F	Lymphoid depletion 5/10 for females
					Neuro	150		300	Male mice were ataxic, female mice were irritable

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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)	Effects
					Repro		300 M		Decrease in right testes weight and its ratio with body weight
NTP 1983a									

[a] The number corresponds to the entries in Figure 2-3
[b] Used to derive an acute MRL of 0.05 mg/kg/day; the data for methemoglobin was used to conduct dose response modeling, resulting in a $BMDL_{1SD}$ of 4.7 mg/kg/day divided by an uncertainty factor of 100 (10 for animal to human, 10 for human variability)
[c] Used to derive an intermediate MRL of 0.02 mg/kg/day; the data for methemoglobin was used to conduct dose response modeling, resulting in a $BMDL_{1SD}$ of 1.8 mg/kg/day divided by an uncertainty factor of 100 (10 for animal to human, 10 for human variability)

BI = biochemical changes, BC = blood chemistry; Bd wt or BW = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; G = gavage; Gastro = gastrointestinal; GGT = gamma-glutamyl transferase; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE= lethality; LOAEL = lowest-observed-adverse-effect level; LD_{50} = lethal dose, 50% kill; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurological function NS = not specified; OP= ophthalmology, OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; UR = urinalysis

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

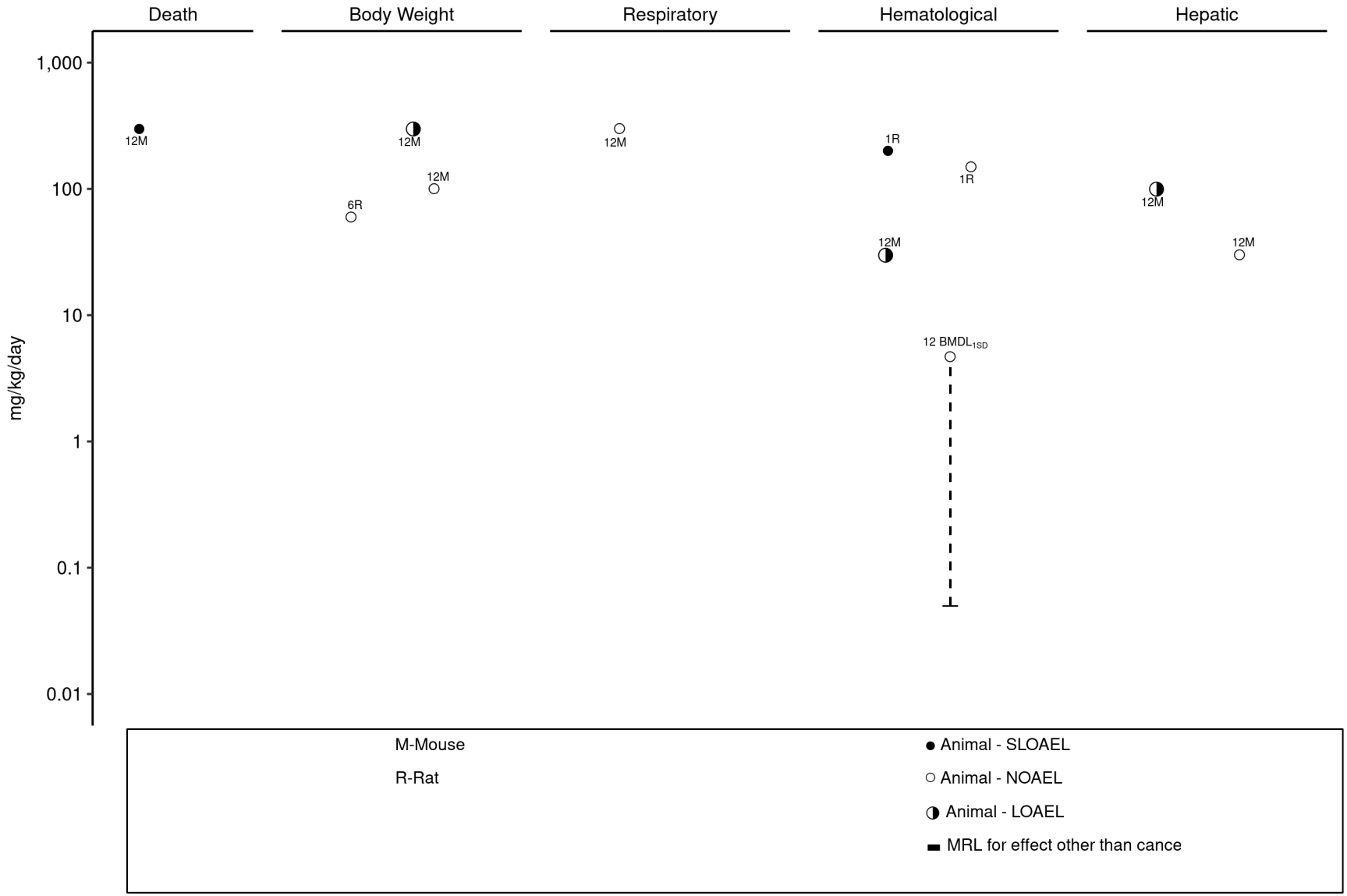


Figure 2-4. Levels of Significant Exposure to Nitrobenzene - Oral

Acute (≤ 14 days)

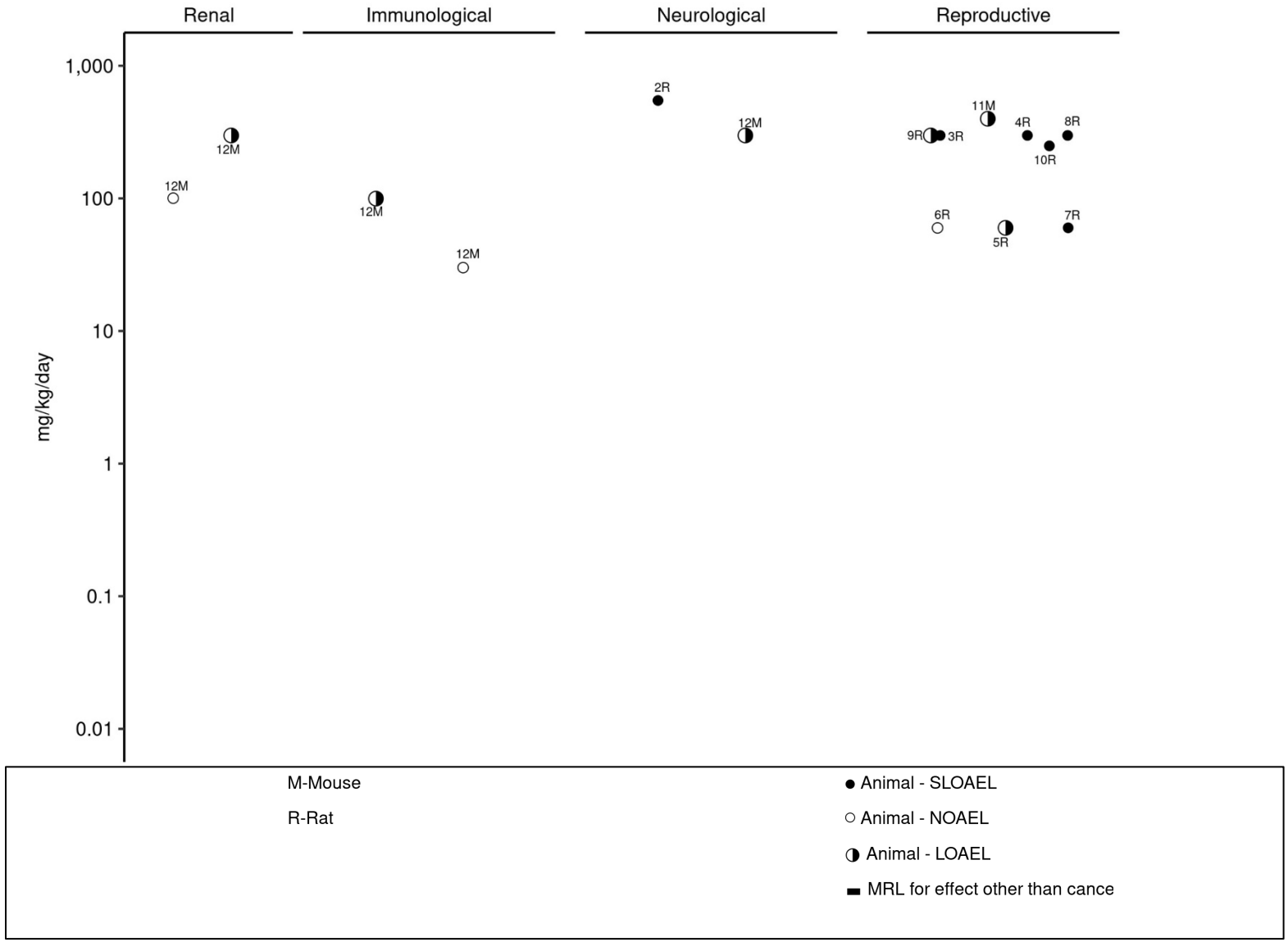


Figure 2-3. Levels of Significant Exposure to Nitrobenzene - Oral

Intermediate (15-364 days)

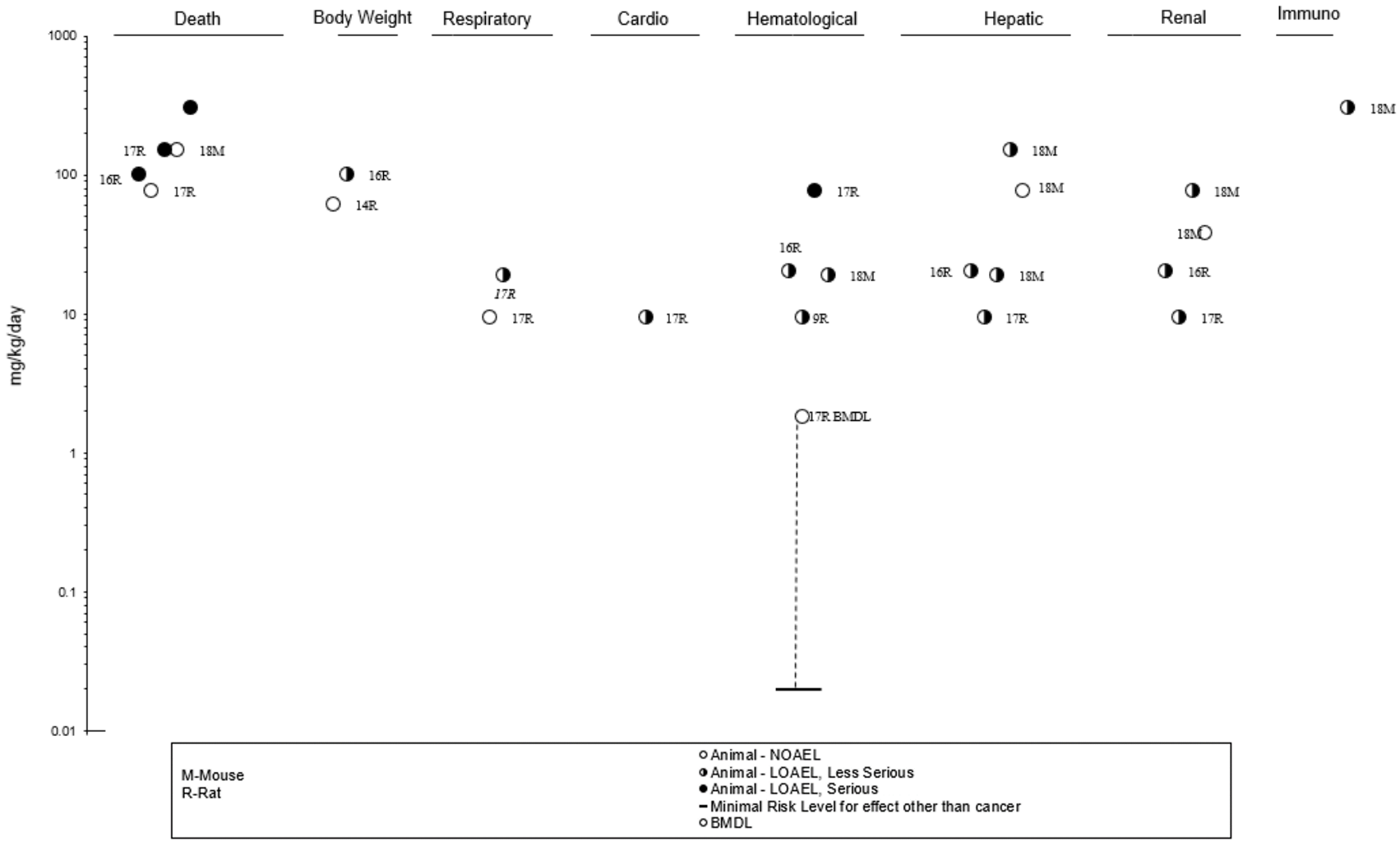


Figure 2-3. Levels of Significant Exposure to Nitrobenzene - Oral
Intermediate (15-364 days)

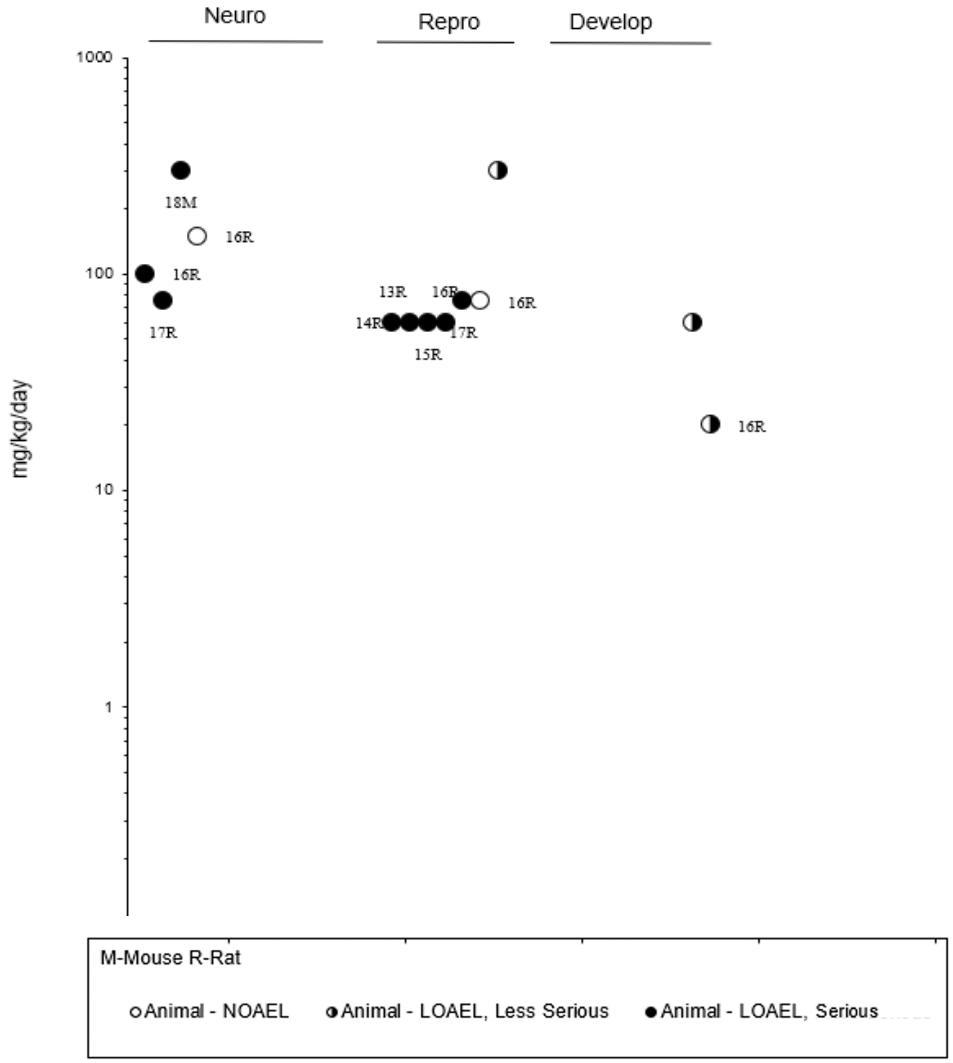


Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
RAT (Fischer-344) 30M 30F	Once / day, 12 days	0, 0.2, 0.4, 0.8, 1.6, 3.2 g/kg	CS, GN, HE, OW, BW, LE, HP	Death	0.8 g/kg		1.6 g/kg	5/5 males and 5/5 females
				Bd wt	3.2 g/kg			
				Resp	0.8 g/kg	1.6 g/kg		Varying degrees of lung congestion
				Hemato			0.2 g/kg	Males: hemoglobin decreased to 14 g/dl from 15.8 g/dL in controls, hematocrit decreased to 7.23% from 8.8% in controls, red blood cells decreased from 8.8 $x10^6$ to 7.2 $x10^6$, reticulocytes increased from 3.4% in control to 11.4%, methemoglobin increased from 0.5% in controls to 7.7%. All changes were significant Females: hemoglobin decreased from 15.9 g/dL to 14.3 in controls g/dL; hematocrit decreased from 43.3 to 38.3% in controls, RBCs decreased from 8.072 $x10^6$ in controls to 6.7 $x10^6$; reticulocytes increased to 11.62% from 1.66%, methemoglobin levels increased from 0.6% to 6.9%. All changes with the exception of RBC levels were significant.
				Hepatic		0.2 g/kg		18% increase in ratio of liver weight to final body weight in males, 22% increase in ratio of liver weight to final body weight in females
				Hepatic	0.2 F g/kg			
				Hepatic	0.8 M g/kg			
				Renal	0.8 F g/kg	1.6 F g/kg		Renal cortical tubule degeneration
				Neuro	0.4 g/kg		0.8 g/kg	Inactivity, ataxia, prostration

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
				Repro	0.4 M g/kg		0.8 M g/kg	Seminiferous tubule atrophy
NTP 1982								
MOUSE (B6C3F1) 30M 30F	Once / day, 12 days	0, 0.2, 0.4, 0.8, 1.6, 3.2 g/kg	CS, GN, HE, OW, BW, LE, HP	Death	0.8 g/kg		1.6 g/kg	5/5 males and 5/5 females
				Bd wt			0.2 g/kg	36% decrease in body weight in males, 31% decrease in females
				Hemato	0.2 g/kg	0.4 g/kg		For males and females there was statistically significant decrease in hemoglobin concentration (males: 14.9 g/dL in controls to 14.2 g/dL; females: 14.72 to 13.5 g/dL) and hematocrit (males: 48.4% in controls to 44.6%; females: 47.7%in controls to 39.8%) and statistically significant increase in reticulocytes (males: 3.32% in controls to 5.1 %; females: 3.75% in controls to 10.56%) and methemoglobin (males: 0.42% in controls to 1.9% in males; females: 0.43% in controls to 2.16%). Female mice also had significantly increased read blood cell counts (9.43 x 10^6 to 7.28 x10^6).
				Hepatic	0.2F g/kg	0.4 F g/kg		8% increased liver weight, 9% increase in liver weight to final body weight and liver weight to brain weight ratios
				Hepatic	0.8 M g/kg			
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) 60M 60F	90 days	0, 0.05, 0.1, 0.2, 0.4, 0.8 g/kg	BW, CS, GN, HP, LE, RX, FI, HE	Death	0.4 g/kg		0.8 g/kg	10/10 males and 10/10 females
				Bd wt	0.8 g/kg			
				Hemato		0.05 g/kg		Males: Significant increase in methemoglobin levels (from 0.5% in controls to 0.99%) Females significant increase in methemoglobin (from 0.67% in controls to 1.57%) and significant decrease in RBCs (from 8.37 $X10^6$ in controls to 7.99 $x10^6$)
				Hepatic		0.05 M g/kg		10% increased ratio of liver weight to final body weight
				Renal	0.05 F g/kg	0.1 F g/kg		7% increase in ratio of kidney weight to final body weight
				Renal	0.1 M g/kg	0.2 M g/kg		3% increase in ratio of kidney weight to body weight
				Neuro	0.2 F g/kg		0.4 F g/kg	Hemorrhages in the brain
				Neuro	0.4 M g/kg		stem 0.8 M g/kg	Hemorrhage in the brain
				Repro	0.2 M g/kg	0.4 M g/kg	stem	Atrophic seminiferous tubules
NTP 1983b								
MOUSE (B6C3F1) 60M 60F	90 days	0, 0.05, 0.1, 0.2, 0.4, 0.8 mg/kg	BW, CS, GN, HP, LE, RX, FI, HE	Death	0.4 mg/kg		0.8 mg/kg	9/10 males, 8/10 females died
				Bd wt	0.8 mg/kg			

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
				Hemato		0.05 mg/kg		Males: There was a significant increases reticulocytes (from 2.46% in controls to 3.18%) and a significant decreased in hemoglobin (from 15.15 g/dL in controls to 14.66 g/dl); Females: there was a significant increase in methemoglobin (from 1.36% in controls to 2.36%)
				Hepatic	0.2 mg/kg	0.4 mg/kg		9% liver weight increase in males, 13% liver weight increase in females
				Renal	0.2 F mg/kg	0.4 F mg/kg		Kidney congestion
				Renal		0.2 M mg/kg		9% right kidney weight increase
				Immuno	0.4 mg/kg	0.8 mg/kg		Atrophy of the thymus gland
				Neuro	0.4 F mg/kg		0.8 F mg/kg	Prostrate, inactive, circling, leaning to one side
				Neuro		0.2 M mg/kg		10% decreased ratio of brain weight to final body weight
				Repro		0.8 M mg/kg		Decreased size of testes
NTP 1983b								

BI= biochemical changes, BC = blood chemistry; Bd wt or BW = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; G = gavage; Gastro = gastrointestinal; GGT = gamma-glutamyl transferase; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE= lethality; LOAEL = lowest-observed-adverse-effect level; LD_{50} = lethal dose, 50% kill; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NX = neurological function NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; UR = urinalysis

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. For example, Martinez et al. (2003) reported on a fatal poisoning where the presence of nitrobenzene was analytically confirmed. Additionally, there are reports of fatal poisonings in humans where the ingested substance is known to be nitrobenzene (Gupta et al. 2012; Gupta et al. 2000; Kumar et al. 2017). In a case presented by Kumar et al. (2017), a young girl with a clear history of nitrobenzene ingestion was treated for suspected acute methemoglobinemia. After sixteen days of treatment, 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. Gupta et al. (2012) and Martinez et al. (2003) also present cases of death following nitrobenzene ingestion and severe methemoglobinemia. In the case presented by Martinez et al. (2003), 250 mL of an unknown substance were ingested, and analysis of blood sample after 48 hours, while the subject was alive determined there was 3.2 μg/mL of nitrobenzene in the blood. Gupta et al. (2012) also did not report the amount of nitrobenzene ingested, but notes that the lethal dose in adults ranges from 2 to 6 g after obtaining the blood samples for analysis within 1 hour of nitrobenzene ingestion while the subject was alive. Given that death in both studies happened after 3-4 days of nitrobenzene ingestion, the differences in blood levels between the two reports are likely due to the different timepoints the blood samples were collected and lack of information regarding the ingested amount of nitrobenzene (Martinez e al. 2003; Gupta et al. 2012).

Deaths have been reported in laboratory animals following acute, intermediate and chronic duration exposure studies.

In an acute inhalation study, Fischer 344 and Sprague-Dawley rats and B6C3F1 mice exposed to nitrobenzene vapors of 10, 35, and 125 ppm demonstrated species differences in response to nitrobenzene exposure (Medinsky and Irons 1985). All B6C3F1 mice exposed to 125 pm of nitrobenzene experienced early morbidity necessitating sacrifice between 2 and 4 days after exposure. Five male and three female Sprague-Dawley rats died four days after exposure, resulting in a 40% rate of lethality. The cause of death in Sprague-Dawley rats exposed to 125 ppm nitrobenzene was presumed to be perivascular hemorrhage in the cerebellar peduncle frequently accompanied by varying degrees of edema and malacia. Fischer-344 rats, however, tolerated this level for 2 weeks without any adverse clinical signs.

In another acute inhalation study, male Sprague-Dawley rats were exposed to 439, 514, 542, 555, 578, and 714 ppm of nitrobenzene four hours at a time (Dupont 1981). No rats died in the two groups exposed to the two lowest concentrations, 1 out of 10 rats exposed to 542 ppm died, 7 out of 10 rats exposed to 555 ppm

died, 8 out of 10 rats exposed to 578 ppm died, and all 10 rats exposed to 714 ppm died. With the exception of one rat exposed to 555 ppm of nitrobenzene, all rats that died did so 1 to 2 days post-exposure. The LD50 (lethal dose, 50% kill) was calculated to be 556 ppm. An acute inhalation study on female New Zealand white rabbits treated with 10, 40, or 100 ppm nitrobenzene for 6 hours a day during the day 7-19 gestation interval resulted in no mortality in the control or low (10 ppm) dose groups. In the mid dose group (40 ppm), one female died post-mating on day 3, one female was killed in a moribund condition and one female aborted. In the high-dose group (100 ppm), all but two females survived to term sacrifice; one was killed in a moribund condition and one was sacrificed after a premature delivery (Biodynamics 1984). Another acute inhalation study on female New Zealand white rabbits investigated the toxicity of nitrobenzene administered via inhalation at targeted concentrations of 10, 40, and 80 ppm. There was no mortality in the control, low- or high-dose groups. One female was killed in a moribund condition on day 14 of gestation in the mid-dose group after failing to improve after a back injury while being bled (Biodynamics 1983).

A chronic inhalation study found that B6C3F1 mice exposed to 0, 5, 25, or 50 ppm nitrobenzene and Fischer 344 and Sprague-Dawley rats exposed to 0, 1, 5, or 25 ppm nitrobenzene for six hours/day did not experience significantly higher rates of mortality than control groups based on trend test and pairwise comparison (Cattley et al. 1994). Cattley et al. (1994) refers to Sprague-Dawley rats as CD rats, but this profile calls them Sprague-Dawley rats for consistency

In an acute-duration oral toxicological evaluation in female B6C3F1 mice 8.5% of the mice given 300 mg/kg nitrobenzene in corn oil for 14 days died (Burns et al., 1994). Nitrobenzene was orally administered via repeated dose gavage to Fischer 344 rats and B6C3F1 mice during a 90-day intermediate study. Dose levels ranged from 9.375 to 150.0 mg/kg for rats and 18.75 to 300 mg/kg for mice. At the highest dose level in rats (150 mg/kg), nine of ten male rats and three of ten female rats died prior to study completion. Three male mice at the highest dose level (300.0 mg/kg) died prior to study completion. Other deaths occurred in this study but were not related to nitrobenzene exposure (NTP 1983a).

Oral administration of 100 mg/kg nitrobenzene via gavage during an intermediate study resulted in the deaths of 2 of 10 male Sprague-Dawley rats during an intermediate study; oral administration of the same dose to female Sprague-Dawley rats resulted in seven of ten females dying during the gestation period and two more dying during the lactation period. One of ten females given 60 mg/kg and one of ten females given 20 mg/kg also died during the lactation period (Mitsumori et al. 1994). Approximately 80% fewer pups were born to dams in the 200 mg/kg dose group.

During an acute study, Fischer 344 rats and B6C3F1 mice were exposed to nitrobenzene via dermal application for 12 days. All rats of both sexes given 1.6 and 3.2 g/kg of nitrobenzene either died or were sacrificed moribund (NTP 1982).

In a 13-week intermediate study of dermal toxicity, rats and mice were administered nitrobenzene at dose levels ranging from 0.05 to 0.80 g/kg. All rats, nine male mice, and eight female mice given 0.80 g/kg died before the end of the study. (NTP 1983b). In a study evaluating the LD50 of nitrobenzene (Smyth Jr. et al. 1969) female albino rats weighing between 90 and 120 g were intubated with chemicals, including nitrobenzene. An LD_{50} of 600 mg/kg in rats was observed in Smyth et al. (1969).

2.3 BODY WEIGHT

No human studies were located evaluating body weight effects of nitrobenzene exposure following inhalation, oral or dermal exposure. However, experimental animal studies have reported on this effect. For example, in a 30-day acute inhalation study in rabbits treated with 10, 40, or 100 ppm nitrobenzene for 6 hours a day during days 7-19 of gestation resulted in comparable values for mean body weight during gestation between the control and treated groups. Mean weight change during days 0-7 pre-treatment interval of gestation were comparable between the control, 10 ppm, 40 ppm, and 100 ppm groups. The mean weight gain in the 40 ppm group was significantly less than that of the control during pre-treatment. During the day 7-19 gestation interval, there was a slight weight gain comparable to the control in the 10 ppm and 40 ppm groups. There was a slight mean weight loss observed in the high-dose group; this was not statistically significant (Biodynamics 1984). Male Sprague-Dawley rats exposed to 439 to 714 ppm of nitrobenzene for a single 4 hour period experienced an 8 to 21% weight loss in 1 to 4 days after exposure; the surviving rats experienced normal weight gain after (Dupont 1981).

In an acute inhalation study on the toxicity of nitrobenzene in pregnant New Zealand white rabbits, nitrobenzene was administered via inhalation at concentrations of 10, 40, and 80 ppm for 6 hours per day. Mean body weight was similar throughout gestation between the control and treated groups. Mean weight change differences between the control and treated groups during gestation was not statistically significant. Mean weight gain during the pre-treatment gestation interval was similar between the control, low-, and high-dose groups. Mean weight gain during this same interval in the 40 ppm group was slightly lower than mean weight gain in the control group. Mean weight gain during the day 7-20 gestation interval was similar between the control, low- and high-dose groups. There was a slightly higher mean weight gain in the mid-dose group compared to the control, but it was not statistically significant (Biodynamics 1984).

In a study investigating chronically inhaled nitrobenzene in B6C3F1 mice and Fischer 344 and Sprague-Dawley rats, neither mice nor rats exposed to 50 ppm nitrobenzene for 90 days for six hours per day

exhibited reductions in body weight (Cattley et al. 1994). Statistically significant fluctuations in mean body weight occurred over the course of the two-year study but were inconsistent and never deviated more than 10% from the means. Male B6C3F1 mice exposed to 50 ppm, female and male Fischer 344 rats exposed to 1 ppm, and male Fischer rats exposed to 25 ppm nitrobenzene from 16 weeks until final euthanasia had consistent mild body weight depression.

Female B6C3F1 mice which were administered nitrobenzene for 14 days via gavage with corn oil displayed a 12% increase in body weight (Burns et al., 1994). The authors hypothesized this increase in body weight was likely due to fluid retention in the high dose group. Female mice receiving 100 mg/kg in the same study did not demonstrate a significant increase in body weight (Burns et al., 1994). Male Sprague-Dawley rats given 100 mg/kg nitrobenzene orally during an intermediate study showed decreased body weight gain at day 21 of exposure, but this was accompanied by a decrease in food consumption (Mitsumori et al. 1994). Females given the same dose also showed depressed body weight gain on day 21 of pregnancy and during the lactation period. Females given the 60 mg/kg showed an inhibition of body weight gain during the lactation period, accompanied by a decrease in food consumption (percentage not reported).

Nitrobenzene was orally administered via repeated dose gavage in corn oil to Fischer 344 rats and B6C3F1 mice during a 90-day study. Dose levels ranged from 9.375 to 150.0 mg/kg for rats and 18.75 to 300 mg/kg for mice. Although, male rat weight change was depressed in dose dependent manner at the 37.5 mg/kg dose level, mean final body weight was not significantly different at any dose level for either rats or mice when compared to controls. Additionally, the only surviving male rat in the high dose group did have a significant decrease in body weight (>10% compared to control) (NTP 1983a).

During a 12-day dermal exposure study, Fischer 344 rats and B6C3F1 mice were exposed to 1.6 and 3.2g/kg of nitrobenzene via dermal application for 12 days. There was no significant change in body weight in surviving rats (NTP 1982).

In a 13-week intermediate study of dermal toxicity, rats and mice were administered nitrobenzene at dose levels ranging from 0.05 to 0.80 g/kg. Mean final body weight and body weight change of the rats was not significantly different at any dose level when compared to controls. Male rat weight gain in the highest dose group (0.80 g/kg) was slightly depressed over the duration of the study. Mean final body weight of mice was not significantly different at any dose level compared to controls. Male body weight change in mice was significantly increased at the 0.10 to 0.80 g/kg dose levels (NTP 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 2 week inhalation study of nitrobenzene toxicity on male and female F344 and Sprague-Dawley rats and B6C3F1 mice (Medinsky and Irons 1985), researchers observed 8/10 mice with moderate bronchiolar epithelial hyperplasia after 125 ppm for 6 hours per day nitrobenzene exposure and hyperplasia presenting in animals examined 3 days after an exposure of 35 ppm. Further, in dead or moribund Sprague-Dawley rats exposed to 125 ppm nitrobenzene, perivascular edema and vascular congestion was found. Vascular congestion was also observed in half (5/10) the male Sprague-Dawley rats (Medinsky and Irons 1985). In an additional acute study, Biodynamics (1984) observed a high incidence of discolored lungs in New Zealand White rabbits in all exposure groups (doses ranged from 10 to 80 ppm).

Hamm Jr. et al. (1984) observed treatment related lesions in the lungs of F-344 and Sprague-Dawley rats and B6C3F1 mice. Specifically, there was a minimal to slight hyperplasia observed in the bronchial epithelium after 50 ppm exposure for 90 days in male and female rats and mice. In male Sprague-Dawley rats, rhinitis associated with epithelial and goblet cell hyperplasia was observed in nasal turbinates after exposure to 16 and 50 ppm nitrobenzene. Similar effects were observed in female rates exposed to 50 ppm nitrobenzene (Hamm Jr. et al. 1984).

In a two-year study of the toxicity of nitrobenzene Cattley et al. (1994) observed a marked increase in bronchiolization of alveolar walls at all exposure concentrations in B6C3F1 mice (doses in mice ranged from 5-50 ppm). Specifically, with only 5 ppm exposure to nitrobenzene 58/67 male mice and 55/60 female mice displayed alveolar bronchiolization at 50 ppm, the highest dose in the study, 62/66 male and 62/62 female mice displayed this effect. No control mice of either sex exhibited this lesion (Cattley et al. 1994). Further, mice also experienced an increase in degeneration of the olfactory epithelium. Specifically, both male and female mice had an increased incidence of degeneration in the 25 and 50 ppm exposure groups. Females also experienced degeneration at 5 ppm (Cattley et al. 1994).

Further, in Cattley et al. (1994) at 1 ppm exposure to nitrobenzene male and female F344 mice displayed a significant increase in pigment deposition in the olfactory epithelium. The presence of the deposition increased with increasing doses. Male Sprague-Dawley rats displayed the same effect starting at 5 ppm. There was also an increase in the incidence of inflammation, suppurative exudate (25-ppm exposure group), and squamous epithelial hyperplasia (in all exposure groups) in the anterior nose were also increased in male and female F344 mice (Cattley et al. 1994).

In Burns et al., (1994) researchers observed a significant increase in absolute lung weight with 30 mg/kg nitrobenzene exposure in B6C3F1 mice gavaged with nitrobenzene for 14 days. However, the same increase was not observed considering relative lung weight. Therefore, the authors hypothesized that the mechanism leading to fluid retention which was considered the culprit for increasing body weight may have also contributed to the increases in lung weight (Burns et al. 1994). NTP evaluated the toxicity of nitrobenzene with both dermal and oral exposure routes (NTP 1982, 1983a, 1983b). In the oral study B6C3F1 mice and F344 rats of both sexes were dosed via gavage with nitrobenzene in corn oil. For mice doses ranged from 18.75 mg/kg to 300 mg/kg and 9 mg/kg to 150 mg/kg for rats for once a day for 90 days. In this study lung weight ratios were significantly increased in the 18.75, 75 and 150 mg/kg dose groups in rats (NTP 1983a). Similar effects were not observed in mice, indicating likely species differences in the toxicity of orally administered nitrobenzene in the lungs. In the dermal toxicity study, NTP, observed congestion in the lungs in F344 rats and B6C3F1 mice after skin painting (NTP 1982, 1983b). Specifically, this was observed at the 1.6 g/kg dose level in rats and mice of both sexes after 12 days of exposure. With 90 days of dermal exposure 10/10 and 9/10 male and female F344 rats experienced lung congestion with 0.8 g/kg dermal nitrobenzene. Investigators also observed lung congestion in 10/10 male and 8/10 female mice with 0.4 g/kg exposure.

2.5 CARDIOVASCULAR

No relevant studies were located regarding cardiovascular effects in humans or animals after inhalation exposure to nitrobenzene. One case study, Agrawal et al. (2011), reported on a 32-year-old male exposed orally to nitrobenzene who experienced methemoglobinemia and cardiogenic pulmonary edema. The amount of nitrobenzene ingested was not known. These effects were reversed by treating the patient with methylene blue.

NTP evaluated oral (NTP 1983a) and dermal (NTP 1982, 1983b) exposure effects from nitrobenzene exposure in B6C3F1 mice and F344 rats. Increased absolute and relative heart weights were observed in F344 female rats at all dose levels after 90 days of oral exposure. The lowest dose in the study was 9.375 mg/kg/day cardiovascular effects after 90 days of exposure mice are less clear. A significant difference between all heart weights in male rats treated with 18.75 mg/kg nitrobenzene and controls was observed, however there was a lack of dose response. Similarly, there was a lack of dose response in relative heart rate in male mice. In female mice there was a slight, non-significant dose-response for absolute heart weight and no dose-response for relative heart weight. A similar effect was seen in rats after 90 days of 0.40 g/kg dermal exposure to nitrobenzene (NTP 1983b).

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans after inhalation, oral or dermal exposure to nitrobenzene. In Cattley et al. (1994) the researchers observed an increase in mononuclear cell infiltrate in the pancreas of female B6C3F1 mice exposed to 50 ppm nitrobenzene for 2 years. No studies evaluating gastrointestinal related effects in animals or humans after oral or dermal exposure to nitrobenzene were located.

2.7 HEMATOLOGICAL

In general, when considering the toxicological effects of nitrobenzene exposure via ingestion, inhalation or dermal routes, methemoglobinemia is the most commonly observed systemic adverse health outcome. Specifically, nitrobenzene exposure affects hemoglobin by converting the iron component from the ferrous state to the ferric state (oxidized) resulting in abnormally high levels of methemoglobin, which is not capable of releasing oxygen to the tissues of the body. MetHb occurs naturally in people, at levels around 1 to 4 percent in blood (Klaassen 2019). However certain chemicals, including nitrobenzene, have been shown to increase the amount of metHb present, lowering oxygen capacity, and causing hypoxia which is generally associated with cyanosis and fatigue, weakness, dyspnea, headache, and dizziness. A distinct cyanosis or slate-blue coloration is noted at 15-20% metHb in blood, while at 30-50% levels, the patient becomes symptomatic, with lethargy, vertigo, headache and weakness: with modest depression of the cardiovascular and CNS while at greater than 60%, develops stupor and respiratory depression and needs immediate treatment (Goldfrank et al. 1998). In the otherwise healthy person, cyanosis may be clinically evident with a methemoglobin as low as 10%. The classic appearance of "chocolate brown blood" can be present at as low as 15% methemoglobin levels (Ludlow et al. 2021). As the percentage of methemoglobinemia approaches 20%, the patient may experience anxiety, light-headedness, and headaches. At methemoglobin levels of 30-50%, there may be tachypnea, confusion, and loss of consciousness. Approaching 50%, the patient is at risk for seizures, dysrhythmias, metabolic acidosis, and coma. Levels above 70% are often fatal (Ludlow et al. 2021).

Case studies reported elevated metHb levels in occupationally exposed workers to nitrobenzene via inhalation (Ikeda and Kita 1964; Lee et al. 2013). Levels of nitrobenzene exposure in these studies was not provided. 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. 2012; Gupta et al. 2000; Kumar et al. 2017; Martínez et al. 2003). Mallouh and Sarette (1993) reported on a case of dermal poisoning of a

two-month-old from hair oil containing 1% nitrobenzene. This resulted in a metHb level of 31.5%, which gradually dropped over three days without treatment. Additionally, Ewert et al. (1998) described an attempted suicide with the injection of "India ink" which contained nitrobenzene and resulted in methemoglobinemia.

In addition to the human case studies several experimental animal studies have evaluated the hematological effects of nitrobenzene inhalation. As with humans, an increase in the amount of metHb is seen in through all routes of exposure and it has been observed to occur with acute, intermediate and chronic duration exposure studies in experimental animals (Cattley et al. 1994; CIIT 1993; Hamm Jr. et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b). In considering the results of the rodent studies the differences between human and rodent metHb levels and metabolism should be considered. The amount of metHb in blood is mainly controlled by the level of the enzyme metHb reductase (Smith, 1993). The amount of innate metHb reductase activity in rodents is greater than that in humans. Specifically, it is estimated that the rat has about 2-to 5- times as much metHb reductase activity compared to humans and the mouse about 10-times as much as a human (Smith, 1993). This means that the transformation of metHb to hemoglobin, which is facilitated by metHb reductase, occurs more quickly in rodents than in humans (Bloom and Brandt 2006). Therefore, it is likely that the increases in metHb concentration observed at a given dose of nitrobenzene in a mouse is less than that which would be observed in a rat which is less than that would be observed in a human at the same dose. That is, rats are more sensitive to nitrobenzene toxicity than mice and humans are more sensitive to nitrobenzene toxicity than either rats or mice. Further, although a 1-4% metHb level is without adverse effects in humans it is not known what level of metHb begins to cause adverse effects in rodents.

In acute, 2-week, inhalation exposure studies in rats and mice, other effects were observed, in addition to the increase in metHb. These include concentration-dependent increases in absolute and relative spleen weight, with relative spleen weights increased as much as three times that of the control rats (male and female Fischer 344 rats and female Sprague-Dawley rats). The significant increase in spleen weight was seen at 35 ppm and higher concentrations (Medinsky and Irons 1985). In Medinsky and Irons (1985) splenic lesions were also present in all animals and dose groups (target concentrations: 10, 35 and 125 ppm; analytically measured concentrations: 9.1, 35.8 and 124.5 ppm) exposed to nitrobenzene for two weeks. At 124.5 ppm 90 to 100 percent 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 Jr. et al. 1984). Increases in metHb were

seen in male F344 rats at concentrations as low as 5 ppm. Female F344 rats and male Sprague-Dawley rats showed increases at 16 ppm and female Sprague-Dawley rats at 50 ppm. Additionally, in all mice and rats evaluated there was a demonstrated increase in spleen weights at 50 ppm and male F334 Rats and female Sprague-Dawley rats experienced increased spleen weights at 16 ppm. Additionally, Hamm Jr. et al. (1984) noted dose-related hematologic changes in rats that are consistent with hemolytic anemia. This effect was not observed in mice. Further, after exposure to 50 ppm nitrobenzene there was an increase in treatment related lesions in both rat species and mice in the spleen. These lesions were similar to what was seen in the acute study with acute sinusoidal congestion and moderate increases in both extramedullary hematopoiesis and the number of hemosiderin-laden macrophages in red pulp (Hamm Jr. et al. 1984).

The splenic lesions observed in the acute and intermediate duration studies were not observed in a chronic, 2 year study of the same species of experimental animals. In this study rats were exposed to 0, 1, 5 and 25 ppm of nitrobenzene and mice to 0, 5, 25 and 50 ppm nitrobenzene (Cattley et al. 1994; CIIT 1993). However, there were still significant changes in metHb levels at both a 15 month interim sacrifice and after 2 years of exposure with doses as low as 25 ppm in female mice and male and female rats. In addition, there was a statistically significant increase in hypercellularity in femur bone marrow in mice exposed to 50 ppm nitrobenzene (Cattley et al. 1994; CIIT 1993).

Smith et al. (1967) argued that nitrobenzene is a “poor methemoglobin-forming agent” in female CD1 mice. They noted that within an hour of exposure to a single dose of nitrobenzene up to 10.0 m-mole/kg of metHb levels were lower in female mice compared to female Sprague-Dawley rats.

Methemoglobinemia has also been observed in acute and intermediate duration oral studies of nitrobenzene exposure in rats. For example, Goldstein et al. (1984) observed a LOAEL of methemoglobinemia with a single 200 mg/kg dose of intubated nitrobenzene in F334 male rats fed pectin-containing diets, but not in those fed pectin-free diet, with a NOAEL of 150 mg/kg in F334 male rats in all groups regardless of diet. A significant dose-response was observed in methemoglobinemia in male rats fed with only pectin-containing diets. In male rats fed pectin-free diet, methemoglobinemia was increased 2.4-fold of controls at 400 mg/kg dose, although a significance criterion was not reported. Additionally, Mitsumori et al. (1994) observed significant effects in male Sprague-Dawley rats in hematological biochemical markers of effect including dose-dependent decreases in red blood cell and hemoglobin concentration and dose-dependent increases in metHb, mean corpuscular volume, mean erythrocyte hemoglobin concentration, reticulocytes and erythroblasts with 54 days of oral exposure to nitrobenzene at doses ranging from 20 to 100 mg/kg/day. In addition, similar to the inhalation studies,

Mitsumori et al. (1994) also observed increased extramedullary hematopoiesis and hemosiderin deposition in the spleen and increased hematopoiesis in bone marrow.

In an acute-duration oral exposure study B6C3F1 female mice were exposed to nitrobenzene via corn oil gavage for 14-day (Burns et al., 1994) at doses of 30, 100 and 300 mg/kg. Burns et al. (1994) observed results consistent with nitrobenzene's hematological toxicity. In this study the most sensitive effect observed was changes in the bone marrow where the number of cells in the femur bone marrow, DNA synthesis, and the number of colony forming units for granulocyte monocyte progenitor cells were increased in a statistically significant manner following a dose-response trend, starting at 30 mg/kg/day. The immature erythrocytes (reticulocyte) resulted in compensatory increases in mean corpuscular volume and mean corpuscular hemoglobin starting at doses of 100 mg/kg. In addition, the percentage of reticulocytes increased dose-dependently, also starting at doses of 100 mg/kg. Hepatomegaly and splenomegaly were observed by the researchers as were pathological observations consistent with extramedullary hematopoiesis.

In an intermediate duration oral toxicity studies in rats and mice conducted by NTP (1983a) dosed B6C3F1 mice and F344 rats of both sexes with doses ranging from 9.375 to 150 mg/kg for rats and 18.75 to 300 mg/kg for mice. Doses were administered via gavage with corn oil. NTP (1983a) observed effects in on the hematological, hepatic, renal, and respiratory system. In both sexes of F344 rats there were significant increases in metHb levels and absolute reticulocytes and decreases in hemoglobin followed by significant dose-response in effects at doses. These effects were accompanied by a decrease in hematocrit and increase in relative reticulocytes in female rats and decrease in MCV in male rats. Effects were significantly dose-dependent. Because only one male rat survived in the 150.0 mg/kg dose group, results are not conclusive from this group. At the 18.75 mg/kg dose level, male rats exhibited elevated relative reticulocytes and slight anisocytosis accompanied by decreased hematocrit and RBC. Slight polychromasia was observed in male and female rats. Hematological health effects suggest regenerative anemia in the rat. B6C3F1 mice also displayed dose related increase in reticulocytes (beginning at 37.5 mg/kg in males and 18.75 mg/kg in females), RBC indices (at 37.5 mg/kg in males and 150 mg/kg in females), and m metHb (at 18.75 mg/kg in males and females).

In addition, dermal toxicity studies have demonstrated very similar effects to those seen in inhalation and oral studies. For example, NTP (1982) noted significant increases in the same hematological biomarkers of effect after 0.2 g/kg (males) and 0.4 g/kg (females) when nitrobenzene was applied to the intrascapular region of F344 rats for 12 days. B6C3F1 mice of both sexes also demonstrated increases in metHb and other hematologic biomarkers at 0.4 mg/kg and above and there were noted increases in MCV in male

mice at 0.2 g/kg and reticulocytes in female mice. Congestion of the spleen was also noted in both species.

In a 90-day dermal study with both F344 rats and B6C3F1 mice, the female mice showed a dose-related increase in metHb while the male mice did not. Both male and female mice had increased reticulocytes. Additionally, both male and female rats exhibited increased metHb and reticulocytes and decreased red blood cells and hemoglobin with 0.05 mg/kg exposure (NTP 1983b).

The action of bacteria normally present in the small intestine and gut is an important element in the formation of metHb resulting from nitrobenzene exposure (Goldstein et al. 1984b; Reddy et al. 1976). Germ-free rats do not develop methemoglobinemia when orally administered nitrobenzene (Reddy et al. 1976). This observation leads to the hypothesis that a nitrobenzene metabolite such as aniline (which is formed by the bacterial reduction of nitrobenzene in the intestines of rats) may be involved in metHb formation. Currently, there are no inhalation and/or dermal exposure studies to nitrobenzene in germ-free rats. In addition, Goldstein et al. (1984b) evaluated the effect of diet, specifically the amount of pectin consumed, may have a role in formation of methemoglobinemia. The investigators found that the presence of cereal-based pectin in the diets of rats resulted in an increased the ability of orally administered nitrobenzene to induce methemoglobinemia (Goldstein et al. 1984b).

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or animals after inhalation, oral or dermal exposure to nitrobenzene.

2.9 HEPATIC

There is some evidence that the human liver is affected after chronic inhalation exposure of 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. They also reported the results of liver function tests such as retention of BSP and increases in icterus index and indirect bilirubin level. Nitrobenzene exposure levels were not measured or estimated. Additionally, Gupta (2012) presented a case of a 17-year-old female that died by suicide by consuming an unknown quantity of nitrobenzene, resulting in severe methemoglobinemia and the demonstrated hepatic centrilobular necrosis on autopsy. No studies were located regarding hepatic effects in humans after dermal exposure to nitrobenzene.

Medinsky and Irons (1985) reported liver lesions in rodent studies that included centrilobular necrosis superimposed on a severe centrilobular hydropic in male F344 rats exposed to nitrobenzene via inhalation of 35 ppm for 2 weeks. However, similar necrotic lesions were not found in female rats. Degenerative

non- necrotic changes in hepatocytes, were noted in both sexes of mice exposed to via inhalation of 35 ppm nitrobenzene. These were similar to necrotic lesions observed in rats and B6C3F1 male mice, but at a less severe degree (Medinsky and Irons 1985).

In a dose-range finding study in New Zealand white rabbits the only reported hepatic effect after exposure to nitrobenzene was increased mean absolute liver weight, however these changes were not significant (Biodynamics 1983). The same liver effect was seen in Sprague-Dawley rats exposed to 10 ppm nitrobenzene for 10 days (Tyl et al. 1987)

In an intermediate duration study increased liver weight, hepatocyte hyperplasia, and multinucleated hepatocytes were observed in male B6C3F1 mice exposed to 16 ppm nitrobenzene via inhalation for 90 days (Hamm Jr. et al. 1984). Additionally, in this same study both sexes of F-344 rats exposed to 50 ppm nitrobenzene via inhalation demonstrated centrilobular necrosis and disorganization of hepatic cords and Sprague-Dawley rats displayed centrilobular hepatocyte hypertrophy.

In a two-year study on nitrobenzene's toxicity via inhalation exposure a significant increase of centrilobular hepatocytomegaly and multinucleated hepatocytes was observed in male mice at all concentrations, with the lowest dose for mice being 5 ppm (Cattley et al. 1994). The same effects were seen only at the 50 ppm exposure level in female mice. In rats, centrilobular hepatocytomegaly occurred at 25 ppm in male F344 rats and at 5 and 25 ppm in Sprague-Dawley rats. Further, the incidence of F344 rats with eosinophilic foci increased in the 5 and 25 ppm groups in males and 25 ppm group in females. The occurrence of spongiosis hepatis was also increased in 25-ppm nitrobenzene-exposed Sprague-Dawley rats. The same effect was not seen in female rats, indicating a potential difference in response by sex. In addition, 5 and 25 ppm nitrobenzene exposure resulted in an increased incidence of centrilobular hepatocytomegaly. The incidence of rats with Kupffer cell pigmentation was increased at all nitrobenzene exposure concentrations, with the lowest dose being 1 ppm (Cattley et al. 1994).

Oral exposure to nitrobenzene in Sprague-Dawley rats and B6C3F1 mice also demonstrated increased liver weight with acute and intermediate-duration exposure (Burns et al., 1994; Mitsumori et al. 1994; NTP 1983a). In Burns et al. (1994) relative liver weight increased dose-dependently starting at 100 mg/kg in B6C3F1 female mice dosed for 14 days. In addition, ALT was significantly increased with 300 mg/kg exposure in the same study. Further, at 300 mg/kg the liver displayed minor histopathological changes including mild hydropic degeneration around focal central veins.

NTP 1983a observed, in male and female rats, liver weights that increased in a dose-dependent manner, with significant increases in liver weight and the ratio of liver weight to final body starting at 9.375 mg/kg. Because only one male rat survived in the 150.0 mg/kg dose group, results are not conclusive

from this group. Further, NTP observed congested livers with 150 mg/kg of nitrobenzene in rats. F344 rats also experiences a loss of cytoplasmic basophilia and a reduction of centrilobular hepatocytes at the same dose. In B6C3F1 mice, hepatocytes in the centrilobular zone were enlarged and nuclei had coarse or stippled chromatin and a large nucleolus with 300 mg/kg exposure (NTP 1983a). Mitsumori et al. (1994) also observed increased centrilobular swelling of hepatocytes, brown pigmentation in Kupffer cells, and extramedullary hematopoiesis starting at 20 mg/kg in male rats.

In one study, 12-days of dermal exposure resulted in increased liver weight and liver congestion in B6C3F1 mice in a dose dependent manner with doses ranging from 0.2 to 1.6 g/kg (NTP 1982). As with the oral exposure studies, many F344 rats that were treated dermally with nitrobenzene experienced a loss of cytoplasmic basophilia, vacuoles or eosinophilic globules in cells in the centrilobular zone. These lesions were seen in after both acute (12-days) and intermediate duration (90-days) exposure. In the 90-day dermal toxicity study male and female F-344 rats also had congested livers and branches of the hepatic artery, portal and collecting veins, and sinusoids that were filled with blood after 0.80 g/kg exposure (NTP 1982, 1983b).

2.10 RENAL

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

Dose-related increases in kidney weights were observed in Fischer-344 rats (both sexes), but not in Sprague-Dawley rats exposed to nitrobenzene via inhalation at 10 to 125 ppm up to 14 days (Medinsky and Irons 1985; Tyl et al. 1987). In Medinsky and Irons (1985) the increase in relative kidney weight was observed at the acute sacrifice (i.e., 3 days post exposure) but not after the 14-day recovery period. At 125 ppm, hydropic degeneration of the cortical tubular cells was observed only in Sprague-Dawley rats (20% of males; 90% of females), and hyaline nephrosis only in Fischer-344 rats (100% of males; 20% of females) (Medinsky and Irons 1985). Renal effects reported in B6C3F1 mice in this study included minimal to moderate multifocal degenerative changes in tubular epithelium of males exposed to 35 ppm for 2 weeks. However, neither hydropic degeneration of the cortical tubular cells nor hyaline nephrosis was seen in mice even at the highest exposure level (125 ppm). Using the same three animal models exposed to nitrobenzene at 5 to 50 ppm for 90 days, dose-related real lesions were observed in both rat strains but not in mice (Hamm Jr. et al. 1984). Additionally, no effects were seen in rabbits exposed up to 81 ppm (Biodynamics 1983) in an acute duration study.

In a study of nitrobenzene's toxicity after chronic inhalation exposure, Cattley et al. (1994) found a significant increase in cysts on the kidney with 50 ppm exposure in B6C3F1 mice and F344 rats. They also observed an increase in the severity of nephropathy with increasing doses of nitrobenzene exposure in male and female F344 rats.

In an acute duration exposure study B6C3F1 mice exposed to nitrobenzene for 14 days via gavage experienced a 10% increase in absolute but not relative kidney weight with 300 mg/kg exposure. There was not a significant increase in kidney weight at 100 mg/kg (Burns et al., 1994). Oral exposure studies in experimental animals demonstrated changes in kidney weights in both mice and rats (NTP 1983a) with intermediate-duration exposure. Specifically, NTP (1983a) observed consistent increases (but not a clear dose response) in right kidney weight, ratio of right kidney weight to final body weight, and ratio of right kidney weight to body weight at all dose levels in male F344 rats. Additionally, pale green hyaline globules were observed in rat cortical tubular cells in the 75mg/kg and 150mg/kg dose groups. In male mice, kidney weights were increased at 75 and 300 mg/kg nitrobenzene. In female mice, relative kidney increased from controls at the 300 mg/kg dose level. Brown pigmentation was observed in male Sprague-Dawley rats exposed to 20, 60, 100 mg/kg nitrobenzene (Mitsumori, 1994). In dermal studies kidney weights were noted to increase with acute and intermediate duration studies.

Additionally, in females dosed with 1.6 g/kg there was some evidence of degeneration in renal cortical tubules after acute exposure (NTP 1982). Additionally, in an intermediate duration dermal application study, kidneys in male and female Sprague-Dawley rats with 0.4 g/kg exposure or more had congestion in the kidneys (NTP 1983b).

2.11 DERMAL

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

In one acute duration studies in which nitrobenzene was applied directly to the skin of the animal no significant effects were seen (NTP 1982). In addition, in an intermediate dermal study NTP noted that male Sprague-Dawley rats had brown patches at the site of application. No additional observations were summarized on other sexes or animals in this study (NTP 1983b)

2.12 OCULAR

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

In data submitted to EPA, slight corneal clouding was observed with single dose of 514 ppm exposure to nitrobenzene and exposure of 555 ppm also resulted in lacrimation and slight corneal clouding in Sprague-Dawley rats (Dupont 1981).

2.13 ENDOCRINE

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

In experimental animal studies via both inhalation and oral exposure with an intermediate exposure duration cellular vacuolization of the zona reticularis was seen in the adrenal gland in female mice (Hamm Jr. et al. 1984; NTP 1983a). In the inhalation study this effect was seen in doses as low as 5 ppm and increased in severity with increasing dose. Additionally, in a 2-year study on the effect of chronic nitrobenzene inhalation thyroid follicular cell hyperplasia was increased in male rats and mice at 25 and 50 ppm (Cattley et al. 1994).

In an in vitro study using porcine thyroid cells Zasada et al. (2015) evaluated the lipid peroxidation potential of nitrobenzene. In this assessment nitrobenzene increased lipid peroxidation in the thyroid cells with increasing dose. Zasada et al. (2015) also observed that this increase in peroxidation could be prevented with melatonin. In addition, in a recombinant yeast estrogen screening test and nitrobenzene did not demonstrate estrogenic activity (Mestankova et al. 2016).

2.14 IMMUNOLOGICAL

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

In the experimental animal studies that evaluated nitrobenzene's toxicity as it relates to immune effects the main effect reported was alterations in immune cells counts. For example, in an acute duration inhalation study Medinsky and Irons (1985) reported an elevation in granulocytes and lymphocytes. However, this was only seen in male Sprague-Dawley rats. The same effect was not seen in female rats nor F344 rats nor B6C3F1 mice. Additionally, in an intermediate exposure duration study there was an accumulation of lymphocytes and macrophages beneath the splenic capsule and there were also

proliferative changes seen in the lymph nodes of animals exposed to nitrobenzene. In a chronic inhalation study by Cattley et al. (1994), the immune effects reported include involution of the thymus by 22% in female B6C3F1 mice after 50 ppm exposure for 505 days. The thymus is known to shrink with age so this finding should be interpreted within that context (Aspinall and Andrew 2000) and an increase in the incidence of rats with Kupffer cell pigmentation was increased at all nitrobenzene exposure concentrations with doses as low as 1 ppm.

In an acute duration oral exposure study, B6C3F1 female mice were administered 0, 30, 100 or 300 mg/kg of nitrobenzene in corn oil via gavage (Burns et al., 1994). In this study Burns et al. (1994) observed immunotoxicity with nitrobenzene exposure including decreases in IgM response in the spleen to T-dependent antigens, alterations in phagocytic activity of macrophages and decreased activity of natural killer cells starting at 100 mg/kg exposure. Host resistance to microbial infection was not impacted by exposure to nitrobenzene in this study. However, there was a trend toward increased susceptibility of the mouse when the host's defense is dependent on T-cell functioning as reflected in the IGM response to nitrobenzene in a sheep red blood cell assay. In addition, alterations in bone marrow activity were observed including increases in the number of colony forming units for granulocyte monocyte progenitor cells were increased in a statistically significant manner following a dose-response trend, starting at 30 mg/kg/day (Burns et al., 1994).

In an intermediate duration oral exposure study, the only reported immune effect was an increase in thymus weight (NTP 1983a). Lymphoid depletion was observed in five out of 10 female rats in the 300.0 mg/kg dose level. The same effect was also seen in an intermediate duration dermal study (NTP 1983b). In the dermal study, thymus glands were found to be atrophic after 0.8 g/kg doses of nitrobenzene. Further there was a marked depletion of lymphocytes.

2.15 NEUROLOGICAL

Neurological effects have been 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 oral ingestion of nitrobenzene (Carter 1936; Leader 1932; Myslak et al. 1971). Additionally, cerebral lesions in the corpus callosum and centrum semiovale have been reported as direct toxic effects of nitrobenzene ingestion (Kumar et al. 2017; Boukobza et al. 2015). Additionally, 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 cannot be reliably

estimated in most of the case studies from which these descriptions have been derived. No studies on the effects on the neurological system in humans after dermal exposure were located.

When Sprague-Dawley rats and B6C3Fl mice were exposed to nitrobenzene at 125 ppm daily for two 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 Fischer rats exposed to the same levels. The reason for these strain differences under similar conditions is not apparent. In an additional acute study, with the goal of determining the LD_{50} for nitrobenzene male CRL:CD rats (Dupont 1981) exposure to 439 ppm nitrobenzene resulted in hyperactive an aggressive behavior in and at 555 ppm rats exhibited tremors. In a 90-day study neurologic signs were not observed in B6C3F1 mice or F344 and Sprague-Dawley rats exposed to 5, 16, or 50 ppm nitrobenzene in air. These animals were observed twice daily for clinical abnormalities, no discussion was presented in this study on histopathological observations of the brain (Hamm Jr. et al. 1984).

In an acute duration oral exposure study brain relative brain weight decreased in B6C3F1 mice exposed to nitrobenzene via gavage at doses of 300 mg/kg. The same difference did not occur with 100 mg/kg exposure (Burns et al., 1994). With oral exposure around 100 ppm Sprague-Dawley rats experienced torticollis (a condition in which the neck muscles are contracted causing the head to tilt to one side), circling movement and abnormal gait (Mitsumori et al. 1994). Additionally, in this same study at both 60 and 100 ppm neuronal necrosis/gliosis in certain nuclei in the cerebellar and medulla and pons were observed (Mitsumori et al. 1994).

Ataxia was observed in ten out of ten female mice exposed to 75 mg/kg and ten out of ten male mice exposed to 150 mg/kg compared to zero out of ten controls rats for both sexes. Other clinical and necropsy neurological findings for male and female rats and mice included head tilt, trembling, immobility, circling and lethargy. These effects were seen at 75 mg/kg and 300 mg/kg in rats and mice, respectively. Seven out of 10 and five out of ten female rats in the 150 mg/kg exposure group exhibited brain stem hemorrhage and vacuolization compared to four out of ten and 6 out of ten control animals, respectively. While two out ten male rats exhibited hemorrhage of the brain stem at 150 mg/kg exposure levels compared to zero out of ten control rats. Male control rats had an incidence of seven out of 10 instances of brain stem vacuolization. Because of alteration in histopathologic observation in control animals (brain stem hemorrhage and vacuolization in female animals and brain stem vacuolization in male animals) no neurological NOAEL was established. Other rats exposed to 150 mg/kg had various degenerative effects observed in the pons, dentate nucleus, and cerebellar peduncle/olivary nucleus, which varied in severity and were characterized by spongy degeneration, necrosis, karyorrhexis,

neutrophils, lymphocytes, plasma cells, and fusiform cells NTP (1983a). Ataxia was observed only at the 300 mg/kg dose in male (9/10) and female mice (1/10), while irritability was observed only in female mice at the 300 mg/kg dose (9/10). Indicated a species difference on the potential neurological effects from nitrobenzene exposure. Brain lesions were reported after a single oral administration of nitrobenzene at 550 mg/kg to male rats (Morgan et al. 1985). Observations included petechial hemorrhages in the brain stem and cerebellum and malacia (cell breakdown) in the fourth ventricle.

In the dermal assessment of nitrobenzene's potential toxicity with 12 days of exposure male Fischer F-344 rats displayed dose-related increases in inactivity, ataxia, prostration and dyspnea with doses ranging from 0.2 to 3.2 g/kg. All animals were dead or moribund with treatment of 1.6g/kg or greater by the end of the experiment. Two out of 5 male and 1 out of five female mice displayed small foci of hemorrhage in both the cerebral and cerebellar cortex after 1.6 g/kg exposure.

In a follow-up 90-day study male and female rats displayed lethargy and ataxia with 0.8g/kg exposure. Although some mice showed similar effects, they were not affected as severely as the rats. Hemorrhage, most frequently in the brain stem, was also apparent in rats treated with 0.40 g/kg and 0.8 g/kg dose for females and 0.80 g/kg dose for males (NTP 1983b).

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 effects (Allenby et al. 1990; Allenby et al. 1991; Linder et al. 1992). However, the evidence that nitrobenzene effects female reproduction in experimental animals is limited.

In a study of New Zealand white rabbits dosed with target concentrations of 10, 40 and 100 ppm (analytically measured average concentrations: 9.9, 41 and 104) of nitrobenzene at days 7-19 of gestation potential for reproductive effects were observed. Specifically, at 40 ppm the mean number of resorption sites, the mean percentage of resorptions to implants and the incidence of resorptions were higher than control. However, these differences from control were not statistically significant. Additionally, the mean percent of resorptions in the high-dose group (14.3%) was higher than that in the control (5.8%). Though the authors stated this effect was not statistically significant (Biodynamics 1984). In the dose range finding study conducted prior to the 1984 by Biodynamics, the authors stated that the mean number of

resorption sites and mean percentage of resorptions were slightly lower in the control compared to the mid (40 ppm) and high (80 ppm) dose groups (Biodynamics 1983).

Evidence of testicular toxicity is seen in acute, intermediate and chronic duration studies via inhalation, oral and dermal exposure. For example, in an acute study in Fischer 344 rats a decrease in testicular weight and size was observed in rats exposed to 125 ppm for 2 weeks via inhalation (Medinsky and Irons 1985). The decrease in testes weight was observed 3 days and 14 days post exposure. Further, testicular lesions were also observed in these rats which consisted of an increase in multinucleated giant cells, Sertoli cell hyperplasia and severe dysspermiogensis. Few sperm with arrested maturation at primary and secondary spermatocyte 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 though the Sertoli cell hyperplasia and the increased numbers of multinucleated giant cells were less severe.

Additionally, in a two-generation inhalation study in Sprague-Dawley rats, 10 weeks of nitrobenzene exposure resulted in a decrease in fertility indices at 40 ppm for F_0 and F_1 generations, while other reproductive parameters were unaltered (Dodd et al. 1987). The study data suggested that the decrease in fertility was caused by males. Specifically, Dodd et al. (1987) observed atrophy of seminiferous tubules, spermatocyte degeneration and reduced testicular and epididymal weights in the F_0 and F_1 generations with 40 ppm exposure. Maternal toxicity was not observed. Hamm Jr. et al. (1984) reported that both F-344 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 Jr. et al. 1984).

Cattley et al (1994) conducted a two-year chronic inhalation study in Sprague-Dawley male rats and male and female F344 rats and B6C3F1 mice and observed similar reproductive effects in male rodents as in the acute and intermediate duration exposure studies. For example, in male mice exposed to nitrobenzene for two years at 50 ppm there was an increase in epididymal hypospermia. The same effect was also observed in Sprague-Dawley male rats exposed to 25 ppm nitrobenzene including an increased atrophy of the testes (Cattley et al. 1994).

Acute and intermediate oral studies in experimental animals indicate similar outcomes as those seen in inhalation studies (Levin et al. 1988; Linder et al. 1992; Mitsumori et al. 1994; NTP 1983a). For example, Levin et al. (1988) administered a 300 mg/kg dose of nitrobenzene in corn oil (gavage). Histological

examination showed that pachytene spermatocytes and step one and two spermatids underwent degeneration and giant cell formation as early as 3 days after treatment. Testicular and spermatogenesis repair was substantial by 3 weeks after treatment. Similar effects were seen in a study with a single dose of 300 mg/kg of nitrobenzene in Sprague-Dawley rats (Linder et al. 1992). Specifically, Linder et al. (1992) observed degenerating and missing pachytene spermatocytes in stages seven through fourteen, some multinucleated giant cells, testicular debris, and an increase in the number of morphologically abnormal sperm.

Severe atrophy of the seminiferous tubules along with decreased sperm concentrations were also observed in Kawaguchi et al. (2004) after 18 days of exposure to 60 mg/kg/day nitrobenzene once per day in Sprague-Dawley rats. In addition, there were significant effects on the sperm with increased presence of detached sperm heads and less vigorous sperm movement. Three out of eight exposed rats had decreased sperm concentrations in the caput/corpus while eight out of eight exposed rats had decrease in sperm concentration in the cauda compared to one of eight control rats. Similar effects were not seen after 3 days of exposure. Additional evidence of testicular toxicity from nitrobenzene exposure was reported by Kawashima et al. (1995) who exposed male Sprague-Dawley rats to nitrobenzene for 7-70 days. As with other studies, the rats experienced changes in testicular weight, sperm counts, motility and vitality when exposed to nitrobenzene for 14 to 21 days. In an assessment of the cytotoxic effects of nitrobenzene on Sprague-Dawley rats Iida et al. (1997) reported that 60 mg/kg/day of nitrobenzene exposure for 1-3 weeks had little effect on spermatocytes prior to the early pachytene stage but haploid cells (spermatids and spermatozoa) were degraded and secondary spermatocyte meiosis was suspended. The effect of nitrobenzene was reversible once exposure stopped, except in the case of testicular weight. Histopathological alterations were observed including decreased relative testis weight at weeks 2 and 3 and decrease relative epididymis weight at week 3 in treated animals compared to control animals.

In an intermediate duration oral exposure study Mitsumori et al. (1994) dosed Sprague-Dawley rats from pre-mating through day four of lactation, for a total of 54 days. Animals were dosed with 0, 20, 60 or 100 mg/kg/day nitrobenzene via gavage. At necropsy there was a significant decrease in relative epididymis and testis weight when compared to controls for the 60 and 100 mg/kg/day dose groups. Histopathological observations revealed the presence of atrophy of the seminiferous tubules, hyperplasia of Leydig cells, and loss of intraluminal sperm in the epididymis in the 60 and 100 mg/kg/day dose groups. Mitsumori et al. (1994) did not observe any differences in fertility or copulation indices. However, many of the pregnant mice (8/10) died before term in the high dose group (Mitsumori et al. 1994). NTP also observed reproductive effects in male rats. In the study, 1/10 rats displayed atrophy in the 37.5 mg/kg dose group and 9/10 displayed atrophy in the 75 mg/kg dose group. Ten out of ten rats

had multinucleated giant cells at the 75 mg/kg dose. Spermatocytes, and spermatids or spermatozoa were sloughed into the lumina of a few tubules in the testes in rats exposed to 9.375 mg/kg (incidence not provided). Male mice showed atrophied testes. At least one mouse displayed atrophy in the testes in all dose groups with the exception of 75 mg/kg. At 150 mg/kg and 300 mg/kg 5/10 mice displayed atrophy in the testes. Right testis weight and its ratio to body weights were significantly decreased in mice at 300 mg/kg dose. In addition, both male animal species experienced hypospermatogenesis, with the rats displaying a greater sensitivity to this effect compared to mice. Specifically, 10/10 rats experienced this effect at 75 mg/kg whereas no mice displayed hypospermatogenesis at this dose. 4/10 mice display hypospermatogenesis at 300 mg/kg.

There are two studies which evaluated nitrobenzene's toxicity after dermal exposure, one acute (12 day) repeated-dose study (NTP 1982) and one intermediate (13 weeks) study (NTP 1983b) in B6C3F1 mice and F344 rats. Both studies found similar effects on the testes as the inhalation and oral studies. Specifically, NTP (1982) observed atrophic seminiferous tubules unrecognizable spermatids and spermatozoa, and presence of multinucleated giant cells at dose levels of 0.8 g/kg and greater. Additionally, at 1.6 g/kg dose level necrotic debris was present in the lumen of the epididymis. These effects were not as severe in mice. In the intermediate duration study, the right testis weight ratio with final body weight was significantly decreased at the 0.40 g/kg dose level in both the rat and the mouse. In rats, multinucleated giant cells were present in 9/10 and 10/10 rats at 0.4 and 0.8 g/kg dose levels respectively. Spermatocytes, spermatids and/or spermatozoa, were sloughed into the lumen of seminiferous tubules in the testes of rats exposed to 0.2 g/kg nitrobenzene. Testes of all male mice of the 0.8 g/kg dose group were smaller than those of the controls and treated mice of other groups. Additionally, seminiferous tubules were atrophied but open. Sertoli cells, spermatogonia, spermatocytes, a few spermatids and no spermatozoa were in the tubules. Multinucleated giant cells were present in some tubules. Sloughing of spermatocytes, spermatids, and spermatozoa into the tubular lamina was the only change in the testes of a few mice in the group dosed with 0.2 g/kg. In addition, 6/10 female rats and 5/10 female mice displayed atrophy in the uterus at 0.8 g/kg in the acute duration exposure study. The study authors also reported that the ovaries of a few rats were congested, however the exact number of rats experiencing this effect was not stated.

The mechanism(s) by which nitrobenzene induces its testicular toxicity is unknown but it may be by potentially affecting Sertoli cells, the cells which control spermatogenesis through the secretion of various proteins based on the stage of spermatogenesis. According to Allenby et al. (1990; 1991) and McLaren (1993a) alterations in the hormones and proteins such as immunoactive inhibin, lactate, pyruvate and other proteins released from Sertoli cells may be responsible for the adverse effects seen on the testes.

Further, McLaren (1993b) demonstrated that there are major age-dependent differences in the secretions of total and specific proteins in the Sertoli cells that can be affected by nitrobenzene exposure and that immature rats may not experience the same effect from nitrobenzene exposure as mature rats. In addition, Shinoda et al. (1998) reported that germ cell degeneration in rat spermatocytes was due to apoptosis in adult Sprague-Dawley rats, which may be induced by changes in secretion of Sertoli cell factors. Richburg and Nanez (2003) aimed to elucidate the molecular mechanism by which nitrobenzene induces testicular toxicity by examining the Fas/Apo-1/CD95 and Fas ligand (FasL) signaling system. However, their research indicated this pathway was not responsible for germ cell apoptosis; they did observe that a dysfunctional Fas-signaling system increases the sensitivity of mice to the germ cell apoptosis following nitrobenzene exposure. Ohkkuma et al. (1999) also evaluated the potential mechanism by which nitrobenzene may adversely affect the reproductive system and determined that nitrosobenzene, a metabolite of nitrobenzene, may cause DNA damage in the presence of NAHD and Cu (II), concluding that oxidative DNA damage may play a role in nitrobenzene's reproductive toxicity.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans after inhalation, oral or dermal exposure to nitrobenzene.

Inhalation studies have indicated that nitrobenzene is not a teratogen (Dodd et al. 1987; Tyl et al. 1987). However, Dodd et al. (1987) did observe a 12% decrease in mean body weight in the offspring exposed to 40 ppm nitrobenzene in an intermediate duration study.

Tyl et al. (1987) evaluated skeletal malformations of Sprague-Dawley rats exposed to nitrobenzene in utero when the mother was exposed via inhalation from gestational day 6 through 15. Although they observed a significant increase in the incidence of total malformations (but not in the incidence of any individual malformations or malformations by category) this does not appear to be related to nitrobenzene as this occurred at the lowest exposure group, 1 ppm, but not at 10 or 40 ppm. The incidence of litters with one or more fetuses with external variations was elevated at 40.0 ppm for ecchymosis (discoloration of the skin due to blead underneath) on the trunk (but not on the head or extremities). There was also increasing incidences of these malformations in the fetuses with increasing doses, however this was not statistically significant. In addition, there was a significant increase the number of litters with animals having holes in the parietal skull plate, with 73 percent of litters in the 40 ppm group displaying this anomaly compared to 32 percent in the control group. It is possible that at the time of evaluation the parietal bone may not have been completely ossified and these effects may reflect delayed ossification (DeSesso and Scialli 2018; Fritz and Hess 1970) In Biodynamics (1984) no dose-related malformations in

fetuses were observed with maternal exposures up to 100 ppm on days 7-19 of gestation in New Zealand rabbits.

There are limited studies examining reproductive effects from oral exposure. Decreased body weights of Sprague-Dawley rat offspring were observed after oral maternal nitrobenzene exposure of 20 mg/kg/day in male offspring and 40 mg/kg/day for female offspring (Mitsumori et al. 1994).

2.18 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, the U.S. EPA has deemed nitrobenzene to be a likely carcinogen by any route of exposure (IRIS 2009). The National Toxicology Program has determined that nitrobenzene is reasonably anticipated to be a human carcinogen. The International Agency for Research on Cancer concluded nitrobenzene is possibly carcinogenic in humans (IARC 2019; NTP 2016).

Carreón et al. (2014) assessed a cohort of workers occupationally exposed to o-toluidine, aniline and nitrobenzene at a rubber chemical manufacturing plant in New York. Compared to the general population excess bladder cancer was observed in workers that were categorized as "definitely exposed" and those that had highest cumulative exposure rank. Given the mixture of chemicals these workers were exposed to it is not possible to determine what chemical (or chemicals) are causing the increased risk in cancer. The authors propose the increase may be due to o-toluidine given it has shown to be a more potent animal carcinogen than aniline or nitrobenzene and there have been previous cohort studies showing increased bladder cancer rates in works exposed to o-toluidine. However, the data available from this study are not amenable to testing this hypothesis.

One two-year chronic bioassay evaluated the effect of nitrobenzene on cancer risk in experimental animal studies was published in Cattley et al. (1994). In this study, nitrobenzene was determined to be carcinogenic, and the responses observed varied based on sex, species and strain of the animal tested. Specifically, in this study male and female B6C3F1 mice and F344 and Sprague-Dawley rats were intermittently exposed (5 days/week, 6h/day) to nitrobenzene via inhalation for 2 years. Mice were exposed to 0, 5, 25 or 50 ppm nitrobenzene while rats received 0, 1, 5 or 25 ppm nitrobenzene. Male F344 rats displayed a significant increase in combined adenomas and carcinomas in liver, kidney and thyroid, with effects increasing with increasing dose. Sprague-Dawley rats also showed a significant increase in the incidence of adenomas and carcinomas in liver (5/23 in 25 ppm rats versus 0/23 in controls). Liver, kidney and thyroid adenomas or carcinomas were not observed in female F344 rats.

However, the female F344 rats did experience a dose-dependent increase in endometrial polyps (19/49 in rats exposed to 25 ppm versus 9/48 in controls). The toxicological significance of the endometrial polyps in rats for understanding potential human health effects is uncertain (Davis 2012) Female mice exposed to nitrobenzene formed adenomas in the mammary glands and experienced marginal increases in the incidence of hepatocellular adenomas. Male mice displayed an increased incidence of combined adenomas and carcinomas of the lungs in males and follicular cell adenomas in the thyroid (Cattley et al. 1994).

It is unclear how nitrobenzene exposure may increase cancer risk. As described in the genotoxicity section (see 2.20), the evidence is fairly conclusive that nitrobenzene does not cause point mutations, as the results of many Ames tests, with and without S9 activation (Anderson and Styles 1978; Assmann et al. 1997; Bonnefoy et al. 2012; Dellarco and Prival 1989; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Hughes et al. 1984; Vance and Levin 1984). However, other genotoxic mechanisms may be plausible with nitrobenzene exposure and cancer in certain organs, but the evidence is more limited. For example, Li, Wang, et al. (2003) demonstrated that nitrobenzene can form adducts with hepatic DNA in mice and several studies demonstrated dose-dependent increases in chromosomal aberrations and increases in micronuclei (Huan et al., 1995, 1996; Bonacker et al., 2004; Robbiano et al, 2004). However, unscheduled DNA synthesis was not observed in genotoxicity evaluations of nitrobenzene (Butterworth et al., 1989; Mattiolo et al. 2006), whereas Ohkuma and Kawanishi (1999) found that nitrosobenzene, a metabolite of nitrobenzene, can cause DNA damage in the presence of NADH and Cu^{2+} in an in vitro study using calf thymus DNA. However, additional research is needed to demonstrate if these in vitro findings are relevant to humans, or even whole animal systems. Therefore, it is difficult to know if nitrobenzene's carcinogenicity occurs through a genotoxic mode of action. Other potential modes of action for nitrobenzene's carcinogenicity include oxidative stress or cytotoxicity followed by increased cell proliferation. However, the data to support these hypotheses are limited to non-existent.

2.19 OTHER NONCANCER

No studies were located regarding other noncancer effects of nitrobenzene exposure.

2.20 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). In general, the current evidence indicates nitrobenzene does not have genotoxic effects. However, there is some evidence which indicates nitrobenzene exposure may result in chromosomal aberrations or other DNA damage, but this remains to be confirmed.

Mutagenicity

Many assays have evaluated nitrobenzene's potential for mutagenicity, in various *S. typhirmurim* strains histidine reversion tests, both with and without S9 activation (Anderson and Styles 1978; Assmann et al. 1997; Bonnefoy et al. 2012; Chiu et al. 1978; Dellarco and Prival 1989; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Hughes et al. 1984; Suzuki et al. 1983; Suzuki et al. 1987; Vance and Levin 1984). None of these studies showed a positive result for nitrobenzene (see Table 2-4). Two studies (Suzuki et al. 1983; Suzuki et al. 1987) looked at nitrobenzene in combination with the co-mutagen norharman (9H-pyrido[3,4-b] indole). On their own neither nitrobenzene nor norharman were mutagenic in strains TA98 or TA100. However, when in Ames assay together with S9, reverse mutations were induced in the TA98 strain but not in the TA100 strain. The authors concluded that nitrobenzene may be able to induce mutagenicity in the presence of a co-mutagen. Further, Suzuki et al. (1987) demonstrated that the presence of nitro reductase-deficient isolate TA98 NR was negative for reverse mutations but only in the presence of co-mutagen in the tester strain, could induce reverse mutations.

Clastogenicity and Aneugenicity

While the evidence for a lack of mutagenicity of nitrobenzene, without co-mutagens, is strong, the data on clastogenicity are less so, with the majority of the evidence suggesting a potential genotoxic effect on the chromosome. For example, Bonacker et al. (2004) investigated the induction of micronuclei using V79 hamster lung fibroblasts possibly by affecting tubulin assembly and spindle apparatus. In micronucleus assays nitrobenzene exhibited a weak but positive test result in micronucleus counts. Minimal-effect-concentrations of nitrobenzene appeared to be as low as 0.01 μM and no-effect-concentrations were between 0.001 and 0.005 μM. CREST (Calcinosis, Raynaud's phenomenon, Esophageal dysfunction, Sclerodactyly, and Telangiectasias; a staining method to evaluate between aneugens and clastogens) analysis suggested the micronucleus effects were aneugenic, not, clastogenic. Additionally, Robbiano et al. (2004) observed a dose-dependent increase in micronucleated kidney cells in rats due to broken and detached chromosomes separated from the spindle apparatus in rats treated with 300 mg/kg nitrobenzene via gavage. In addition, a statistically significant increase in clastogenic effects in human kidney cells exposed to 0.250-0.50 mM nitrobenzene was also observed (Robbiano et al. 2004). Bonnefoy et al. (2012) also found in an in vitro analysis using human blood cells that when exposed to varying concentrations of nitrobenzene (0.01 to 10 μg/ml) about 13 to 14% of cells had observed micronuclei.

This was an increase above control (which was approximately 6 to 8%) but it was not high enough to reach statistical significance. Further, Huang et al. (1995; 1996) observed an increase in the percent of chromosomal aberrations in human lymphocyte cells from 12.4 to 33.2% as doses increased from 0.05 to 0.80 mmol/L. However, a cytogenetic analysis of lymphocytes in the peripheral blood or in splenic blood of rats exposed to nitrobenzene at 5 to 50 ppm for 6 hours per day 5 days per week for 21 days did not reveal an increase in sister chromatid exchange (SCE) or chromosome breakage (Kligerman et al. 1983). Similarly, no induction of unscheduled DNA synthesis in vivo or in vitro hepatocyte DNA repair test was observed (Kligerman et al. 1983). Further, nitrobenzene did not induce structural chromosomal aberrations in human spermatozoa incubated with 500 ug/mL for 120 minutes without activation by S9 fraction (Tateno et al., 1997)

DNA Damage

There is mixed evidence as to whether or not nitrobenzene may cause DNA damage with Comet assays finding consistent positive evidence of damage. For example, Robbiano et al. (2004) observed a dose-dependent increase in the DNA fragmentation as measured by Comet assay. This observation was made in both human and rat kidney cells incubated with concentrations of 0.125 to 0.50 mM nitrobenzene for 48 hours. Additionally, Mattioli et al. (2006) evaluated DNA fragmentation with Comet assays performed on human thyroid cells and also found a dose-dependent increase in tail length and tail moment. According to the authors, DNA damaging concentrations ranged from 1.25 mM to 5 mM. In an in vivo analysis presented in Mattioli et al. (2006) Sprague-Dawley rats were treated with half of the LD50, a single dose of 620 mg/kg, and found that nitrobenzene induced a significant increase in DNA fragmentation in thyroid, liver and kidney cells, with the greatest damage being seen in the liver and the kidney, with less damage observed in the thyroid. However, in two studies that evaluated unscheduled DNA synthesis both in vivo (Mirsalis et al. 1982) and in vitro with human and rat cells, no increase in induced DNA repair response was observed (Butterworth et al. 1989). Lastly, Li, Wang et al. (2003) and Li, Cheng et al. (2003) observed that nitrobenzene can form adducts with hepatic DNA in male mice. After administering nitrobenzene intraperitoneally in corn oil at doses of 0.1-100 ug/kg and 10 mg/kg animals were sacrificed and the authors observed a dose-response relation with hepatic DNA adducts and nitrobenzene dose within 2 hours of exposure at all dose levels (Li, Wang et al., 2003). In addition, in a time course study, mice were exposed to 4.1 ug/kg dose of nitrobenzene and animals were sacrificed between 4 hours and 21 days after exposure (Li, Wang et al., 2003). Authors observed that adducts disappeared with a half-life of 10 hours for the initial 3 days. Thereafter, adducts disappeared with a half-life of 6.5 days in 21 days. These findings appear to point to a genotoxicity potential of nitrobenzene. However, the DNA adducts were neither characterized nor identified.

In conclusion, overall, results of nitrobenzene genotoxicity studies are mixed, and it appears to be a weak genotoxicant based on negative results in *Salmonella* assays as well as negative clastogenic findings from in vivo sister chromatid exchange assays, unscheduled DNA synthesis and mixed results of chromosomal aberrations, DNA breakage and micronucleus data. Results of some DNA binding and adduct studies remain unconfirmed.

Table 2-4. Genotoxicity of Nitrobenzene In Vitro

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
S. typhimurium strains TA98, TA100, TA1535, TA 1538	Mutagenic Activity	-	ND	Anderson and Styles (1978)
S. typhimurium strains TA98, TA100	Mutagenic Activity	-	-	Assman et al. 1997
S. typhimurium strains TA98, TA100	Mutagenic Activity	-	ND	Chiu et al. 1978
S. typhimurium strains TA98, TA100	Mutagenic Activity	-	-	Dellarco and Prival 1989
S. typhimurium strains TA1538, TA98, TA100	Mutagenic Activity	-	-	Garner and Nutman (1977)
S. typhimurium strains TA98, TA100, TA1535, TA 1537	Mutagenic Activity	-	-	Haworth et al. 1983
S. typhimurium strains TA98	Mutagenic Activity	ND	-	Ho et al. 1981
S. typhimurium strains TA 97, TA98, TA100	Mutagenic Activity	-	-	Hughes et al 1984
S. typhimurium strains TA98, TA Mix	Mutagenic Activity	-	-	Mestankova et al. 2016
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Mutagenic Activity	-	-	Shimizu et al. 1983
S. typhimurium strains TA 98, TA98NR, TA 100, TA100NR, TA97a, TA1535, TA1537, TA1537NR, TA1538	Mutagenic Activity	-	ND	Vance and Levin (1984)
S. typhimurium TA1535	Mutagenic Activity	-	-	Bonnefoy et al. (2012)
Mammalian cells:				
Human lymphocytes	Chromosomal aberrations	ND	+	Huang et al. 1995; 1996
Human thyroid cells	DNA fragmentation (comet assay)	ND	+	Mattioli et al. 2006

Table 2-4. Genotoxicity of Nitrobenzene In Vitro

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Human kidney cells	DNA fragmentation (comet assay)	ND	+	Robbiano et al. 2004
Rat kidney cells	DNA fragmentation (comet assay)	ND	+	Robbiano et al. 2004
V79 hamster lung fibroblasts	Micronucleus Assay	ND	+	Bonacker et al. 2004
Human Kidney Cells	Micronucleus Assay	ND	+	Robbiano et al. 2004
Rat Kidney Cells	Micronucleus Assay	ND	+	Robbiano et al. 2004
Human kidney cells	Micronucleus Assay	ND	+	Robbiano et al. 2004
Rat kidney cells	Micronucleus Assay	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; NA = not applicable; ND = no data

Table 2-5. Genotoxicity of Nitrobenzene In Vivo

Species (test system)	End point	Result	Reference
Male Sprague-Dawley Rats (liver, thyroid and kidney cells)	DNA fragmentation (comet assay) in thyroid, liver, kidney cells	+	Mattioli et al. 2006
Sprague-Dawley Rats (kidney cells)	DNA fragmentation (assay	+	Robbiano et al. 2004
Male F-344 rat hepatocytes	Unscheduled DNA synthesis	-	Mirasalis et al. 1982
F344 rat peripheral blood lymphocyte	Sister chromatid exchange	-	Kligerman et al. 1983
F344 rat isolated spleen lymphocyte	Sister chromatid exchange	-	Kligerman et al. 1983
Kunmig mice	DNA binding	+	Li et al. 2003a, b

+ = positive result; — = negative result; NA = not applicable; ND = no data

No studies were located regarding genotoxic effects in humans or animals after dermal exposure to nitrobenzene.

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

3.1 TOXICOKINETICS

3.1.1 Absorption

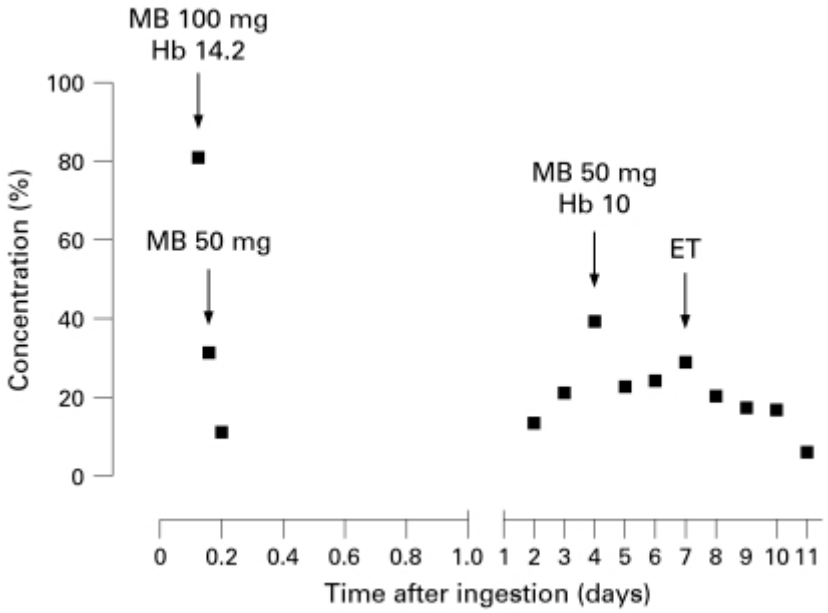
There are no quantitative data regarding the extent of uptake of nitrobenzene by humans after an oral exposure; however, case reports of human poisoning provide indirect evidence of oral absorption. Myslak et al. (1971) reported symptoms of poisoning in a 19-year-old female about 30 minutes after ingesting about 50 mL of nitrobenzene. Detection of the presence of high levels of metabolites of nitrobenzene, p-amino- and p-nitrophenol, in patient's urine (see section 3.3.1) demonstrates absorption from the gastrointestinal tract (Myslak et al. 1971). Martinez et al. (2003) reported a fatal case of severe methemoglobinemia (70%) observed in an 82-year-old male who had ingested about 250 mL of nitrobenzene in the previous 24 h. Forty-eight hours after ingestion, a blood sample was collected, and 3.2 pg/mL of nitrobenzene was detected (Martínez et al. 2003). Patel et al. (2008) reported a case of extremely high levels of methemoglobin (MetHb) (66.7%) in a 20-year-old male 16 h after ingesting about 75 mL nitrobenzene. The authors described administering methylene blue (100 mg via an I.V) but metHb rose again 18 hours after administration. The authors suggested this may be due to the release of nitrobenzene from adipose tissue.

Another case of recurrent methemoglobinemia was reported in a 25-year-old female on days 3 and 5 after ingestion of 100 mL of 22% nitrobenzene. Initial metHb levels were 81% by Perera et al. (2009) the time course of metHb levels from this case are presented in Figure 3-1. The authors speculated that due to massive ingestion of nitrobenzene, metabolism of the parent compound and its active metabolites was saturated. This may have led to prolonged exposure to the active metabolite (Perera et al. 2009).

Available in vivo and in vitro evidence suggests extensive intestinal absorption of nitrobenzene in experimental animals. Rickert et al. (1983) administered single oral doses of 22.5 or 225 mg/kg [^{14}C]-labeled nitrobenzene to male F344 (CDF[F344]/CrlBR), Sprague-Dawley (Crl:CD[SD]BR), and male B6C3F1 (B6C3F1/Crl/BR) mice (225 mg/kg only). A significant absorption of nitrobenzene from the gastrointestinal tract appeared in Fischer-344 and Sprague-Dawley rats with the recovery of 72- 88% in 72 h, and lesser in B6C3F1 mice with 54% in urine. Six metabolites were found in the bile. The authors also examined the role of bacterial gastrointestinal nitroreductases in absorption of nitrobenzene. This was done by using axenic (bacteria-free) CDF(F344)/CrlGN rats given similar doses of [^{14}C]-nitrobenzene. By comparing the difference in absorption with conventional animal the authors concluded that the

metabolites produced through nitro-reduction in conventional rats were initiated in the intestines, based on their observation of the absence of the nitrobenzene metabolites in the bile of axenic rats (Rickert et al. 1983).

Figure 3-1. Methemoglobin Concentration Following Nitrobenzene Poisoning.



MB = Methylene Blue, Hb = hemoglobin (g/L), Concentration (%) = Methemoglobin (%), ET= Exchange Transfusion
Source: Perera et al. 2009

Albrecht and Neumann (1985) also provide evidence of absorption after oral administration of [14C]-nitrobenzene (25 mg/kg) in female Wistar rats. Within a week after administration, 80% of radioactivity dose was recovered from the body, through urine (65%) and feces (15%). This indicates significant absorption of nitrobenzene from the gastrointestinal tract (Albrecht and Neumann 1985). Parke (1956), using oral administration of 250 mg/kg [14C]-nitrobenzene in rabbits, recovered 78% of administered [14C]-nitrobenzene 8 days after dosing in exhaled air (1.6%), urine (58%), and feces (11.3%) (Parke 1956) (detailed information is in section 3.1.4). Quantitatively, the available experimental animal studies indicate gastrointestinal tract absorption of orally administered nitrobenzene of over 60%.

An in vitro study used brush border membrane vesicles isolated from the small intestines of Sprague-Dawley rats to examine the nitrobenzene absorption (Alcorn et al. 1991). Brush border membrane vesicles isolated from human intestinal epithelial cells are purified and used to evaluate drugs and their toxicokinetics for intestinal transport studies (Patel and Misra 2011). Since nitrobenzene was well absorbed by all segments (i.e., proximal third, middle third, and distal third) of the small intestine, the

compound's lipophilicity and lipid composition of gut membrane were suggested to be the main determinants of the absorption (Alcorn et al. 1991).

In humans, nitrobenzene was well absorbed through the lung, with quantitative estimates of nitrobenzene's pulmonary absorption available from some clinical studies. During a 6-hour exposure of seven human research subjects (adult males, age unstated) to nitrobenzene (5–30 μg/L; 1–6 ppm) found absorption to average 80%, varying from a mean value of 87% in the first hour to 73% in the sixth hour (Salmowa et al. 1963). The efficiency of uptake was dose dependent and showed considerable individual variation (Salmowa et al. 1963). Piotrowski (1967) also estimated about 80% pulmonary absorption in four adult male volunteers (age unstated) exposed to a range of nitrobenzene concentrations in air (5–30 μg/L; 1–6 ppm) 6 hours daily for 4-7 days. Beauchamp Jr. et al. (1982) estimated that 18.2–24.7 mg of nitrobenzene would absorb through the lungs of humans exposed to an airborne nitrobenzene concentration of 10 mg/m^3 for 6 hours (Beauchamp Jr. et al. 1982).

In animal studies, no quantitative data on absorption of nitrobenzene after inhalation were located. However, nitrobenzene appears to be well absorbed through the lung, based on observations of toxic responses and pathological findings in treated animals (Medinsky and Irons 1985).

The toxicokinetics of dermal exposure have not been well studied in animals.

Nitrobenzene has been found to be capable to penetrate human dermal barrier in several studies. Piotrowski (1967) exposed human subjects to nitrobenzene vapor through a chamber for 6 hours, while receiving fresh air through a breathing tube and mask. The highly variable (0.23–0.30 mg/hour per μg/L) absorption rate of nitrobenzene was dependent on either the nitrobenzene concentration in the chamber (5–30 μg/L) or whether the subject was dressed or naked. For instance, naked subjects exposed to a chamber concentration of 10 μg/L nitrobenzene had an estimated absorbed dose from 10– 19 mg, while wearing normal working clothes reduced absorption of nitrobenzene by 20–30%. The absorption rate of vapor through the skin is much less than that of liquid, which can reach about 2 mg /cm^2/ hr (Salmowa et al. 1963) and the main source of danger in industry is the contamination of skin and clothing with liquid (Piotrowski 1967). Feldmann and Maibach (1970) applied 21 [14C]-labeled organic compounds, including nitrobenzene, in acetone (4 μg/cm2) as liquid to a 13 cm^2 circular area of the ventral forearm surface of six human subjects (age and sex not stated). The skin site was not protected and not washed for 24 hours. For the purpose of skin absorption estimates, the cumulative amounts of radiolabel measured in urine over 5 days. The absorption rate of the [14C]-labeled nitrobenzene, estimated as percent dose per hour, was the highest (0.022%/hour) in the first 24-hours. The excretion was still measurable (0.006%/hour) in the urine between 96 and 120 hours after application, indicating the redistribution of

nitrobenzene or its metabolites from adipose tissue rather than continued absorption. The authors estimated a factor of 2.6% for dermal absorption (Feldmann and Maibach 1970).

Bronaugh and Maibach (1985) measured percutaneous absorption of nitrobenzene and other nitroaromatic compounds in vivo and in vitro in the humans and monkeys. Rapid penetration was observed with maximum absorption occurring in the first few hours in both species. Significant differences in absorption were found in different procedures of human study with higher values in vitro (7.8 vs. 1.5%). The relative volatility was measured by the loss of compound from applied epidermal discs at various time intervals. The greatest loss of the compound occurred in the first minute. The in vivo data shows that percutaneous absorption of nitrobenzene was greater in monkeys than in humans. The in vivo and in vitro studies suggest a monkey skin as a good model for human skin for percutaneous absorption through their comparable absorption rate. In monkeys, 81.4% of dose was excreted as unchanged nitrobenzene in urine (Bronaugh and Maibach 1985). Deutsche Forschungsgemeinschaft *(*DFG) (2012) estimated the absorption of 25 mg per day by persons exposed to an air concentration of 1 ml/m^3 nitrobenzene at work through skin (one third) and inhalation (two thirds) where working week exceeded 40 hours (DFG 2012).

The toxicokinetics of dermal exposure have not been well studied in animals.

3.1.2 Distribution

No studies of the distribution of nitrobenzene or its metabolites after inhalation and dermal exposure by humans or animals were found in the literature. Studies of oral exposure indicate wide distribution of nitrobenzene in tissues, particularly in fatty tissues, a pattern of distribution likely related to nitrobenzene's lipophilicity.

Nitrobenzene was found in stomach, liver, brain and blood with the highest concentration in liver (0.124 mg/kg tissue), and brain (0.164 mg/kg tissue) in autopsies of 5 patients that had died from nitrobenzene poisoning (Wirtschafter and Wolpaw 1944). Forty-eight hours after ingestion, 3.2 pg/mL of nitrobenzene was still detectable in blood (Martínez et al. 2003) (see section 3.1.1). Delayed rise in the MetHb levels in a case of severe methemoglobinemia (67%) after a dose of methylene blue led Patel et al. (2008) to suggest possible attribution of the release of nitrobenzene stores from the adipose tissue. The delayed release of nitrobenzene from gastrointestinal tract is seen, in addition to stores in the adipose tissue after severe poisoning (Gupta et al. 2000).

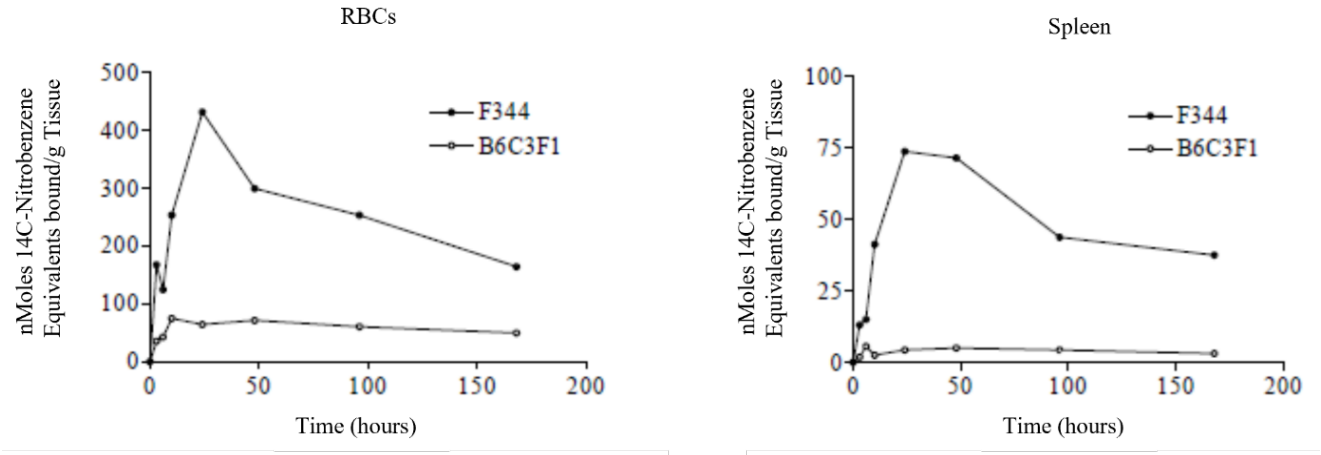
Piotrowski (1977) found that the ratio of the concentration of the compound in adipose tissue to that in the blood was about 10:1 at an hour after an intravenous dose in rats.

Radiolabel studies in experimental animals indicate wide distribution of nitrobenzene and/or its metabolites in the body. Freitag et al. (1982) found 0.43% of the radioactive dose in the liver and 2.3% in the remaining carcass at necropsy. No radiolabeled nitrobenzene was observed in abdominal adipose tissue or in the lungs 8 days after exposure of three male Wistar rats to 1 mg nitrobenzene/kg-body weight via stomach tube for three days. Albrecht and Neumann (1985) measured [^{14}C]-nitrobenzene in blood, liver, kidney, and lung at the first day and a week after exposure of female Wistar rats to 25 mg/kg (0.20 mmol/kg) radiolabeled by gavage. Recovery of radioactive label (radioactivity in tissue [pmol/mg]/dose [μmol/kg]) from tissues at first day after exposure was as following order: blood (229 ± 48) > kidney (204 ± 27) >> liver (129 ± 9.5) >> lung (62 ± 14). Tissue levels of radioactive label decreased 4 to 5-fold in liver and kidney, and ~2-fold in blood and lung (Albrecht and Neumann 1985). Parke (1956) found 44.5% of radioactivity dose in tissues, i.e., in kidney fat (15.4%), skeletal muscle (12%) and intestinal fat (11.6%) (excluding stomach and intestinal contents), in one and a half days after administration of 250 mg [^{14}C]-nitrobenzene/kg of body weight of a rabbit by stomach tube. In 8 days after dosing, radioactivity came down to 7.5%, with the highest levels of 5.4% in fat deposits, consistent with nitrobenzene lipophilicity. The amount of nitrobenzene in the blood was not measured.

Albrecht & Neumann (1985) also reported higher binding affinity of [14C]-nitrobenzene metabolites to hemoglobin (Hb) (1.03 ± 0.137 μmol/mg/dose after one day) and plasma proteins (0.136 ± 0.034) compared to [14C]-acetanilide (0.177 ± 0.014 in Hb and 0.07 ± 0.007 in plasma proteins). In both cases the reactive metabolite was thought to be nitrosobenzene, and aniline was bound to protein sulfhydryls via a sulfinic acid amide bond (Albrecht and Neumann 1985).

Goldstein and Rickert (1984) measured covalent binding of radiolabeled nitrobenzene in male CDF (F344)/CrlBR rats and B6C3F1/CrlBR mice fed a single oral dose of 10 or 40 μCi [14C]-nitrobenzene in corn oil (doses ranges of 75–300 mg/kg). Both species showed a dose dependent increase in the covalent binding of radiolabeled nitrobenzene to red blood cells (RBCs) and spleen proteins, while total and bound levels of radiolabeled nitrobenzene were significantly greater in RBCs from rats (6–13 times) at all doses (Figure 3-2).

Figure 3-2. Time Course of Covalently Bound [14C]-Nitrobenzene in RBCs and Spleen of Rats and Mice.



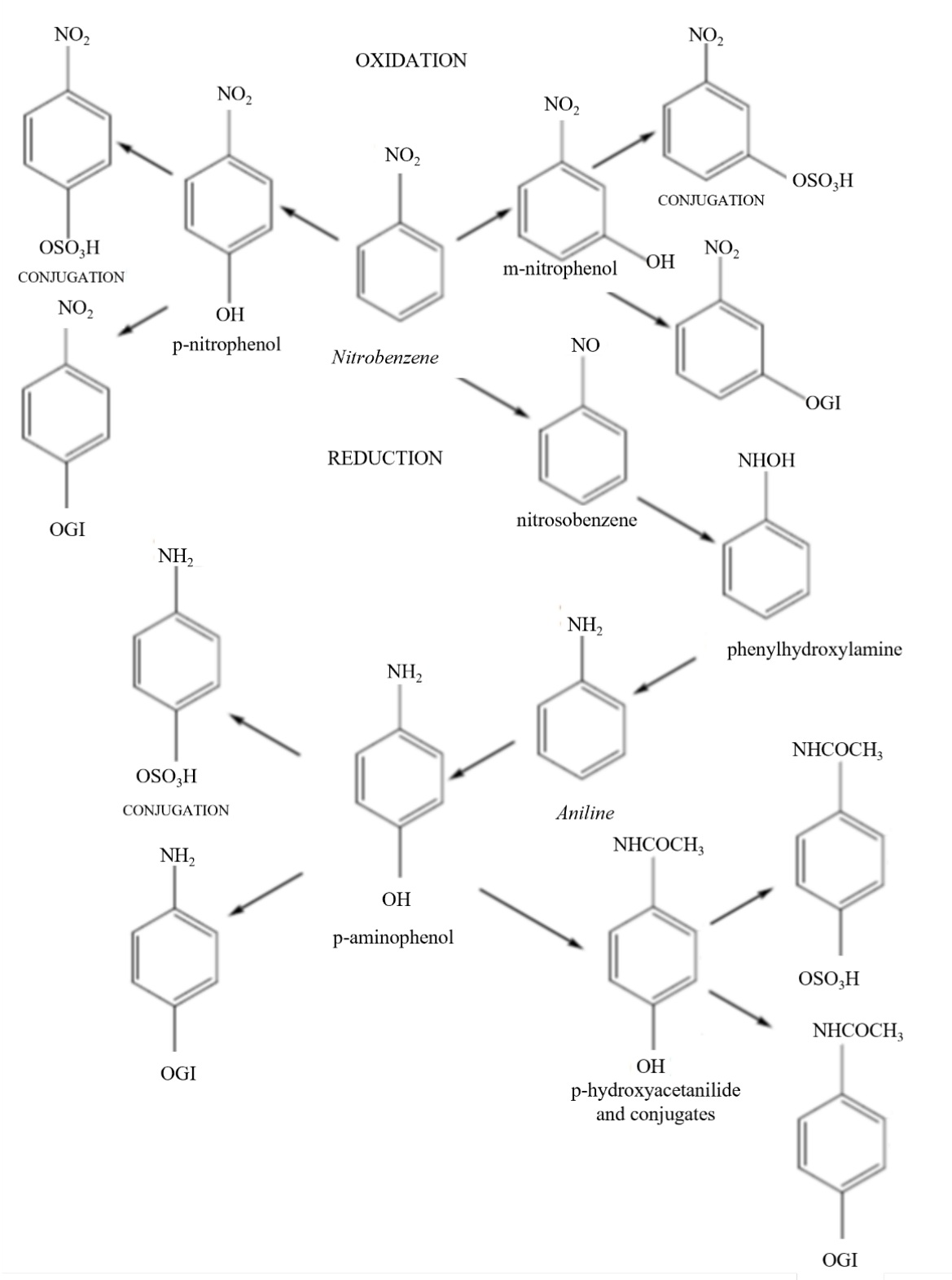
Note: Animals were administered 200 mg/kg [14C]-nitrobenzene and sacrificed at various time points. Each point represents the mean ± standard error of the mean of three to four determinations. Statistically significant differences between the F344 rat and B6C3F1 mouse were noted at all doses tested.

Source: Adapted from Goldstein and Rickert 1984

3.1.3 Metabolism

Nitrobenzene is metabolized in mammals by both reductive and oxidative pathways. The metabolites of nitrobenzene are likely to be associated with many toxicological effects. Reduction of the nitro group yields nitrosobenzene, phenylhydroxylamine, and aniline, and was followed by phase II reactions involving the replacement of a nitro group by glutathione, and the formation sulfated or glucuronidated conjugates. Nitrobenzene is oxidized by either hydroxylation of the benzene ring (usually at positions 3 or 4) to nitrophenols or after initial nitroreduction of the exocyclic nitro group to the amine by oxidation to phenylhydroxylamine. Figure 3-3 shows a schematic of oxidative and reductive metabolisms of nitrobenzene (Rickert 1987).

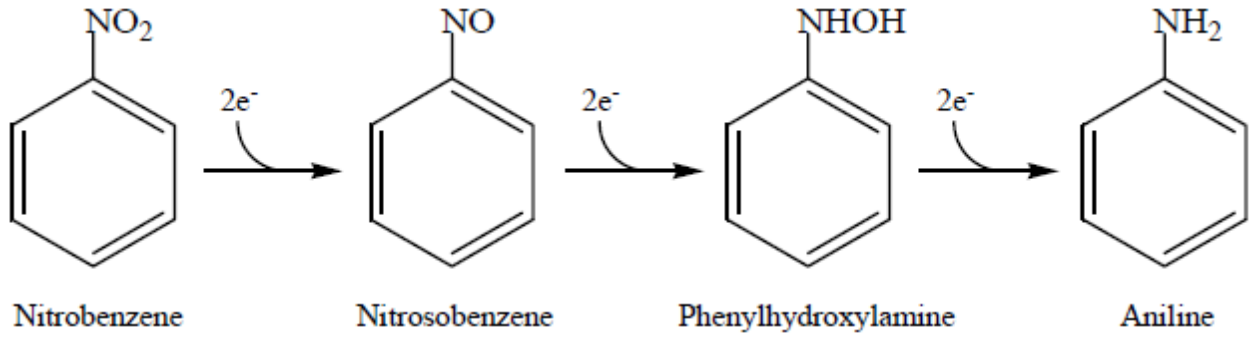
Figure 3-3. Outline of the Metabolism of Nitrobenzene: a Substrate for Oxidation and Reduction Reactions.



Source: Modified from Rickert 1987

Reduction of nitrobenzene to nitroxide intermediates is thought to be primarily mediated by endogenous intestinal microbes. Evidence suggests that nitrobenzene reduction to aniline is a result of a three-step, two-electrons per-step transfer with the intermediates in this process are nitrosobenzene and phenylhydroxylamine (Albrecht and Neumann 1985; Bryant and DeLuca 1991; Reddy et al. 1976) and appears to be catalyzed by type I Nitroreductase (also known as nicotine adenine dinucleotide phosphate [NADPH] dehydrogenase). Figure 3-4 illustrates the mechanism of the three-step, two-electrons-per-step reduction of nitrobenzene in the intestinal microflora.

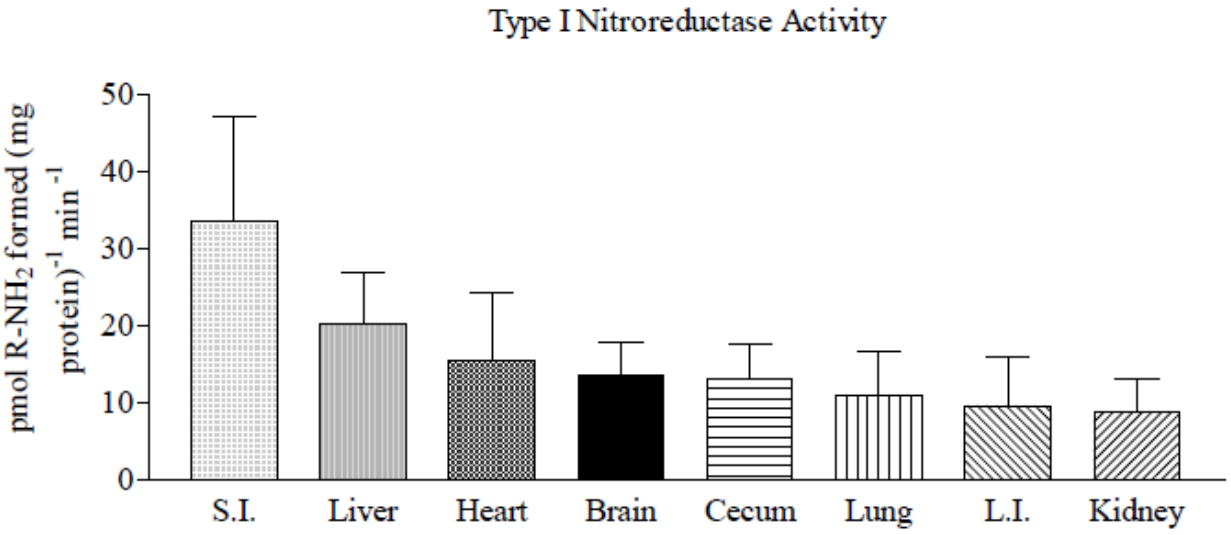
Figure 3-4. Mechanism of Bacterial Nitrobenzene Reduction to Aniline.



Source: Adapted from Holder 1999

Although organ-specific activities have been reported, the intestinal microflora in the small intestines expressed the highest enzymatic activity of type I nitroreductase in male Sprague-Dawley rats (Figure 3-5) (Ask et al. 2004).

Figure 3-5. Type I Nitroreductase Activity in Male Sprague-Dawley Rats.



Source: Adapted from Ask et al. 2004

Note: Results are expressed as pmol of reduced nilutamide (R-NH2) formed per milligram protein per minute (mean ± SEM; n ≥ 4). S.I. = small intestine contents, L.I. = large intestine contents.

Goldstein et al. (1984) also investigated the role of microflora in metabolism of radiolabeled nitrobenzene in vitro and observed metabolites, including aniline, nitrosobenzene, and azoxybenzene, with larger amounts in the presence of pectin-enriched gut contents (Table 3-1) (Goldstein et al. 1984).

Table 3-1. Formation of Metabolites of Nitrobenzene in the Presence of Cecal Contents *In Vitro*: Influence of Diet

		Metabolite formation (percent total radioactivity)[a]			
Diet	Pectin (%)	Aniline	Nitrosobenzene	Azoxybenzene	Nitrobenzene
NIH-07	8.4	36 ± 10[b]	7 ± 0[b]	7 ± 1[b]	34 ± 11[b]
AIN-76A	5 (added)	11 ± 4	3 ± 2	3 ± 2	78 ± 11
AIN-76A	0	3 ± 1	0 ± 0	0 ± 0	95 ± 2

[a] Values are means ± SEM of four determinations.
[b] Significantly different from AIN-76A.
Source: Goldstein et al. 1984

To investigate the influence of gut microflora on the metabolism of nitrobenzene *in vivo*, normal or antibiotic-treated male F344 (COBS CDF/CrlBR) rats were kept in metabolic cages for up to 72 hours after treatment with 225 mg/kg nitrobenzene (containing 0.1 µCi/mg [14C]-nitrobenzene) by gavage (Levin and Dent 1982a). In antibiotic-treated rats, p-hydroxyacetanilide (a reductive metabolite of nitrobenzene) significantly decreased, while p- and m-nitrophenol (oxidative metabolites) slightly increased versus controls, indicating the antibiotic-induced inhibition of total nitrobenzene metabolism (Table 3-2).

Table 3-2. Urinary Metabolites of [14C]-Nitrobenzene Excreted Within 72 Hours After Gavage

Metabolite	Percent of total[a]	
	Control rats	Antibiotic-treated rats
p-Nitrophenol	22.4 ± 0.9	26.5 ± 3.8
m-Nitrophenol	11.4 ± 0.6	16.1 ± 2.0
p-Hydroxy-acetanilide	16.2 ± 1.7	0.9 ± 0.0[b]
Unidentified peak I	4.5 ± 0.3	5.5 ± 0.9
Unidentified peak II	3.7 ± 0.6	0.5 ± 0.1[b]
Total recovered	58.2	49.5

[a]Values are means ± standard deviations for three animals/group.
[b]Significantly different from controls.
Source: Levin and Dent 1982

Ample evidence is available on the mechanism of methemoglobinemia caused by the interaction of Hb with the products of nitrobenzene reduction (i.e., nitrosobenzene, phenylhydroxylamine, and aniline). Earlier evidence suggests that the intestinal microflora in the rat is an important element in the formation metHb resulting from nitrobenzene exposure (Reddy et al. 1976). The authors demonstrated that 30–40% of the hemoglobin (Hb) in the blood was converted to metHb within 1–2 hours after intraperitoneal (i.p.) administration of nitrobenzene (200 mg/kg of body weight in sesame oil) in normal Sprague-Dawley rats, in contrast to no measurable metHb formation, even when measured up to 7 hours after treatment of the same dose administered to germ-free or antibiotic-pretreated rats. The nitroreductase activity was negligible in gut contents from germ-free rats and greatest in control rats, when compared the rate of synthesis of aniline in homogenates of liver, kidney, gut wall, and gut contents prepared from various treatment animals (Table 3-3). Therefore, metHb formation involves a nitrobenzene metabolite such as aniline, which is formed by the bacterial reduction of nitrobenzene in the intestines of rats.

Table 3-3. Reduction of Nitrobenzene by Various Rat Tissue Homogenates

Tissue	Aniline formation (nmol/mg protein/hour)[a]		
	Bacteria-free	Bacteria-free (acclimatized)	Control
Liver	2.0 ± 0.2	2.5 ± 0.4	3.3 ± 0.4
Kidney	0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.4
Gut wall	2.0 ± 0.4	2.0 ± 0.6	2.4 ± 1.0
Gut contents	0.2 ± 0.0	15.2 ± 2.7	11.1 ± 3.3

[a]Results are means ± standard error of the means (SEM) of determinations in three animals/group, with all determinations in triplicate.
Source: Reddy et al. 1976

Facchini and Griffiths (1981) investigated methemoglobinemia-inducing capacity of nitrobenzene in both normal and antibiotically pretreated rats in vivo, and in vitro on incubation with rat blood. An in vitro incubation of blood with nitrobenzene demonstrated little or no metHb formations (Facchini and Griffiths 1981). *In vivo* findings with axenic animals (Table 3-4) confirm the importance of microbial reductive

metabolism in the formation of metHb, specifically through the formation of nitrosobenzene, phenylhydroxylamine, or aniline.

Table 3-4. MetHb Formation in the Blood of Rats Dosed Intraperitoneally with 200 mg/kg Nitrobenzene in Corn Oil

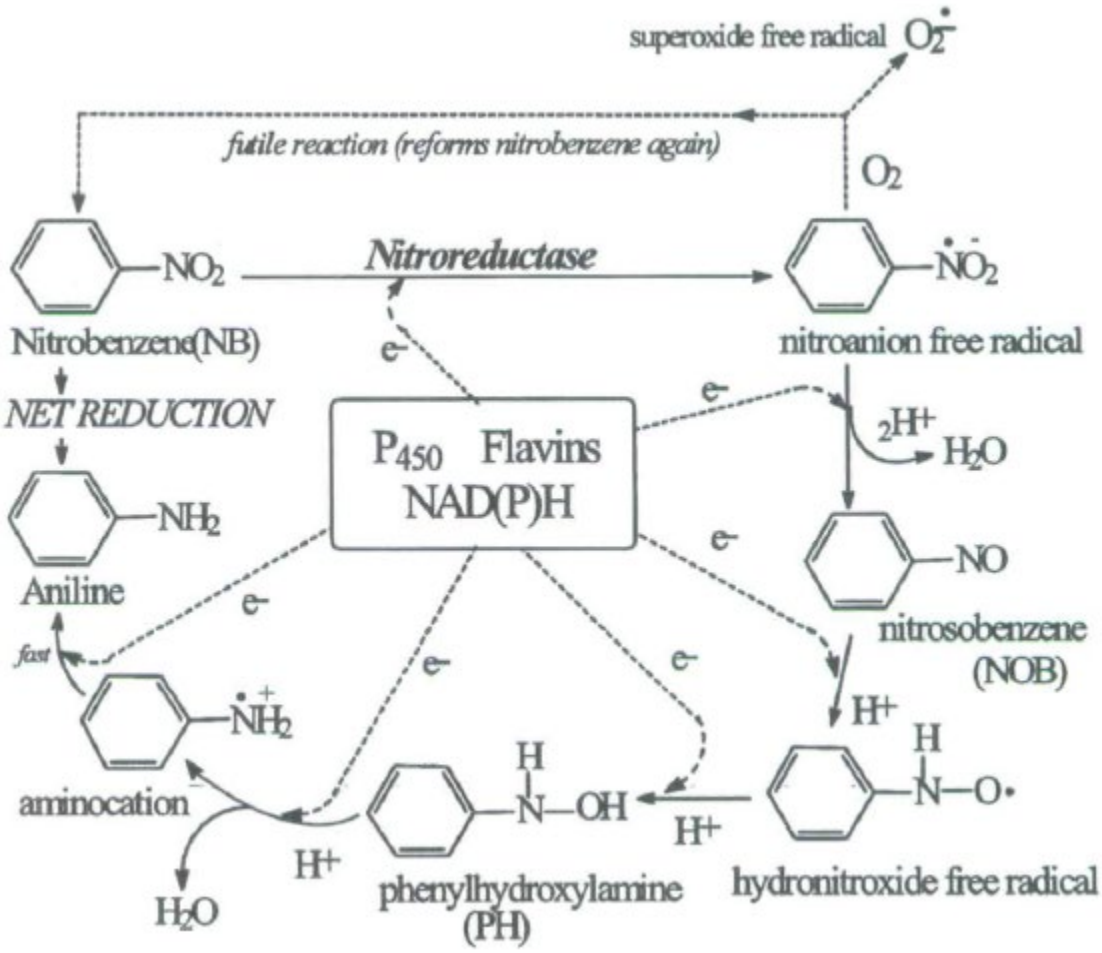
	MetHb formation (%)[a]	
Time after dosing (hours)	Control rats	Antibiotic-treated rats
1	18.2 ± 5.0	1.7 ± 0.4
2.5	24.7 ± 4.2	2.1 ± 0.2
5	32.7 ± 5.0	1.9 ± 0.4
8	9.9 ± 2.3	0.4 ± 0.1

[a]Results are means ± SEM, three animals/group. Source: Facchini and Griffiths 1981

The nitrobenzene metabolites, nitrosobenzene and phenylhydroxylamine have been found to bind with hemoglobin in the blood of orally exposed mice and rats (Goldstein and Rickert 1984) (see section 3.1.2). Goldstein et al. (1984b) observed metHb level in blood at 1, 2, 4, 8, and 24 hours after dosing and observed levels peaking at the 4-hour time point. In addition, they found metHb levels associated with pectin-containing diet in male CDF(F344)/CrlBR rats. Specifically, Goldstein et al. (1984b) pretreated male CDF(F344)/CrlBR rats with diets containing pectin (a carbohydrate with nutritional value for microflora) or cellulose (a metabolically inert carbohydrate) for 28 days prior to a single 200 mg/kg dose of [^{14}C]-nitrobenzene via gavage. Levels of metHb were monitored in the blood. Rats receiving cellulose-containing diet had no metHb formed in the blood while those fed the pectin diet had elevated methHb levels.

Hepatic microsomes and erythrocytes are also important sites of reduction of nitrobenzene to aniline, which produces reactive intermediates, including a nitro anion free radical, nitrosobenzene, a hydronitroxide free radical, phenylhydroxylamine, and a theoretical amino-cation free radical (Levin and Dent 1982; Reddy et al. 1976; Holder 1999). Some intermediates are reversible (i.e., aniline can oxidize back to nitrobenzene or any step in between). The nitro anion free radical may undergo a reverse reaction to reform nitrobenzene through nonenzymatic reactions with tissue oxygen. This so-called “futile loop” also generates a superoxide anion (Sealy et al. 1978), which may be dismutated to molecular oxygen and hydrogen peroxide by superoxide dismutase (Holder 1999; Mason and Holtzman 1975a, 1975b). These reactions forming superoxide and hydrogen peroxide may further perturb the local redox balance, potentially contributing to oxidative stress (Gutteridge 1995). Holder (1999) summarized the six one-electron reduction steps of microsomal nitrobenzene reduction (Figure 3-6).

Figure 3-6. Mechanism of Microsomal Nitrobenzene Reduction.

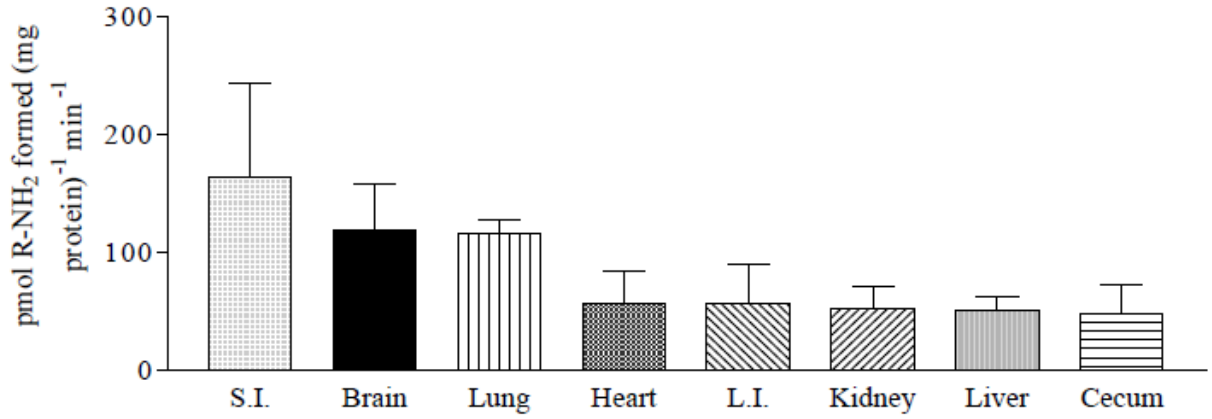


Source: Adapted from Holder 1999

The scheme shows the production of reactive free radicals to form aniline, starting with a nitro anion free radical, which in turn is reoxidized to nitrobenzene with the formation of a superoxide anion. Alternatively, further nitroreduction to nitrosobenzene produces hydronitroxide free radical and further reduction to phenylhydroxylamine which may produce an amino cation free radical (Figure 3-6).

Reduction of nitrobenzene by the one-electron reductive pathway is catalyzed by a type II (oxygen-sensitive) nitroreductase, which has highest activity in the microflora of the intestinal tract of male Sprague-Dawley rats but is also active in other tissues (Figure 3-7).

Figure 3-7. Type II Nitroreductase Activity of Male Sprague-Dawley Rats.

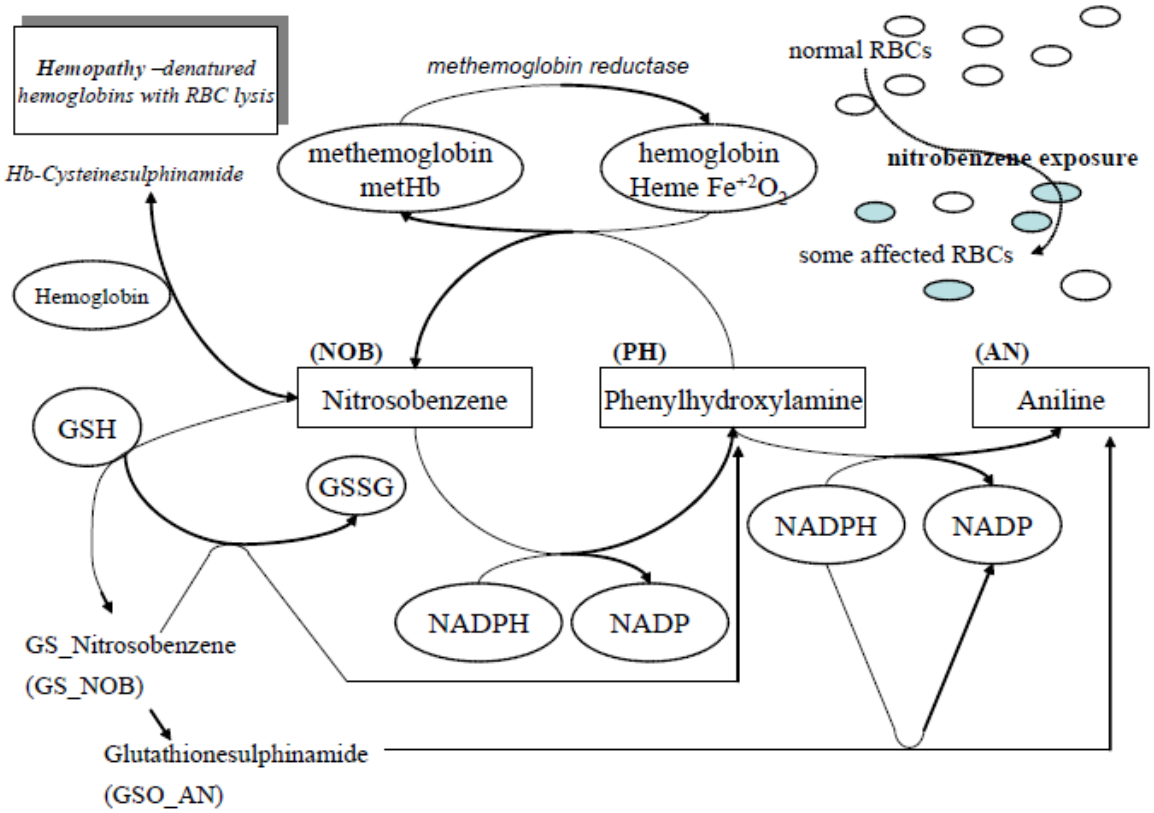


Note: Results are expressed as pmol of reduced nilutamide (R-NH2) formed per milligram protein per minute (mean ± SEM; n ≥ 4). S.I. = small intestine; L.I. = large intestine.

Source: Adapted from Ask et al. 2004.

Nitrobenzene also undergoes reductive metabolism in erythrocytes, and the associated redox chemistry is of particular importance due to its association with nitrobenzene-induced methemoglobinemia. However, because *in vitro* incubation of RBCs with nitrobenzene alone does not result in the metHb formation (Facchini and Griffiths 1981), the cycling of the reductive products of nitrobenzene within RBCs cause oxyhemoglobin (oxyHb) to be formed to metHb (Figure 3-8).

Figure 3-8. Cycling of Nitrosobenzene and Phenylhydroxylamine in RBCs, Resulting in the Formation of metHb.



Note: GSH = reduced glutathione, GSSG = oxidized glutathione; GS = glutathionyl conjugate.
Source: Adapted from Holder (1999).

Therefore, the cycling between phenylhydroxylamine and nitrosobenzene is the primary metabolic event in the formation of metHb. Reduction of nitrosobenzene to phenylhydroxylamine can occur nonenzymatically by endogenous reducing agents or enzymatically by NADH-cytochrome b5 reductase, thereby the redox cycle ended up with the overall expenditure of NADH and the accumulation of metHb (Maples et al. 1990). Nitrosobenzene has detrimental effect on metabolic balance of RBCs through its metabolism. That is the binding affinity of nitrosobenzene to the heme moiety of Hb is 14-fold higher than molecular oxygen (Eyer and Ascherl 1987). Another potential hazard of nitrosobenzene is its binding to peptides and proteins carrying cysteine residues, including Hb and reduced glutathione (GSH), which produces sulfhemoglobin (Eyer 1979). Furthermore, the excessive cycling of nitrosobenzene may result in an overall depletion of GSH.

Maples et al. (1990) used ESR spin trapping technique *in vivo* in rats dosed with nitrobenzene to detect the formation of DMPO/hemoglobin thiol free radical adduct in blood. This study demonstrated that

nitrobenzene is metabolized to phenylhydronitroxide radical that oxidizes thiols within erythrocytes (Maples et al. 1990). Continuous recycling of phenylhydroxylamine and nitrosobenzene to these highly reactive moieties may lead to increased fragility of erythrocytes membranes and further damage in the spleen (CIIT 1993; Goldstein and Rickert, 1984).

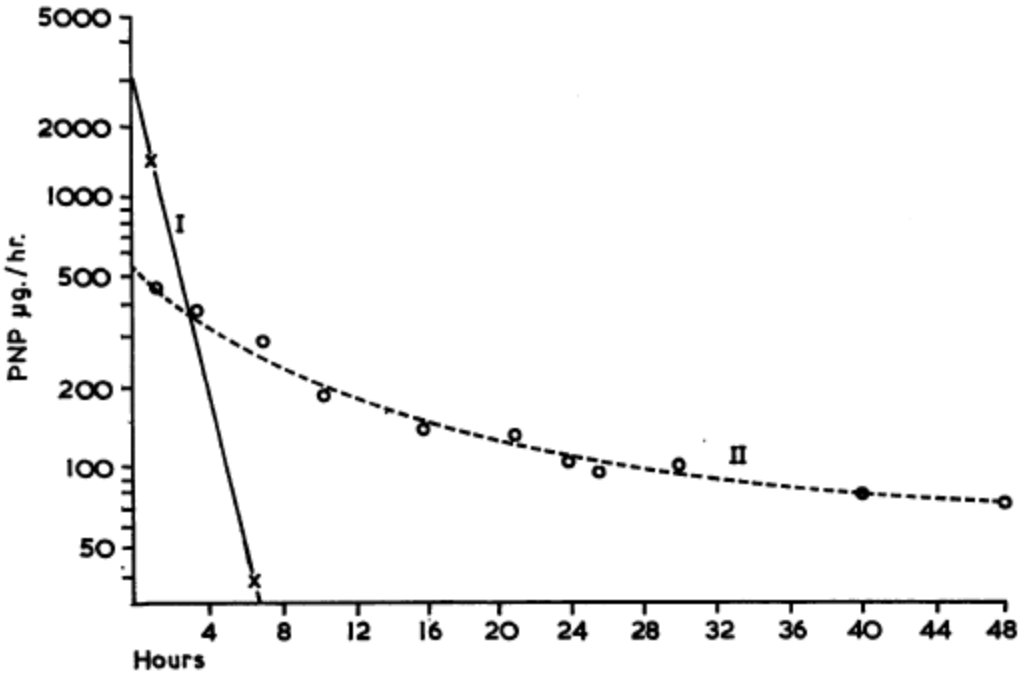
Various hepatic microsomal oxygenates from the cytochrome P450 family of enzymes can catalyze oxidation of nitrobenzene, forming p- and m-nitrophenols and derivative aminophenols, which are subsequently conjugated by phase II enzymes. Oxidation of nitrobenzene generally occurs via ring oxidation in a process that is generally slower than microsomal reduction of nitrobenzene. Subsequent conjugation to metabolites excreted in urine is discussed below in Section 3.1.4. Although N-hydroxylation to nitroxides is a minor pathway of oxidation, it is potentially important toxicologically because of the nitroxide intermediates that can be formed (Blaauboer and Van Holsteijn 1983; Kiese 1966; Mason 1982; Miller 1970; Verna et al. 1996; Weisburger and Weisburger 1973).

3.1.4 Excretion

The major route of excretion after oral exposure to nitrobenzene in humans and animals is urinary excretion of metabolites.

In most cases of human poisoning, p-aminophenol and p-nitrophenol are the observed nitrobenzene metabolites excreted in the urine (Myslak et al. 1971; Von Oettingen 1941). Myslak et al. (1971) reported an extensive excretion of those metabolites in the urine of a subject who ingested about 50 mL of nitrobenzene. The level of nitrobenzene metabolites reached a maximum on day 2 (198 mg/day for p-aminophenol) and 3 (512 mg/day for p-nitrophenol). Piotrowski (1967) examined the excretion rates of p-nitrophenol in a volunteer after intake of a single oral dose of 5 mg p-nitrophenol and 30 mg nitrobenzene, separately. The excretion of p-nitrophenol after exposure to p-nitrophenol was very rapid; in contrast, excretion was slow after exposure to nitrobenzene (Figure 3-9). It is thought that the accumulation observed does not depend on the behavior of the metabolite but is due to the slow rate of metabolism of nitrobenzene. The initial half-time of elimination of p-nitrophenol after exposure to nitrobenzene was around 5 h, with a late-phase half-time of >20 h (estimated from Figure 3-9), while all p-nitrophenol was eliminated by 8 h when subjects were exposed to nitrophenol directly. (JK Piotrowski 1967).

Figure 3-9. Excretion Rates (µg/hr) of p-Nitrophenol After Oral Intake of p-Nitrophenol (I) and Nitrobenzene (II), as a Function of Time After Ingestion.



Source: JK Piotrowski 1967.

The excretion of nitrobenzene observed in urine was 50 ± 10% and in feces about 4% of the dose within 24 h, increased to 65% and 15.5%, respectively in a week after a single oral administration of 25 mg [14C]nitrobenzene/kg of body weight in rats via gavage, appeared in the urine and in the. Collectively, about 80% of the dose are in the excreta in this study (Albrecht and Neumann 1985). As discussed in Section 3.1.3, Levin and Dent (1982) observed fecal, urinary, and exhalatory excretion of nitrobenzene metabolites in normal or antibiotic-treated male F344 rats after exposure to 225 mg/kg nitrobenzene (containing 0.1 µCi/mg [14C]-nitrobenzene via gavage with). Specifically, the recovery of the radiolabel in feces and expired air were 16.4 ± 2.2% and 2.3 ± 0.5% for control rats and 12.5 ± 3.6% and 3.4 ± 1.5%, for antibiotic-treated animals, respectively. Freitag et al. (1982) observed the 7-day excretion of 59.3% of the dose in the urine and 15.4% in the feces after 3-day oral exposure of male Wistar rats to 1 mg nitrobenzene/kg body weight via stomach tube. Not all sources of elimination of label were measured (e.g., carbon dioxide in expired air), and 22.6% of the administered dose was not accounted for (Freitag et al. 1982).

Rickert et al. (1983) compared the metabolism across species and strains through its excreted metabolites in male F344 and Sprague-Dawley rats, and male B6C3F1 mice in their experiment mentioned in section

3.1.1. The disposition of radiolabeled products among feces, urine, and expired air in animals housed in metabolic cages for 72 hours after dosing is shown in Table 3-5. Urine is shown as a primary route of excretion in all exposed groups. In rats, approximately 72- 88% of the administered dose was recovered in 72 h, of which about 80% was in urine. In B6C3F1 mice, recovery (54%) appeared lesser than in two rat strains, of which about 35% in urine (compared with 57- 63% in rats at the same dose) and about 19% in feces (compared with 14% in rats). The degree of conjugation to different metabolites showed species and strain differences (Table 3-6). In F344 rats, all detectable nitrobenzene metabolites were sulfates, consistent with Levin and Dent (1982). On the other hand, Sprague-Dawley rats and B6C3F1 mice excreted both sulfate and glucuronide conjugates, in addition to unconjugated metabolites. Moreover, the urinary metabolite p-Aminophenol was only detected in mice.

Similar results were found in other non-human species. In the giant chinchilla rabbit, Robinson et al. (1951) also reported urine as the major excretion pathway, with 45% of the radioactivity excreted in urine within 72 hours after oral exposure to a [14C]-nitrobenzene. Parke (1956) administered both [^{14}C] labeled and unlabeled nitrobenzene at doses of 200 mg/kg and 250 mg/kg to rabbits by stomach tube and kept the animals in cages for 30 hours after dosing. Urine and feces were collected for up to 10 days, in addition to expired air. After 250 mg/kg oral administration, 78% of administered radioactivity was recovered from the body within 8 days; 1.6% was in exhaled air, 58% in urine, 12.2% in feces and in gastrointestinal tract contents. A fraction eliminated from the body in the expired air includes 0.5% (1.3 mg) of unchanged nitrobenzene (up to 30 hours), a trace (<0.1%) of aniline, and 1% of the radioactivity of CO_2; about 58% were excreted in urine as metabolites, including 31% of p-aminophenol and 9% each of m- and p-nitrophenols, 4 and 3% of m- and o-aminophenols, and other minor metabolites, and only <0.1% (0.25 mg) excreted as unchanged nitrobenzene; and 6 out of 9% of metabolites eliminated in the feces as p-aminophenol (up to 4–5 days) (Parke 1956).

Table 3-5. Recovery of Radiolabel in F344 and Sprague-Dawley Rats and B6C3F1 Mice 72 Hours After Exposure to a Single Oral Dose of [14C]-Nitrobenzene[a]

	Percentage of dose recovered					
	F344 rat			Sprague-Dawley rat		B6C3F1 mouse
Excretory product	225 mg/kg oral	225 mg/kg i.p.	22.5 mg/kg oral	225 mg/kg oral	22.5 mg/kg oral	225 mg/kg oral
Urine	63.2 ± 2.1	56.8 ± 0.9	65.8 ± 2.4	60.8 ± 1.1	64.5 ± 0.8	34.7 ± 4.8
Feces	14.2 ± 0.7	13.7 ± 1.8	21.4 ± 1.8[b]	11.8 ± 1.1	11.5 ± 0.1	18.8 ± 0.4[b]
Expired air	1.6 ± 0.1	1.4 ± 0.1	1.0 ± 0.6	2.5 ± 0.3	0.8 ± 0.2	0.8 ± 0.1
Total	79.0 ± 2.2	71.9 ± 2.6	88.2 ± 1.8[b]	75.1 ± 1.1	76.8 ± 1.0	54.3 ± 4.7[b]

[a]Values are mean + SEM for three animals.
[b]Significantly different from F344 rats given 225 mg/kg orally.
Source: Rickert et al. (1983).

Table 3-6. Urinary Excretion of Nitrobenzene Metabolites in Male Rats and Mice Gavaged with a Single Oral Dose of [14C]-Nitrobenzene

		Percentage of dose[a]				
		F344 rat (mg/kg)		Sprague-Dawley rat (mg/kg)		B6C3F1 mouse (mg/kg)
Compound	Free/ conjugate	225	22.5	225	22.5	25
p-Hydroxyacetanilide	Free	–[b]	–	1.3 ± 0.2	0.9 ± 0.2	0.4 ± 0.0
	Glucuronide	–	–	1.8 ± 0.6	1.1 ± 0.1	3.1 ± 0.3
	Sulfate	19.0 ± 0.9	19.8 ± 2.8	5.8 ± 1.2	1.7 ± 0.9	0.4 ± 0.1
p-Aminophenol	Free	–	–	–	–	0.1 ± 0.1
	Glucuronide	–	–	–	–	0.2 ± 0.2
	Sulfate	–	–	–	–	9.4 ± 1.3
p-Nitrophenol	Free	–	–	2.2 ± 0.6	0.7 ± 0.2	0.8 ± 0.1
	Glucuronide	–	–	0.5 ± 0.1	0.6 ± 0.0	0.1 ± 0.1
	Sulfate	19.9 ± 1.1	23.3 ± 2.1	10.3 ± 2.9	5.6 ± 1.8	6.3 ± 1.1
m-Nitrophenol	Free	–	–	1.2 ± 0.4	0.4 ± 0.1	0.1 ± 0.1
	Glucuronide	–	–	0.5 ± 0.2	0.5 ± 0.1	–
	Sulfate	10.2 ± 0.6	11.6 ± 1.4	6.2 ± 1.7	3.8 ± 1.2	6.1 ± 1.2
Unidentified peak I	Total	9.8 ± 0.7	9.0 ± 0.5	25.3 ± 1.2	31.1 ± 2.1	4.8 ± 0.7
Unidentified peak II	Total	–	–	5.7 ± 4.0	16.4 ± 5.6	2.6 ± 0.2

[a]Values are means ± standard error of the means for three animals/group over a 72-hour period.
[b]– = not detected.
Source: Rickert et al. (1983).

As discussed in section 3.1.1, Ikeda and Kita (1964) measured the urinary excretion of p-nitrophenol and p-aminophenol in a woman who was exposed to nitrobenzene, primarily by inhalation, in an occupational setting for 17 months. The rate of excretion of these two metabolites was similar and paralleled the level of metHb in blood. However, Salmowa et al. (1963) detected p-nitrophenol, but not p-aminophenol, in the urine of human research subjects (7 volunteers) exposed to 1-6 ppm nitrobenzene via inhalation for 6 hours with diverse and dose dependent urinary excretion rates which varied between individuals. Although, p-nitrophenol could be detected for as long as 100 hours after exposure to 6 ppm, rapid excretion was observed in the first two hours which was then followed by a steady state excretion rate (Salmowa et al. 1963).

Piotrowski (1967) exposed four males to nitrobenzene at 10 mg/m^3 via inhalation for 6 h per day several days. On average, 16% of the absorbed dose was excreted in the urine as p-nitrophenol with the rate of excretion increasing for the first few days on repeated exposure followed by steady state starting on the third day. The excretion plateaued by the fourth day. The urinary p-nitrophenol level was only about 40% of the peak value in first day (JK Piotrowski 1967). A case of 17-month occupational exposure of a 47-year-old woman to nitrobenzene found p-nitrophenol and p-aminophenol (1 to half of p-nitrophenol) in the urine, which were gradually eliminated over 2 weeks (Ikeda and Kita 1964).

As described in section 3.1.1, Piotrowski (1967) investigated dermal exposure to nitrobenzene vapor in the air. At 1 ppm, about 8 mg is absorbed through the skin and about 20% is excreted as p-nitrophenol in the urine the first day (section 3.1.1). Feldmann and Maibach (1970) applied [14C]-labeled nitrobenzene dissolved in acetone to the forearm skin of six subjects. After 5 days of exposure, 1.5 ± 0.84% of the applied dose or about 58% of the absorbed dose was excreted in urine. The authors observed 60.5% of the radioactive label in the urine by 20 hours after intravenous administration of [14C]-nitrobenzene (Feldmann and Maibach 1970).

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.

A PBPK/PD model has not yet been developed and validated for nitrobenzene. To date, the only available model is a biophysically based kinetic (BPBK) model of chemical absorption across human skin developed by Guy et al. (1985) and applied to the penetration kinetics of 12 chemicals, including nitrobenzene (Figure 3-10). The first-order linear kinetics was modeled, including rate constants describing penetrant diffusion through the stratum corneum (k_1); further transport across the viable epidermal tissue to the cutaneous blood vessels (k_2); affinity of the penetrant for the stratum corneum over the viable tissue(k_3); and the elimination rate of chemical from blood to urine (k_4). Values of k_1 and k_2 were previously determined for a different chemical and molecular weight corrections were applied for nitrobenzene, while k_4 values alongside F (fraction of the total applied dose that is recovered in the urine) were experimentally measured (Feldmann and Maibach, 1970). k_3 was obtained by fitting the model prediction to the experimental data. The model also estimated ratio k_3/k_2 for an effective partition coefficient of the chemical between stratum corneum and viable tissue, assuming the possible assessment from the corresponding octanol-water partition coefficients. The model resulted in the best fit between the experimental data and the prediction of the model (Figure 3-11). This BPBK model presents the valid prediction of the general percutaneous absorption kinetics of nitrobenzene.

Figure 3-10. Schematic Representation of the Pharmacokinetic Model (Guy et al. 1982)

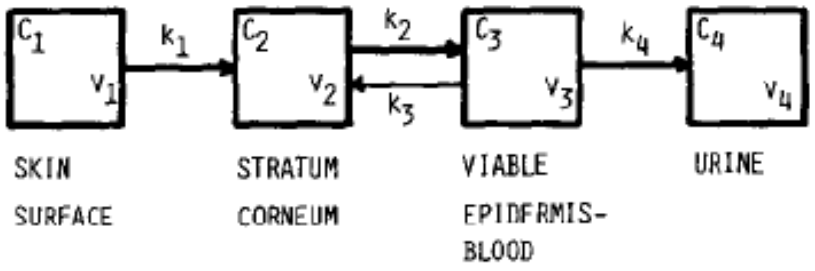
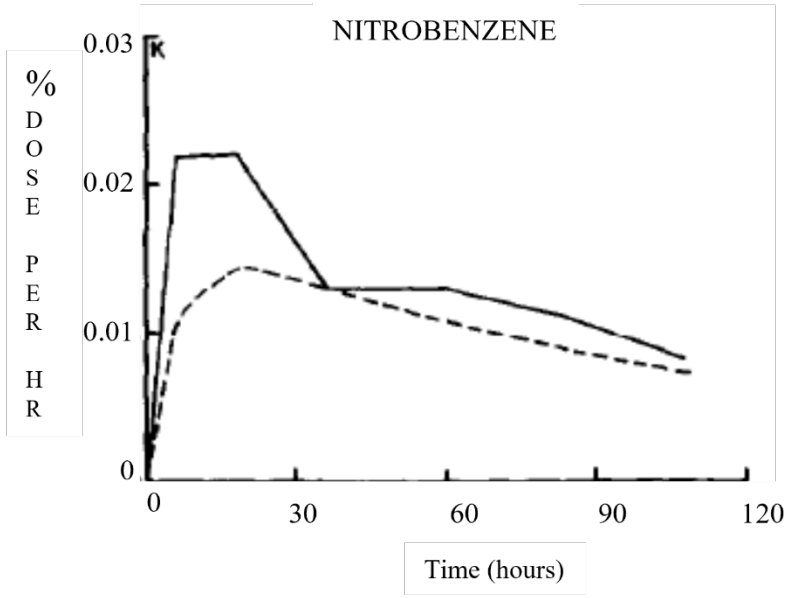


Figure 3-11. Comparison Between Experimental Data (Noncontinuous Line) (Feldmann and Maibach, 1970) and Theoretical Prediction (Broken Line) of Nitrobenzene Based Upon Rate Constants Given in Table 1 from Guy et al. (1985)



Source: Guy et al. 1985.

3.1.6 Animal-to-Human Extrapolations

Nitrobenzene is very lipid-soluble compound. In the rat, the ratio of the concentration of nitrobenzene in adipose tissue to that in the blood was 10:1 an hour after an intravenous dose (Piotrowski 1977). Half of the compound had accumulated unchanged in rabbit tissues within 2 days after intubation of 0.25 ml oral dose of nitrobenzene (Dorigan and Hushon 1976). Nitrobenzene appears to be cumulative, remaining in the human body for about 2-4 days (JK Piotrowski 1967; Piotrowski 1977). Two major metabolites, p-aminophenol and p-nitrophenol, have also been shown persistent in urine of patients after poisoning. Only 20 or 30% of nitrobenzene dose was excreted as its metabolites from humans in the urine (Piotrowski 1977). The slow rate of nitrobenzene metabolism in humans leads to increase in excretion of p-

nitrophenol in urine 2.5 times at about 4 days of exposure. The urinary excretion half-life of p-nitrophenol from humans was around 60 h (Salmowa et al. 1963) and 84 h in females after a single dose of nitrobenzene (Myslak et al. 1971). The excretion rate of nitrobenzene metabolites paralleled the level of metHb in human blood (Ikeda and Kita 1964).

Nitrobenzene is capable of converting hemoglobin to metHb. The current understanding of the responsibility of the nitrobenzene reduction products, including nitrosobenzene, phenylhydroxylamine, and aniline, in the formation of metHb from Hb causing methemoglobinemia is discussed above. It is of great interest to consider the potential of such agents to cause metHb formation in human red blood cells (RBC) and interspecies susceptibilities with regard to metHb formation in the hope of finding an animal model with sensitivities similar to those of the human (Table 3-7) (Calabrese 1991).

Table 3-7. Spontaneous MetHb Reductase Activity of Mammalian Erythrocytes[a]

	Activity in Species/Activity in Humans				
References / Species	Smith and Beutler, 1966	Malz, 1962	Kiese and Weis, 1943	Robin and Harley, 1966	Stolk and Smith, 1966
Pig	0.37	0.37		0.09	
Horse	0.75	0.5		0.64	
Cat		0.5	0.85	1.2	1.0
Cow	0.8	0.75		1.1	
Goat	1.1	0.75			1.0
Dog		0.88	1.4	1.3	
Sheep	1.4	1.0		2.1	5.0
Rat		1.4	1.3	1.9	4.5
Guinea pig		1.2	2.4	1.9	7.5
Rabbit		3.5	3.3	3.8	9.5
Mouse					

[a]Data from various investigators using nitrated red cells with glucose as a substrate have been normalized by making a ratio of the activity of the species to the activity in human red cells.
Source: Calabrese 1991.

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. 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 metHb 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 month 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 metHb 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 metHb 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 to 6 days after nitrobenzene exposure (Gosselin et al. 1984; Von Oettingen 1941).

The presence of susceptible populations in the workplace is obviously of great concern since chronic and potentially high levels of exposure to nitrobenzene combined with a genetic predisposition toward methemoglobinemia can put certain individuals at very high risk (Linch 1974). People who have preexisting or underlying diseases, such as anemia, cardiovascular disease, lung disease, sepsis, or abnormal Hb species (for example, carboxyhemoglobin, sulfhemoglobin, or sickle cell Hb), maybe at greater risk of developing the chemically induced methemoglobinemia at much lower levels of exposure to nitrobenzene (Lewis R. 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 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 pulse oximeter to monitor blood oxygen levels regularly (Ashurst et al. 2010; Mahmood et al. 2019; Toker et al. 2015). Acquired methemoglobinemia can also be caused by malaria medication (Kudale 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).

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.

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.

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. In the latest National Health and Nutrition Examination Survey (NHANES) 2011-2014 subsamples, nitrobenzene was not detected (LOD 0.32 ng/mL) (CDC 2021b). However, 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; JK Piotrowski 1967). About 10 to 20 percent 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 reflect recent exposure to nitrobenzene. However, only levels of p-nitrophenol were reported in urine of U.S. population in the NHANES in 1999-2000, 2001-2002, 2007-2008, 2009-2010, 2011-2012 and 2013-2014. In 1999-2000 and 2001-2002, levels of urinary p-nitrophenol were reported as 4.25 (2.15-10.2) and 2.98 (2.6-3.33) µg/g creatinine at the 95^{th} percentile (95% CI) in total population, respectively (CDC 2021a). In 2007-2008 and 2009-2010, geometric mean (95% CI) of urinary p-nitrophenol in total population was 0.692 (0.632-0.757) and 0.473 (0.430-0.521)

µg/g creatinine; and 95th percentile was 3.57 (2.94-4.39) and 2.62 (2.32-2.91) µg/g creatinine (CDC 2021a). In 2011-2012 and 2013-2014, geometric mean (95% CI) of urinary p-nitrophenol in total population was 0.679 (0.614-0.750) and 0.694 (0.653-0.737) µg/g creatinine; and 95th percentile was 2.97 (2.51-3.45) and 3.04 (2.63-3.38) µg/g creatinine (CDC 2021b). Those levels are similar or slightly lower than those in a nonrandom subsample of NHANES III (1988-1994) participants (CDC 2009; Hill et al. 1995), and those in a small number of samples from two geographically distinct U.S. population (Olsson et al. 2003) and in 482 pregnant females from an agricultural region of California (Eskenazi et al. 2004). The median levels of urinary p-nitrophenol (1.08 µg/g creatinine in 2013-2014) and geometric mean levels (1.09 µg/g creatinine in 2013-2014) are highest in children aged 6-11 years old.

The limitation of using these metabolites as biomarkers of exposure, however, is that they are non-specific. P-nitrophenol is a metabolite of nitrobenzene and other insecticides such as methyl parathion, ethyl parathion, and O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate. Children aged 2.5 to 5.5 (n = 13) from an agricultural region of Washington State were observed to have mean levels of urinary p-nitrophenol at 12.8 µg/g after organophosphate exposure (Kissel et al. 2005). Childhood and adult residents from where methyl parathion was applied indoors in the late 1990s had urinary levels of p-nitrophenol as high as 21,000 µg/g creatinine and 3,415µg/g p-nitrophenol (levels reported by NHANES in 1999-2000 were below the limit of detection of 0.8 µg/L), and many were symptomatic (Barr et al. 2002; CDC 2021a; 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 determine the level of nitrobenzene exposure.

Methemoglobinemia can also indicate exposure to nitrobenzene. The nitrobenzene metabolites, nitrosobenzene and phenylhydroxylamine, have been found to bind with hemoglobin in the blood of orally exposed mice and rats could also 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, although they are difficult to quantify, and assays are expensive.

Fast, sensitive, accurate and reproducible methods to quantify hemoglobin adducts using liquid chromatography have been established by Zhang et al. (2008). Albrecht & Neumann (1985) suggested a means of biological exposure monitoring for nitrobenzene that detects aniline by GC after hydrolysis of hemoglobin adducts. This approach is able to determine the aniline cleavage product in the nanogram range (0.01 ng absolute). The advantages of this method in the measurement of metHb are its high sensitivity and the stability of hemoglobin adducts over time, which may allow the method to estimate cumulative exposure over weeks to months. Rats metabolize nitrobenzene at different rates compared

humans. An *in vitro* study in rat and human erythrocytes incubated with 0.37 mmol/L nitrosobenzene found 3–4 times as much hemoglobin adduct formed in rats compared to humans. Measurement of hemoglobin adducts as a potent indirect measure of macromolecular damage that leads to critical toxicity in other potential target tissues may be used as an internal dosimeter of exposure to nitrobenzene (Neumann 1988). As the metabolism that predisposes the binding may vary individually, the method has the potential to account for individual susceptibility.

3.3.2 Biomarkers of Effect

The presence of methemoglobinemia can indicate exposure to nitrobenzene as well as to any of several other toxic substances. In cases of methemoglobinemia, a systemic adverse health outcome, caused by nitrobenzene exposure via ingestion, inhalation or dermal routes. Nitrobenzene exposure affects hemoglobin by converting the iron component from the ferrous state to the ferric state (oxidized) resulting in abnormally high levels of methemoglobin, which is not capable of releasing oxygen to the tissues of the body. MetHb occurs naturally in people, at levels around 1 to 4 percent in blood (Klaassen 2019). However, chemicals like nitrobenzene increase the amount of metHb present, lowering oxygen capacity, and causing hypoxia that causes cyanosis, fatigue, weakness, dyspnea, headache, and dizziness. A distinct cyanosis or slate-blue coloration is noted at 15-20% metHb in blood, while at 30-50% levels, the patient becomes symptomatic, with lethargy, vertigo, headache, and weakness: with modest depression of the cardiovascular and CNS while at greater than 60%, develops stupor and respiratory depression and needs immediate treatment (Goldfrank et al. 1998). In the otherwise healthy person, cyanosis may be clinically evident with a methemoglobin as low as 10%. The classic appearance of "chocolate brown blood" can be present at as low as 15% methemoglobin levels (Ludlow et al. 2021). As the percentage of methemoglobinemia approaches 20%, the patient may experience anxiety, light-headedness, and headaches. At methemoglobin levels of 30-50%, there may be tachypnea, confusion, and loss of consciousness. Approaching 50%, the patient is at risk for seizures, dysrhythmias, metabolic acidosis, and coma. Levels above 70% are often fatal (Ludlow et al. 2021).

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 LD50) 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 Jr. 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 is given intravenously, there was increased toxicity in rabbits. 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 which operate through a similar mechanism of action in causing increases in methemoglobin such as nitrates and nitrites. Exposure to multiple methemoglobin inducing agents would likely increase the risk of an adverse outcome.

The ethanol extract of *Euphobia hirta* is a suggested antioxidant against nitrobenzene-induced nephrotoxicity (Suganya et al. 2011).

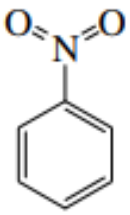
CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

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 dinitrobezenes, 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	Pubchem 2021
Synonym(s) and Registered trade name(s)	Nitrobenzol; essence of mirbane, essence of myrbane; oil of mirbane; Mononitrobenzene; Nitrobenzol; Caswell No.600	Pubchem 2021
Chemical formula	$C_6H_5NO_2$	Lide 2005
Chemical structure		Lide 2005
CAS registry number	98-95-3	Lei 2008; Lide 2005
UNII:	E57JCN6SSY	Pubchem 2021
EPA hazardous waste number	U169	Pubchem 2021
HSDB	104	Pubchem 2021

CAS = Chemical Abstracts Service; HSDB = Hazardous Substances Data Bank

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Nitrobenzene is found in either crystal form or as an oily liquid. 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 2008; Lide 2005
Color	Colorless to greenish-yellow or yellow	Pubchem 2021
Physical state	Crystals or oily liquid	Haynes 2015
Melting point(s)	5.7 °C	Lide 2005
Boiling point(s)	210.8 °C	Lei 2008; Lide 2005
Critical temperature and pressure	720 K and 4.824 MN/M SQ	Pubchem 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)	Pubchem 2021
Odor	Volatile oil almond odor; pungent odor	Pubchem 2021
Odor threshold:		Pubchem 2021
Water	30-110 µg/L	
Air	4.7x10^{-3} ppm – 1.90 ppm	
Solubility:		Haynes 2015
Water	Slightly soluble in water; 2.1 g per kg H_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 2008; Lide 2005
Log K_{oc}	1.94	PubChem 2021
Relative Vapor Density	4.2 (air=1)	Pubchem 2021
Vapor pressure at 25 °C	0.245 mmHg	Pubchem 2021
Henry's law constant	2.3 at 25°C	Lei 2008
Degradation half-life in air via reaction with OH radicals	No data	
Dissociation constants:	pKa = 3.98 at 0°C	Lide 2005
Heat of combustion	-10,420 Btu/lb	Pubchem 2021
Heat of vaporization	55.01 kJ/mol at 25 °C	Haynes 2015
Autoignition temperature	900 °F	HSDB 2010
Flashpoint	88 °C	Haynes 2015
Flammability limits in air	1.8% by volume at 200°F	Lide 2005
Conversion factors:	4.05 mg/m^3 = 1 ppm	NIOSH 2018
Explosive limits	Moderate when exposed to heat or flame	Pubchem 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, $AgClO_4$	Pubchem 2021

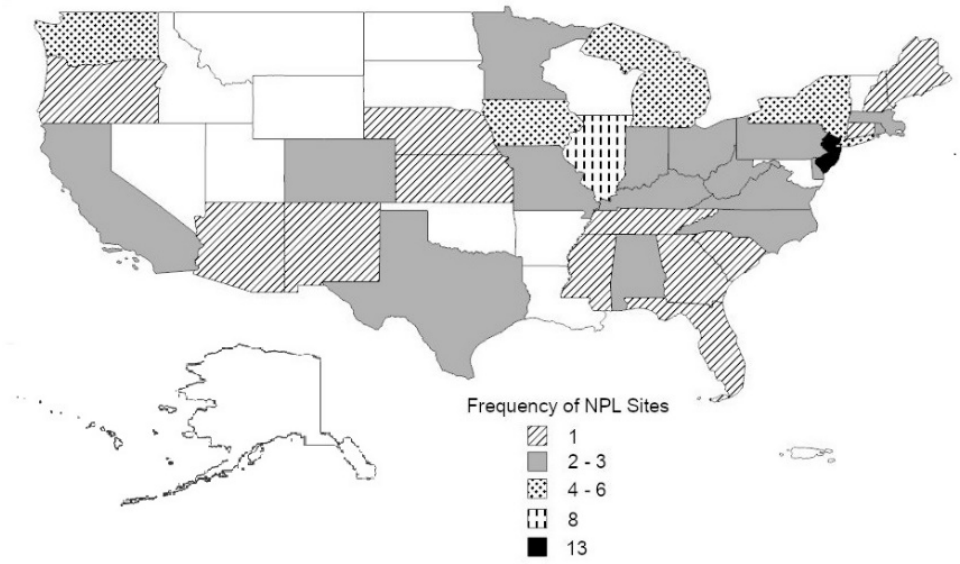
HSDB = Hazardous Substances Data Bank

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Nitrobenzene has been identified in at least 92 of the 1,867 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2019). 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 2019

- The most likely routes of exposure to nitrobenzene are through the skin and through inhalation.
- Populations working in explosive, pharmaceutical, aniline, pesticide, and dye-stuff manufacturing are at a higher risk of exposure to nitrobenzene.
- The general population is exposed to nitrobenzene in the air and possibly in drinking water.
- Nitrobenzene will degrade by photolysis and by biodegradation.

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 suggest 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 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 a 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 time a period of two decades (Bozzelli and Kebbekus 1982; EPA 1985a; Harkov eta l. 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.

Because of the relative ease of measurement of many of nitrobenzene's properties and its ready detectability by both chemical analysis and human olfaction (sense of smell), its release, transport and fate, and the consequent exposure of human beings have been studied since the mid-1900s. Thus, the potential for human exposure to nitrobenzene is better understood than that of many other chemicals.

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 to 65°C and then purified by washing and distilling with steam and redistilling (HSDB 2010).

There was a gradual increase in nitrobenzene production volume in the United States from 73,600 metric tons (kkg) in 1960 to 434,900 kkg in 1986. According to Chemical Market Reporter, the production capacity of nitrobenzene in the United States increased from 1,600 million to 3,015 million pounds per year from 1984 to 2005 (HSDB 2010).

According to the Chemical Data Reporting (CDR) database, five sites in the United States reported data in CDR for nitrobenzene: BASF Corp in Geismar, Louisiana; Rubicon LLC in Geismar, Louisiana; the Chemours company FC, LLC in Nederland, Texas; the Chemours company FC, LLC in Pascagoula, Mississippi; Covestro LLC in Baytown, Texas; and the Chemours company FC, LLC in Baytown, Texas (CDR 2016).

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. The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2018). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility’s North American

Industry Classification System (NAICS) codes is covered under EPCRA Section 313 or is a federal facility; and if their facility manufactures (defined to include importing) or processes any TRI chemical in excess of 25,000 pounds, or otherwise uses any TRI chemical in excess of 10,000 pounds, in a calendar year (EPA 2018).

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

Facility	State[a]	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
BASF CORP	AL	1,000	9,999	1, 13
BASF CORP	LA	1,000,000	9,999,999	1, 3, 4, 6
BUZZI UNICEM USA-CAPE GIRARDEAU	MO	1,000	9,999	12
CALGON CARBON CORP	KY	10,000	99,999	12
CLEAN HARBORS DEER PARK LLC	TX	100,000	999,999	9, 12
CLEAN HARBORS EL DORADO LLC	AR	100,000	999,999	9, 12
COVESTRO LLC	TX	1,000,000	9,999,999	1, 5, 13
FIRST CHEMICAL CORP	MS	1,000,000	9,999,999	1, 3, 4, 6
HERITAGE THERMAL SERVICES	OH	10,000	99,999	12
LEHIGH CEMENT CO	IN	100	999	12
MALLINCKRODT PHARMACEUTICALS	NC	1,000,000	9,999,999	6
NORLITE LLC	NY	100	999	12
ORIENT CORP OF AMERICA	DE	1,000,000	9,999,999	6
ROSS INCINERATION SERVICES INC	OH	1,000	9,999	12
RUBICON LLC	LA	1,000,000	9,999,999	1, 3, 4, 6
THE DOW CHEMICAL CO - BEAUMONT ANILINE	TX	1,000,000	9,999,999	1, 3, 6, 9
TIARCO CHEMICAL	SC	10,000	99,999	6
VEOLIA ES TECHNICAL SOLUTIONS LLC PORT ARTHUR FACILITY	TX	1,000	9,999	12

Source: TRI17 2019; Data are from 2017

[a] Post office abbreviations used.

[b] Amounts on site reported by facilities in each state.

[c] Activities/Uses;

1. Product
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

5.2.2 Import/Export

No recent data documenting import or export volumes of nitrobenzene were located. However, it is estimated that these quantities are negligible, based on the 1978 import volume of 38 kkg and 1980 export volume of 36 kkg, which represent less than 1% of United States production during those years (Collins et al. 1981).

5.2.3 Use

Nitrobenzene is widely used to produce raw materials like aniline, quinolone, 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 flavoring agent, a perfume for soaps and as a solvent for shoe dyes (Collins et al. 1981; Dunlap 1981; EPA 1985a; HSDB 2010).

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 1988a, 1989; HSDB 2010). In the past, the EPA has not believed that releases of nitrobenzene to the environment are substantial (EPA 1984).

5.3 RELEASES TO THE ENVIRONMENT

Most (97% to 98%) of the nitrobenzene produced is retained in closed systems for use in synthesizing aniline and other substituted nitrobenzenes and anilines (Dorigan and Hushon 1976; Chemical Marketing Reporter 1987). 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 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

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 lbs/yr may be released from industrial processes (Dorigan and Hushon 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.

The third principal source of nitrobenzene is the atmospheric photo-chemical reaction of nitrogen oxides with benzene, which presumably is derived from automobile fuels and, to a lesser extent, solvent uses of benzene (Dorigan and Hushon 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.

Estimated releases of 21,663.5 pounds (~9.83 metric tons) of nitrobenzene to the atmosphere from 18 domestic manufacturing and processing facilities in 2017, accounted for about 3.4% of the estimated total

environmental releases from facilities required to report to the TRI (TRI17 2019). 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
LA	2	6740	0	440000	170	No data	446740	170	446910
TX	4	4400.95	0	181305	0	No data	185706	0	185706
NC	1	6746	0	0	0	No data	6746	0	6746
MI	1	1089.8	0	0	1217	No data	1097.8	1209	2306.8
AL	1	1929	0	0	0	No data	1929	0	1929
KY	1	727	0	0	0	No data	727	0	727
MO	1	19	0	0	0	176	19	176	195
SC	1	4.58	0	0	0	No data	4.58	0	4.58
NY	1	2.28	0	0	0	No data	2.28	0	2.28
OH	2	2.068	0	0.007	0.007	No data	2.068	0.014	2.082
DE	1	2	0	0	0	No data	2	0	2
AR	1	0.82	0	0	0	No data	0.82	0	0.82
IN	1	0	0	0	0	No data	0	0	0
Total	18	21663.5	0	621305	1387	176	642976.5	1555	644531.5

Source: TRI17 2019; Data are from 2017

RF = Reporting Facilities; UI = Underground Injection

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 by a given facility.
f The sum of on-site surface water discharges, and off-site transfers to wastewater treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
g The sum of on-site and off-site disposal to underground injection wells (Class I wells and Class II-V).
h The sum of on-site and off-site disposal to: Resource Conservation and Recovery Act (RCRA) subtitle C landfills, other landfills, RCRA subtitle C surface impoundments, other surface impoundments, land treatment, other land disposal.
i Includes the sum of off-site transfers to: storage only, solidification/stabilization (metals only) disposal, other off-site management, waste broker for disposal, unknown.
j Total on-site disposal or other releases of the chemical including emissions to air, surface water discharges, land and underground injection wells.
k Total amount of chemical transferred off-site for disposal or other releases, including to POTWs.

5.3.2 Water

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 (Dorigan and Hushon 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 and plastics industry (Shackelford et al. 1983). Nitrobenzene was detected in one of 33 industrial effluents at a concentration greater than 100 µg/L (Perry et al. 1979). Reported nitrobenzene concentrations in raw and treated industrial wastewaters from several industries range from 1.4 to 91,000 µg/L (EPA 1983a). The highest concentrations are associated with wastewaters from the organic chemicals and plastics industries.

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 one 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 into the Songhua River (Dai et al. 2010b).

Although nitrobenzene is sparingly soluble in water [1,900 ppm at 20°C (Verschueren 1985); 2,090 ppm at 25°C (Banerjee et al. 1980)], its pungent, characteristic odor ["bitter almonds," (Windholz et al. 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 appear unlikely to escape unnoticed. Nitrobenzene was detected in groundwater at 3 of 862 hazardous waste sites at a geometric mean concentration of 1,400 µg/L according to the Contract Laboratory Program (CLP) Statistical Database (CLPSD 1988). Nitrobenzene was not detected in any surface water samples from the 862 sites.

Nitrobenzene has been identified in water samples collected at 12 of the 92 NPL hazardous waste sites at which it was detected in one or more environmental media (ATSDR 2019).

Estimated releases of 0 pounds (0 metric tons) of nitrobenzene to surface water from 18 domestic manufacturing and processing facilities in 2017, accounted for about 0% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2019). The facilities did not report releases to publicly owned treatment works (POTWs) (TRI17 2019). These releases are summarized in Table 5 2.

5.3.3 Soil

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). Nitrobenzene was detected in soil/sediment samples at 10 of 92 hazardous waste sites at which it was detected in one or more environmental media (ATSDR 2019).

Estimated releases of 1387 pounds (~0.63 metric tons) of nitrobenzene to soils from 18 domestic manufacturing and processing facilities in 2017, accounted for about 0.2% of the estimated total environmental releases from facilities required to report to the TRI (TRI17 2019). An additional 621305 pounds (~281.82 metric tons), constituting about 96% of the total environmental emissions, were released via underground injection (TRI17 2019). These releases are summarized in Table 5 2.

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. With a vapor pressure of 0.245 mm Hg at 25 °C, nitrobenzene is unlikely to volatilize from dry soil surfaces (Daubert and Danner 1989). However, with a Henry's Law constant of 2.4 x 10^{-5} atm-cu m/mol, it is highly likely to volatilize from moist soils (HSDB 2010). If released into the air, nitrobenzene exists solely as a vapor in the atmosphere with an estimated half-life for the reaction with photochemically-produced hydroxyl radicals in air of 115 days (HSDB 2010). The vapor density reported for nitrobenzene relative to air is 4.1 to 4.25 (HSDB 2010; WHO 2003). Removal processes for nitrobenzene in air may involve settling of vapor due to its higher density relative to air (Bidleman 1988; Dorigan and Hushon 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 Cupitt (1980) and expressly measured in field releases (Dana et al. 1984). Atmospheric residence time was estimated to be 190 days (Cupitt 1980).

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) note 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 did not volatilize in their microcosms simulating land-application of wastewater but was totally degraded. Enfield et al. (1986) employed a calculated Henry's law constant of 1.30×10^{-3} kPa m^3 mol^{-1}, and arrived at a biodegradation rate coefficient greater than 8 day^{-1}. They predicted that 0.2% of the added nitrobenzene could be accounted for in volatiles. The EXAMS computer model (Burns et al. 1981) 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. (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 is commonly used as a conventional adsorbent to remove nitrobenzene from water (Dai et al. 2010a). Dai et al. (2010a) found that carbon materials resulting from the combustion of woody biomass are also effective adsorbents that can be used to remove nitrobenzene from water.

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 (Pubchem). In another study, nitrobenzene in conjunction with other pollutants were 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 Koc 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 deg 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 Koc and retardation factor for

the sandy soil were 30.6 and 1.27, while the other two organic soils had Koc 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 (Kd) expressed in terms of organic matter (K_{om}), where K_{om} = 100 x K_d (% organic matter), for a wide variety of chemicals and soils, to the octanol-water partition coefficient 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 measured bioconcentration factors (BCF) for nitrobenzene in several aquatic organisms (1.47 to 28.32) indicate minimal bioconcentration potential (HSDB 2010). 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 less than 10 for the golden ore (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-hr exposure. Longer exposures of other species, however, did not increase the value; the EM in snails *(Physa sp*.) was 0.7; in mosquito (*Culex quinquifasciatus*) larvae, 0.8; in Daphnia magna, 0.15; and in alga (*Oedogonium*), 0.03. Bioaccumulation is not expected to be significant in terrestrial animals (EPA 1985). Bioaccumulation from water is not considered significant at values of less than 300 (Trabalka and Garten Jr. 1982).

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. (1987; 1987) and Nolt (1988). 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 1985a). p-Nitrophenol and nitrosobenzene were reported to be the principal photodegradation products of nitrobenzene vapors exposed to UV light in air (Hastings and Matsen 1948). In another study, both o- and p-nitrophenols were found when O_2 was present, and phenol was also found when O_2 was absent (Nojima and Kanno 1977). In the atmosphere, nitrobenzene is degraded by hydroxyl radical oxidation with a half-life of 115 days (Bidleman 1988). 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; HSDB 2010). Nitrobenzene absorbs sunlight in the ultraviolet and blue spectral region, so direct photolysis may degrade nitrobenzene in aqueous systems (Wang et al. 2008). Near the surface of water bodies, nitrobenzene may degrade by direct photolysis with a half-life of 2.5 to greater than 6 days (Zepp, 1987b). Phototransformation of nitrobenzene in natural water has been studied due to public concern after a spill in the Songhua River in China. 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 to 21.5 hours) that indicated indirect photodegradation may have played an important role in the loss of nitrobenzene flux in the river. Under both natural and simulated solar irradiations, the main organic products were 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 (Dorfman and Adams 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 (HSDB 2010). 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.

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 min^{-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 (Mabey et al. 1982); i.e., under environmental conditions. Zepp et al. (1987a) 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. 1987b). Algae do not enhance photolysis of nitrobenzene (Zepp and Schlotzhauer 1983). Photolysis may be an important pathway in natural waters (EPA 1985a), 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 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 readily biodegraded (Piwoni 1986). Under aerobic soil conditions, nitrobenzene has a half-life of 56 days (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. Usually, nitrobenzene was reduced to aniline and the rate of reaction was determined by dissolution rate and mineral solubility (Yu 1992).

Other Media. Nitrobenzene may be almost completely removed by activated sludge treatment (EPA 1983a; Gomolka 1979). Gomolka (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/hr/g dry weight of activated sludge with nitrobenzene as the sole carbon source. Tabak et al. (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; Davis et al. 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 (Barth and Bunch 1979; 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 less than 2 weeks in adapted media. As soon as degradation began, aniline was detected (Hallas and Alexander 1983).

According to results of the biodegradation test of the Japanese Ministry of International Trade and Industry, nitrobenzene is degradation resistant, reportedly due to its insolubility in water and the presence of a nitro group on an aromatic ring (HSDB 2010).

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 negligible. . 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.

Monitoring of nitrobenzene in the environment reveals variably low levels in air, very infrequent occurrence in surface waters, and infrequent occurrence but higher levels in industrial wastewaters. Nitrobenzene may be present in soils at hazardous waste sites.

Table 5-3 shows the limit of detections typically achieved by analytical analysis in environmental media. Presented in Table 5-4 is a summary of the range of concentrations detected in environmental media.

Table 5-3. 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

Table 5-3. Lowest Limit of Detection for Nitrobenzene Based on Standards[a]

Media	Detection limit	Reference
Air	0.5 mg/m^3	NIOSH 1977
Air	130 pptv	Francis et al. 2009
Wastewater	No data	Patil and Shinde 1988
Wastewater	3.6 pg/L	EPA 1982a
Wastewater	5.42 µg/L	Zhang et al. 2007
Water	1.9 pg/L	EPA 1982b
Water	500 ppm	Baby et al. 2000
Groundwater	0.5 µM	Bell et al. 2003
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 pg/kg[b]	EPA 1986d
Soil and solid waste	12.5 Pga[d]	EPA 1986e
Blood	0.32 ng/mL	CDC 2017
Urine	No data	Ikeda and Kita 1964
Urine	No data	Lauwerya 1988
Urine (spiked with nitrobenzene)	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.

Table 5-4. Nitrobenzene Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median	Geometric mean	Geometric SD	Number of quantitative measures	NPL sites
Water (ppb)	21.5	74.88	37.3	24	12
Soil (ppb)	5550	13921.16	30.04	14	10
Air (ppbv)	NA	NA	NA	NA	NA

[a] Concentrations found in ATSDR site documents from 1981 to 2019 for 1,867 NPL sites (ATSDR 2019). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

NA = not applicable; NPL = national priorities list SD = standard deviation

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 ppb in industrial areas, and 0.02 and 0.09 ppb in two residential areas, but in 1982, levels in residential areas were approximately 0.3 ppb or less, while levels in industrial areas were 0.9 ppb or more (Bozzelli and Kebbekus 1982). Again, most of the samples were negative for nitrobenzene. The highest values were 3.5 to 5.7 ppb.

Harkov et al. (1983) reported low levels of nitrobenzene (0.07 to 0.1 ppb) 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 ppb and another of 0.3 ppb; 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 ppb 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. Pellizzari (1978b) found only one positive value of 107 ng/m^3 (20 ppb) at a plant site in Louisiana. EPA data (1985a) showed less than 25% of United States air samples positive with a median concentration of approximately 0.01 ppb.

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 less than 10 ppb in 1980 (Young et al. 1983). Nitrobenzene was not reported in runoff samples in 1982 in a nation-wide 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. Levins et al. (1981) 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). No data were located on occurrence of nitrobenzene in groundwater.

Nitrobenzene is detected more frequently and at higher concentrations in effluents from industrial sources. The STORET database shows that 1.8% of the 1,245 reporting stations on industrial wastewaters have had measurable values (Staples et al. 1985).

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 nitrobenzenes concentration in the Yellow river, concentrations of nitrobenzene ranged from 0.128 – 8.427 µg/L, and nitrobenzene was the predominant contaminant in all locations sampled (He et al. 2006).

Nitrobenzene was found in all 33 water samples taken from the eastern part of the North Sea at concentrations from 0.5 – 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.

5.5.2 Sediment and Soil

The only measurement of nitrobenzene in soil located was a value of 8 ppm detected 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.

5.5.3 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 (C McFarlane et al. 1987; JC McFarlane et al. 1987).

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. 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. Ambient exposure in the winter may be negligible.

Table 5-5. 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) (CDC 2021b)

	Survey	Geometric mean	Selected percentiles (95% confidence interval)				Sample
			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	2013-2014	Not calculated	<LOD	<LOD	<LOD	<LOD	593
	2015-2016	Not calculated	<LOD	<LOD	<LOD	<LOD	537
20 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
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

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

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 ppm), which is the OSHA 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 (the Comprehensive Environmental Response, Compensation and Liability Act), 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.

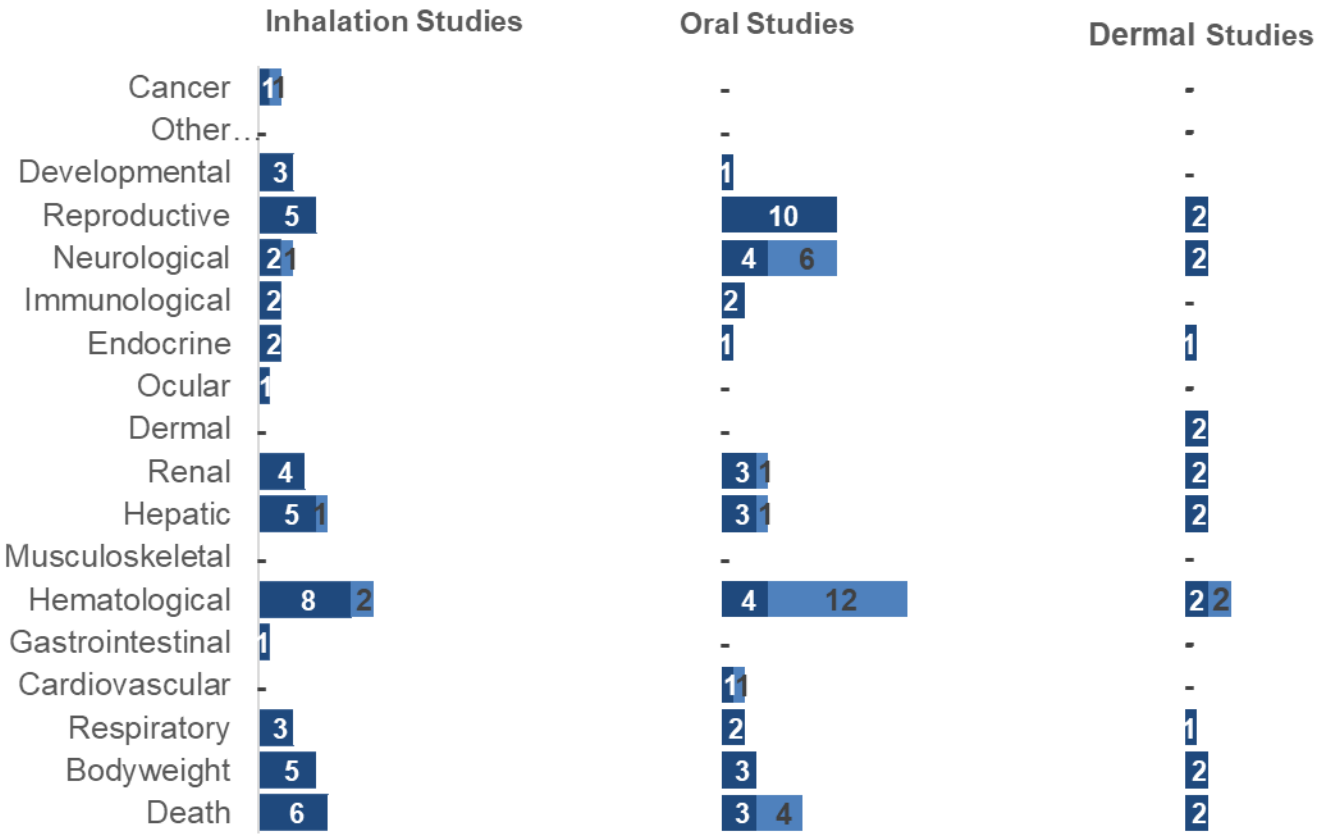
Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will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

6.1 EXISTING 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.

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

6.2 IDENTIFICATION OF DATA NEEDS

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

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

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

Chronic-Duration MRLs. The data were adequate to derive an MRL for inhalation exposure based on the evidence provided in Cattley et al. (1994). However, no studies were located which 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 fairly comprehensive with full toxicological evaluations conducted on acute and intermediate duration inhalation, oral and dermal studies. However, there are no chronic oral or dermal studies, which could improve the adequacy of the database. The database would benefit from studies that are designed to examine health effects of chronic oral and dermal exposure studies.

Respiratory. The available inhalation studies on nitrobenzene demonstrate the potential for nitrobenzene to cause toxicity in the nasal passage and the lungs. For example, there was a significant increase in the bronchiolization of the alveoli in mice exposed to nitrobenzene and increased pigmentation and degeneration of the olfactory epithelium (Cattley et al., 1994). The significant increase in alveolar bronchiolization in mice at the lowest exposure dose made it difficult to elucidate the dose-response relationship given the high incidence of health effect at the lowest exposure dose. It would be beneficial to understand how nitrobenzene may affect alveolar bronchiolization at doses lower than 5 ppm. Otherwise, the available data on nitrobenzene's toxicity to the respiratory system appears to be adequate.

Endocrine. Several studies noted endocrine effects after nitrobenzene exposure. Specifically, both Hamm Jr. et al., (1984) and NTP (1983a) observed cellular vacuolization of the zona reticularis in the adrenal gland. Additionally, chronic nitrobenzene inhalation resulted in thyroid follicular cell hyperplasia in Cattley et al. (1994) which may indicate toxicity to the thyroid 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 papers were located which evaluated developmental outcomes as a result of nitrobenzene exposure (e.g., Tyl et al. 1987 and Dodd et al., 1987). Both authors stated no developmental effects were observed. However, Tyl et al. (1987) did observe an increase of litters with one or more

Sprague-Dawley rat fetuses with external variations at 40.0 ppm for ecchymosis (discoloration of the skin due to bleeding underneath) on the trunk (but not on the head or extremities). Additionally, the incidence of these malformations increased with increasing doses, however this increase was not statistically significant. In addition, there was a significant increase the number of litters with animals having holes in the parietal skull plate, with 73 percent of litters in the 40 ppm group displaying this anomaly compared to 32 percent in the control group.. Since methemoglobinemia is a biomarker of nitrobenzene exposure, studies that examine the effects of methemoglobinemia on developmental parameters need to be conducted to examine the health effects further.

Cancer. A chronic inhalation study for nitrobenzene has been conducted (Cattley et al. 1994). This study has provided the basis for many cancer classifications. However, it is not known if the effects seen in this study occur in humans after nitrobenzene exposure. In the current profile only one epidemiological study was located, which also included exposure to two other chemicals (aniline and o-toluidine) (Carreón et al. 2014). No chronic oral studies assessing nitrobenzene's carcinogenicity potential were located, which would improve the database for this endpoint. Further, epidemiological studies with chronic oral exposure to nitrobenzene would help learn more about 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 have genotoxic effects. However, there is some evidence that nitrobenzene causes chromosomal aberrations based on the positive comet and micronucleus assays. Additionally, researchers have observed nitrobenzene forming adducts with hepatic DNA (Li et al. 2003a; Li et al. 2003b). Therefore, additional research would be beneficial to understand the potential genotoxic effects of nitrobenzene.

Epidemiology and Human Dosimetry Studies.

Only one relevant epidemiology study was located while developing the profile. Additional epidemiological studies on the toxicity of nitrobenzene would improve the database on this chemical in regard to understanding the relevance of the effects seen in experimental animals for humans.

Biomarkers of Exposure and Effect.

Exposure. Urinary levels of p- nitrophenol and aminophenol reflect recent exposure to nitrobenzene. The limitation of using these metabolites as biomarkers of exposure, however, is that they are non-specific. p-Nitrophenol is a metabolite of not only nitrobenzene, but also of the 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 determine the level of nitrobenzene exposure. Nitrobenzene can be biomonitored in urine, but it is only reflective of recent exposure.

Effect. The presence of increased levels of metHb 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.

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 high degree of absorption. In animals, absorption studies using oral and dermal route suggest the 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 concentration was found in the liver, brain, blood and stomach. Delayed rise in the metHb levels in severe methemoglobinemia after antidote administration may be attributed to the release of nitrobenzene stores from the adipose tissue. However, there is 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 understanding 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 inhalation studies using mice and rats. The reason for these differences and the toxicokinetics involved are not understood. Available data suggests dermal absorption of nitrobenzene elicits a more sensitive response in monkeys than in humans. Additional toxicokinetic studies in species other than rodents and attempts to estimate the sensitivity of humans relative to these test species would be valuable aids in interpreting the results of available toxicity studies and in understanding individual differences noted in response to nitrobenzene exposure. 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. 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.

Use. Nitrobenzene is widely used in the workplace to produce raw materials and as a solvent. Information on the uses of nitrobenzene is available in the literature and more information is not needed.

Release. According to the Emergency Planning and Community Right to Know Act of 1986 (EPCRTKA), (§313), (Pub. L. 99-499, Title III, §313), industries are required to submit release information to the EPA. The Toxic Release Inventory (TRI), which contains release information for 2017,

became available in 2019. This database is updated yearly and provides a reliable estimate of industrial production and emissions.

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. Data on the amounts of nitrobenzene disposed was not located in the literature.

Regulatory Information. 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 that information on the nature and quantity of plant metabolites would assist assessment of exposure via that route.

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. Because nitrobenzene is a priority pollutant, extensive data are available on its occurrence in surface waters, sediments, and aquatic animals. It would be useful to have data on its presence in soils and groundwater and correlations of measured air concentrations to soil levels and of plant levels to soil concentrations.

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

need to be performed for local sites and the potentially effected 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

ATSDR is not aware of any ongoing studies related to nitrobenzene toxicity.

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.

Nitrobenzene is on the list of chemicals subject to the requirements of "The Emergency Planning and Community Right-to-Know Act of 1986" (EPCRA) (EPA 2019). Section 313 of Title III of EPCRA, requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually their relation of these chemicals to any environmental media (EPA 1999).

The EPA regulates nitrobenzene under the Clean Air Act (CAA) and it has designated nitrobenzene as a hazardous air pollutant (HAP) (EPA 2017). The manufacture of nitrobenzene is subject to certain provisions for the control of volatile organic compound emissions. The major source of nitrobenzene emissions is other substituted nitrobenzenes and anilines (NTP 2016).

The Clean Water Act (CWA) lists nitrobenzene as a toxic pollutant and designated it as a hazardous substance (EPA 2018). Since nitrobenzene concentrations above trace levels in water are infrequent, according to the WHO, it is unnecessary to derive a formal guideline value (WHO 2017). Health-based values are derived and included in table 7-1 (WHO 2017).

The Resource Conservation and Recovery Act (RCRA) identifies nitrobenzene as a toxic waste with toxicity and a hazardous constituent of waste. Because nitrobenzene is listed as a hazardous substance, the storage, transportation, treatment and disposal of waste nitrobenzene is controlled by EPA. It has been assigned the hazardous waste codes of U169, F004, K083, K103, K104 (NTP 2016). Since nitrobenzene is assigned the hazardous waste code F004, nitrobenzene wastes are prohibited from underground injection unless the waste contains less than 1 percent of nitrobenzene (EPA 2020a). Nitrobenzene is also subject to land disposal restrictions (EPA 2020b).

Table 7-1. Regulations and Guidelines Applicable to Nitrobenzene

Agency	Description	Information	Reference
	Air		
EPA	RfC	9 x 10^{-3} mg/m^3	IRIS 2009
EPA	RfD	2 x 10^{-3} mg/kg-day	IRIS 2009
OSHA	PEL TWA	1 ppm (5 mg/m^3)	NTP 2016
	Water & Food		
EPA	Food and Water Fish or shellfish and water consumption Fish or shellfish consumption only Organoleptic-effect criteria	 10 µg/L 600 µg/L 30 µg/L	NTP 2016
WHO	Drinking water guidelines Long-term exposure Short-term exposure	 8-63 µg/L 30 µg/L	WHO 2017
	Cancer		
EPA	Carcinogenicity classification	Likely to be carcinogenic to humans by any route of exposure	IRIS 2009
IARC	Carcinogenicity classification	Group 2B[a]	IARC 2019
NTP-HHS	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen[a]	NTP 2016
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, shipyards, and construction	1 ppm (5 mg/m^3)	NIOSH 2018
NIOSH	REL (Up to 10-hour TWA)	1 ppm (5 mg/m^3)	NIOSH 2018
	Emergency Criteria		
EPA	AEGLs-air	No data	AEGLs 2018
AIHA	ERPG-1 ERPG-2 ERPG-3	No data	AIHA 2019
DOE	PAC-1 PAC-2 PAC-3	15 100 1,000	DOE 2018

[a] Group 2B: Possibly carcinogenic to humans.

[b] Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2016).

AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; HHS = Department of Health and Human Services; DOE = Department of Energy; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; IARC = International Agency for Research on Cancer; 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

CHAPTER 8. REFERENCES

AEGLs. 2018. Acute Exposure Guideline Levels (AEGLs) values. https://www.epa.gov/aegl/access-acute-exposure-guideline-levels-aegls-values#chemicals

Agrawal A, Gutch M, Arora R, et al. 2011. Acute cardiogenic pulmonary oedema with multiorgan dysfunction--still to learn more about nitrobenzene poisoning. BMJ Case Reports 2011.

AIHA. 2016. Current ERPG Values (2016). Fairfax, VA: American Industrial Hygiene Association. https://www.aiha.org/get-involved/AIHAGuidelineFoundation/EmergencyResponsePlanningGuidelines/Documents/2016%20ERPG%20Table.pdf.

Albrecht W, Neumann H-G. 1985. Biomonitoring of aniline and nitrobenzene: hemoglobin-binding in rats and analysis of adducts. Arch Toxico 571-5.

Alcorn CJ, Simpson RJ, Leahy D, et al. 1991. In vitro studies of intestinal drug absorption: Determination of partition and distribution coefficients with brush border membrane vesicles. Biochem Pharmacol 42(12):2259-2264. https://doi.org/10.1016/0006-2952(91)90228-W.

Allenby G, Sharpe RM, Foster PM. 1990. Changes in Sertoli cell function in vitro induced by nitrobenzene. Fundam Appl Toxicol 14(2):364-375.

Allenby G, Foster PM, Sharpe RM. 1991. Evaluation of changes in the secretion of immunoactive inhibin by adult rat seminiferous tubules in vitro as an indicator of early toxicant action on spermatogenesis. Fundam Appl Toxicol 16(4):710-724.

Anderson D, Styles JA. 1978. The bacterial mutation test. Br J Cancer 37(6):924.

Ashurst JV, Wasson MN, Hauger W, et al. 2010. Pathophysiologic Mechanisms, Diagnosis, and Management of Dapsone-Induced Methemoglobinemia. Journal of American Osteopathic Association 110(January):16-20.

Ask K, Décologne N, Asare N, et al. 2004. Distribution of nitroreductive activity toward nilutamide in rat. Toxicol Appl Pharmacol 201(1):1-9. https://doi.org/10.1016/j.taap.2004.04.006.

Aspinall R, Andrew D. 2000. Thymic involution in aging. J Clin Immunol 20(4):250-256. https://doi.org/10.1023/A:1006611518223.

Assmann N, Emmrich M, Kampf G, et al. 1997. Genotoxic activity of important nitrobenzenes and nitroanilines in the Ames test and their structure-activity relationship. Mutat Res 395(2-3):139-144.

Astier A. 1992. Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene. J Chromatogr B Biomed Appl 573(2):318-322. https://doi.org/10.1016/0378-4347(92)80136-E.

ATSDR. 2019. Full SPL data Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention. http://www.atsdr.cdc.gov/SPL/resources.

Baby RE, Cabezas M, Walsöe de Reca EN. 2000. Electronic nose: a useful tool for monitoring environmental contamination. Sensors Actuators B: Chem 69(3):214-218.

Balwani MR, Bawankule CP, Ramteke V, et al. 2017. Methylene blue induced methemoglobinemia with acute kidney injury in a glucose-6-phosphate dehydrogenase-deficient patient. Indian J Nephrol 27(6):465-467. 10.4103/ijn.IJN_316_16.

Banerjee S, Yalkowsky SH, Valvani SC. 1980. Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility-partition coefficient correlation. Environ Sci Technol 14(10):1227-1229.

Bao Y, Huang Q, Li Y, et al. 2012. Prediction of nitrobenzene toxicity to the algae (Scenedesmus obliguus) by quantitative structure–toxicity relationship (QSTR) models with quantum chemical descriptors. Environ Toxicol Pharmacol 33(1):39-45.

Barr DB, Turner WE, DiPietro E, et al. 2002. Measurement of p-nitrophenol in the urine of residents whose homes were contaminated with methyl parathion. Environ Health Perspect 110 Suppl 6(Suppl 6):1085-1091. 10.1289/ehp.02110s61085.

8. REFERENCES

Barth EF, Bunch RL. 1979. RemoVability, biodegradation and treatability of specific pollutants. Washington, DC, US Environmental Protection Agency.

Beauchamp Jr. RO, Irons RD, Rickert DE, et al. 1982. A critical review of the literature on nitrobenzene toxicity. Crit Rev Toxicol 11(1):33-84. 10.3109/10408448209089848.

Bell LS, Devlin JF, Gillham RW, et al. 2003. A sequential zero valent iron and aerobic biodegradation treatment system for nitrobenzene. Journal Of Contaminant Hydrology 66(3-4):201-217.

Biodynamics. 1983. A range finding study to evaluate the toxicity of nitrobenzene in the pregnant rabbit. Project number 83-2723.

Biodynamics. 1984. Initial submission: An inhalation teratology study in rabbits with nitrobenzene with cover letter dated 082892. EPA/OTS 920007432#88-920007432.

Blaauboer BJ, Van Holsteijn CWM. 1983. Formation and disposition of N-hydroxylated metabolites of aniline and nitrobenzene by isolated rat hepatocytes. Xenobiotica 13(5):295-302. 10.3109/00498258309052267.

Bloom JC, Brandt JT. 2019. Chapter 11. Toxic Responses of the Blood. In: Klassen C, ed. Casarett and Doull's Toxicology: The Basic Science of Poisons. New York, NY: McGraw-Hill.

Bojar RM, Rastegar H, Payne DD, et al. 1987. Methemoglobinemia from intravenous nitroglycerin: a word of caution. Ann Thorac Surg 43(3):332-334. 10.1016/s0003-4975(10)60627-3.

Bonacker D, Stoiber T, Böhm KJ, et al. 2004. Chromosomal genotoxicity of nitrobenzene and benzonitrile. Arch Toxicol 78(1):49-57.

Bonnefoy A, Chiron S, Botta A. 2012. Environmental nitration processes enhance the mutagenic potency of aromatic compounds. Environ Toxicol 27(6):321-331.

Boukobza M, Garnier R, Cleophax C, et al. 2015. CT and MRI findings and follow-up after massive nitrobenzene ingestion. A case report. J Neurol Sci 357(1-2):322-325.

Bozzelli JW, Kebbekus BB. 1982. A study of some aromatic and halocarbon vapors in the ambient atmosphere of New Jersey. Journal of Environmental Science Health Part A 17(5):693-711.

Briggs GG. 1981. Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors, and the parachor. J Agric Food Chem 29(5):1050-1059. 10.1021/jf00107a040.

Bronaugh RL, Maibach HI. 1985. Percutaneous absorption of nitroaromatic compounds: in vivo and in vitro studies in the human and monkey. Journal of Investigative Dermatology 84(3):180-183.

Bryant C, DeLuca M. 1991. Purification and characterization of an oxygen-insensitive NAD(P)H nitroreductase from Enterobacter cloacae. J Biol Chem 266(7):4119-4125.

Burns LH, Cline DM, Lassiter RR. 1981. Exposure Analysis Model System (EXAMS). Athens, GA: US Environmental Protection Agency, Athens Environmental Research Laboratory.

Burns, L.A., Bradley, S.G., White, K.L., McCay, J.A., Fuchs, B.A., Stern, M., Brown, R.D., Musgrove, D.L., Holsapple, M.P., Luster, M.I., Munson, A.E. 1994. Immunotoxicity of Nitrobenzene in Female B6C3F1 Mice. Drug and Chemical Toxicology, 17 (3) 271-315

Butterworth BE, Smith-Oliver T, Earle L, et al. 1989. Use of primary cultures of human hepatocytes in toxicology studies. Cancer Res 49(5):1075-1084.

Calabrese EJ. 1991. Principles of animal extrapolation: CRC Press Taylor Francis Group.

Canton JH, Slooff W, Kool HJ, et al. 1985. Toxicity, biodegradability, and accumulation of a number of Cl/N-containing compounds for classification and establishing water quality criteria. Reg Toxicol Pharmacol 5(2):123-131.

Car BD, Eng VM, Everds NE, et al. 2005. Clinical pathology of the rat. In: Suckow MA, Weisbroth SH, & Franklin CL, ed. The Laboratory Rat 2 ed. Elsevier

Carreón T, Hein MJ, Hanley KW, et al. 2014. Bladder cancer incidence among workers exposed to o-toluidine, aniline and nitrobenzene at a rubber chemical manufacturing plant. Occup Environ Med 71(3):175-182.

Carter FW. 1936. An unusual case of poisoning, with some notes on non-alkaloidal organic substances. Med J Aust 2(17):558-564.

8. REFERENCES

Castle L, Philo MR, Sharman M. 2004. The analysis of honey samples for residues of nitrobenzene and petroleum from the possible use of Frow mixture in hives. Food Chem 84(4):643-649.

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.

CDC. 2009. Fourth report on human exposure to environmental chemicals. Atlanta, GA: U.S: Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.cdc.gov/exposurereport/.

CDC. 2021a. Fourth report on human exposure to environmental chemicals. Updated tables March 2021. Volume one: NHANES 1999-2010. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.cdc.gov/exposurereport/index.html.

CDC. 2021b. Fourth report on human exposure to environmental chemicals. Updated tables March 2021. Volume two: NHANES 2011-2016. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.cdc.gov/exposurereport/index.html.

Chang MJW, Kao GI, Tsai CT. 1993. Biological monitoring of exposure to low dose aniline, p-aminophenol, and acetaminophen. Bull Environ Contam Toxicol 51(4):494-500. 10.1007/bf00192163.

Chemical Marketing Reporter. 1987. Chemical profiles: Nitrobenzene. 50.

Chiu C, Lee L, Wang C, et al. 1978. Mutagenicity of some commercially available nitro compounds for Salmonella typhimurium. Mutat Res 58(1):11-22. 10.1016/0165-1218(78)90090-3.

Chongtham DS, Phurailatpam J, Singh MM, et al. 1997. Methaemoglobinemia in nitrobenzene poisoning. Journal Of Postgraduate Medicine 43(3):73-74.

Chou Lin W, Speece RE, Siddiqi RH. 1978. Acclimation and degradation of petrochemical wastewater components by methane fermentation. Paper presented at the Biotechnol. Bioeng. Symp.;(United States).

CIIT. 1993. Volume 1: A chronic inhalation toxicity study of nitrobenzene in b6c3f1 mice, fischer 344 rats and sprague-dawley (cd) rats. EPA/OTS 0794-0970.

CLPSD. 1988. Contract Laboratory Program Statistical Database. Viar and Company, Management Services Division, Alexandria, VA.

Cole RH, Frederick RE, Healy RP, et al. 1984. Preliminary findings of the priority pollutant monitoring project of the nationwide urban runoff program. 56 J Water Pollut Control Fed898-908.

Collins JV, Mayo DR, Riordan BJ. 1981. Economic impact analysis of proposed test rule for nitrobenzene. Washington, DC: U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances.

Cupitt LT. 1980. Fate of Toxic and Hazardous Materials in the Air Environment. Atmospheric Chemistry and Physics Lab. US Environmental Protection Agency, Research Triangle Park, NC, EPA 600/603-680-084, NTIS No. PB680-221948.

Dai Y, Mihara Y, Tanaka S, et al. 2010. Nitrobenzene-adsorption capacity of carbon materials released during the combustion of woody biomass. J Hazard Mater 174(1-3):776-781.

Dai Y, Terui N, Lin Y, et al. 2010. Determination of nitrobenzene in water and ice samples collected from the Songhua River after an explosion of a petrochemical plant and investigation on enclosing behavior of nitrobenzene into ice. Analytical Sciences: The International Journal Of The Japan Society For Analytical Chemistry 26(4):519-523.

Dana MT, Lee RN, Hales JM. 1984. Hazardous air pollutants: Wet removal rates and mechanisms. NTIS, SPRINGFIELD, VA.

Dangwal SK, Jethani BM. 1980. A simple method of determination of nitrobenzene and nitrochlorobenzene in air and urine. Am Ind Hyg Assoc J 41(11):847-850.

Dasgupta A, Matos J, Muramatsu H, et al. 2018. Nanostructured carbon materials for enhanced nitrobenzene adsorption: Physical vs. chemical surface properties. Carbon 139833-844.

Daubert TE, Danner RP. 1989. Data Compilation, Tables of Properties of Pure Cmpds NY, NY

Davis EM, Murray HE, Liehr JG, et al. 1981. Basic microbial degradation rates and chemical byproducts of selected organic compounds. Water Res 15(9):1125-1127.

Davis EM, Turley JE, Casserly DM, et al. 1983. Partitioning of selected organic pollutants in aquatic ecosystems.

Davis B. 2012. Endometrial stromal polyps in rodents: biology, etiology, and relevance to disease in women. Toxicol Pathol 40(3):419-424. https://doi.org/10.1177/0192623311431466.

Dellarco VL, Prival MJ. 1989. Mutagenicity of nitro compounds in salmonella typhimurium in the presence of flavin mononucleotide in a preincubation assay. Environ Mol Mutagen 13116-127.

DeSesso JM, Scialli AR. 2018. Bone development in laboratory mammals used in developmental toxicity studies. Birth Defects Res 110(15):1157-1187. https://doi.org/10.1002/bdr2.1350.

DFG. 2012. List of MAK and BAT values 2012. Commission for the investigation of health hazards of chemical compounds in the work area.

Dodd DE, Fowler EH, Snellings WM, et al. 1987. Reproduction and fertility evaluations in CD rats following nitrobenzene inhalation. Fundamental Applied Toxicology 8(4):493-505.

DOE. 2018. Table 3: Protective Action Criteria (PAC) Rev. 28A based on applicable 60-minute AEGLs, ERPGs, or TEELs. The chemicals are listed by CASRN. June 2018. Oak Ridge, TN: U.S. Department of Energy. https://sp.eota.energy.gov/pac/docs/Revision_29A_Table3.pdf.

Dong J, Zhao Y, Zhao R, et al. 2010. Effects of pH and particle size on kinetics of nitrobenzene reduction by zero-valent iron. J Environ Sci (China) 22(11):1741-1747.

Dorfman LM, Adams GE. 1973. Reactivity of the hydroxyl radical in aqueous solutions National Standard Reference Data System.

Dorigan J, Hushon JM. 1976. Air pollution assessment of nitrobenzene: Mitre Corporation.

Draper WM, Crosby DG. 1984. Solar photooxidation of pesticides in dilute hydrogen peroxide. J Agric Food Chem 32(2):231-237.

D'sa SR, Victor P, Jagannati M, et al. 2014. Severe methemoglobinemia due to ingestion of toxicants. Clin Toxicol 52(8):897-900.

Dunlap KL. 1981. Nitrobenzene and nitrotoluenes. In: Grayson M, Eckroth D, eds. Kirk Othmer encyclopedia of chemical technology. John Wiley and Sons, Inc 3916-932.

Dupont. 1981. Initial submission: Acute inhalation toxicity of benzene, nitro in male Crl:Cd rats with cover letter dated 090292

Ellis DD, Jone CM, Larson RA, et al. 1982. Organic constituents of mutagenic secondary effluents from wastewater treatment plants. Arch Environ Con Toxicol 11(3):373-382.

Enfield CG, Walters DM, Wilson JT, et al. 1986. Behavior of organic pollutants during rapid-infiltration of wastewater into soil: I. Processes, definition, and characterization using a microcosm. Hazardous Waste and Hazardous Materials 3(1):57-76.

EPA. 1980a. Ambient water quality criteria for nitrobenzene: The Division.

EPA. 1982a. Test method: Nitroaromatics and isophorone - method 609. In: Longbottom JE, Lichtenberg 53, eds. Test methods: Methods for organic chemical analysis of municipal and industrial wastewater. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory EPA-600/604-682-057.

EPA. 1982b. Test method: Base/neutrals and acids - method 625. In: Longbottom JE, Lichtenberg JJ, eds. Test methods: Methods for organic chemical analysis of municipal and industrial wastewater. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory EPA-600/604-682-057.

EPA. 1983a. Nitrobenzene. In: Treatability manual: Volume I. Treatability data. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development EPA-600/602-682-OO601a.

EPA. 1984. U.S. Environmental Protection Agency. Federal Register 49:25013-25017. .

EPA. 1985. Health and environmental effects profile for nitrobenzene. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Research and Development EPA/600/X-685/365 NTIS No. PB688-180500. .

EPA. 1986b. Method 8090: Nitroaromatics and cyclic ketones. In: Test methods for evaluating solid waste. Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. .

EPA. 1986c. Method 8250: Gas chromatography/mass spectrometry for semivolatile organics: Packed column technique. In: Test methods for evaluating solid waste. . Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response.

EPA. 1986d. Method 8270: Gas chromatography/mass spectrometry for semivolatile organics: Capillary column technique. In: Test methods for evaluating solid waste. Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response.

EPA. 1988a. U.S. Environmental Protection Agency: Part II. Federal Register 53:31138-31222.

EPA. 1989. U.S. Environmental Protection Agency: Part II. Federal Register 54:1056-1119.

EPA. 1999. Section 313 of the Emergency Planning and Community Right-to-Know Act. Toxic Chemical Release Inventory. Washington, DC: U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics https://www.epa.gov/sites/production/files/documents/1999chem.pdf. EPA 745-B-99-005.

EPA. 2017. Initial List of Hazardous Air Pollutants with Modifications. https://www.epa.gov/haps/initial-list-hazardous-air-pollutants-modifications

EPA. 2018. Appendix A To Part 423—126 Priority Pollutants. Steam Electric Power Generating Point Source Category. Code of Federal Regulations 40 CFR 423. https://www.govinfo.gov/content/pkg/CFR-2018-title40-vol31/xml/CFR-2018-title40-vol31-part423.xml.

EPA. 2019. Table II. EPCRA Section 313 Chemical List For Reporting Year 2018 (including Toxic Chemical Categories). U.S. Environmental Protection Agency: https://www.epa.gov/sites/production/files/2019-03/documents/ry_2018_tri_chemical_list.pdf.

EPA. 2020a. Hazardous waste injection restrictions. Code of Federal Regulations 40 CFR 148. https://www.ecfr.gov/cgi-bin/text-idx?node=pt40.26.268#se40.29.268_148

EPA. 2020b. Land disposal restrictions. Code of Federal Regulations 40 CFR 268. https://www.ecfr.gov/cgi-bin/text-idx?SID=735d0c65a78b400ddac15114fb63a0ac&mc=true&node=pt40.25.148&rgn=div5

Eskenazi B, Harley K, Bradman A, et al. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 112(10):1116-1124. 10.1289/ehp.6789.

Ewert R, Buttgereit F, Prügel M, et al. 1998. Intravenous injection of India ink with suicidal intent. International Journal Of Legal Medicine 111(2):91-92.

Eyer P. 1979. Reactions of nitrosobenzene with reduced glutathione. Chem-Biol Interact 24(2):227-239. https://doi.org/10.1016/0009-2797(79)90011-5.

Eyer P, Ascherl M. 1987. Reactions of para-Substituted Nitrosobenzenes with Human Hemoglobin. In Biological Chemistry Hoppe-Seyler (Vol. 368, pp. 285).

Facchini V, Griffiths LA. 1981. The involvement of the gastro-intestinal mi̇croflora in nitro-compound-induced methaemoglobinaemia in rats and its relationship to nitrogroup reduction. Biochem Pharmacol 30(9):931-935. https://doi.org/10.1016/0006-2952(81)90036-8.

Feldmann RJ, Maibach HI. 1970. Absorption of some organic compounds through the skin in man. Journal of Investigative Dermatology 54(5):399-404. https://doi.org/10.1111/1523-1747.ep12259184.

Francis GJ, Langford VS, Milligan DB, et al. 2009. Real-time monitoring of hazardous air pollutants. Anal Chem 81(4):1595-1599. 10.1021/ac802510h.

Freitag D, Geyer H, Kraus A, et al. 1982. Ecotoxicological profile analysis: VII. Screening chemicals for their environmental behavior by comparative evaluation. Ecotoxicology Environmental Safety 6(1):60-81.

Fritz H, Hess R. 1970. Ossification of the rat and mouse skeleton in the perinatal period. Teratology 3(4):331-338. 10.1002/tera.1420030409.

Fu W, Fu H, Skøtt K, et al. 2008. Modeling the spill in the Songhua River after the explosion in the petrochemical plant in Jilin. Environmental Science And Pollution Research International 15(3):178-181.

Garner RC, Nutman CA. 1977. Testing of some azo dyes and their reduction products for mutagenicity using Salmonella typhimurium TA 1538. VJ Mutation Research/Fundamental Molecular Mechanisms of Mutagenesis 44(1):9-19.

Gatermann R, Huehnerfuss H, Rimkus G, et al. 1995. The distribution of nitrobenzene and other nitroaromatic compounds in the North Sea. Mar Pollut Bull 30(3):221-227.

Goldfrank L, Lewin N, Flomenbaum N, et al. 1998. Goldfrank's Toxicologic Emergencies / Edition 6 (Vol. 11): McGraw-Hill Professional Publishing.

Goldstein RS, Chism JP, Hamm TJ. 1984. Influence of diet on intestinal microfloral metabolism and toxicity of nitrobenzene. Toxicologist 4143.

Goldstein RS, Chism JP, Sherrill JM, et al. 1984. Influence of dietary pectin on intestinal microfloral metabolism and toxicity of nitrobenzene. Toxicol Appl Pharmacol 75(3):547-553.

Goldstein RS, Rickert DE. 1984. Macromolecular covalent binding of [14C] nitrobenzene in the erythrocyte and spleen of rats and mice. Chem-Biol Interact 50(1):27-37.

Gu D, Shao N, Zhu Y, et al. 2017. Solar-driven thermo- and electrochemical degradation of nitrobenzene in wastewater: Adaptation and adoption of solar STEP concept. J Hazard Mater 321703-710.

Gupta G, Poddar B, Salaria M, et al. 2000. Acute nitrobenzene poisoning. Indian Pediatrics 37(10):1147-1148.

Gupta A, Jain N, Agrawal A, et al. 2012. A fatal case of severe methaemoglobinemia due to nitrobenzene poisoning. Emergency Medicine Journal: EMJ 29(1):70-71.

Gutteridge JM. 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 41(12):1819-1828.

Guy RH, Hadgraft J, Maibach HI. 1985. Percutaneous absorption in man: a kinetic approach. Toxicology Appl Pharmacol 78(1):123-129.

Hallas LE, Alexander M. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. Appl Environ Microbiol 45(4):1234-1241.

Hamm Jr. TE, Phelps M, Raynor TH, et al. 1984. Ninety day inhalation toxicity study of nitrobenzene in F-344 rats, and CD rats and B6C3Fl mice. Research Triangle Park, NC: Chemical Industry Institute of Technology.

Hanley KW, Viet SM, Hein MJ, et al. 2012. Exposure to o-toluidine, aniline, and nitrobenzene in a rubber chemical manufacturing plant: a retrospective exposure assessment update. J Occup Env Hyg 9(8):478-490.

Harkov R, Kebbekus B, Bozzelli JW, et al. 1983. Measurement of selected volatile organic compounds at three locations in New Jersey during the summer season. Journal of the Air Pollution Control Association 33(12):1177-1183.

Harkov R, Kebbekus B, Bozzelli JW, et al. 1984. Comparison of selected volatile organic compounds during the summer and winter at urban sites in New Jersey. Sci Total Environ 38259-274.

Harkov R, Gianti Jr SJ, Bozzelli JW, et al. 1985. Monitoring volatile organic compounds at hazardous and sanitary landfills in New Jersey. Journal of Environmental Science Health Part A 20(5):491-501.

Hastings SH, Matsen FA. 1948. The photodecomposition of nitrobenzene. Journal of the American Chemical Society 70(10):3514-3515.

Haworth S, Lawlor T, Mortelmans K, et al. 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental mutagenesis 5(S1):3-49.

Haynes W. 2015. CRC handbook of chemistry and physics: A ready-reference book of chemical and physical data. 95th ed. Boca Raton, FL: CRC Press.

He M-C, Sun Y, Li X-R, et al. 2006. Distribution patterns of nitrobenzenes and polychlorinated biphenyls in water, suspended particulate matter and sediment from mid- and down-stream of the Yellow River (China). Chemosphere 65(3):365-374.

Ho C-H, Clark BR, Guerin MR, et al. 1981. Analytical and biological analyses of test materials from the synthetic fuel technologies: IV. Studies of chemical structure-mutagenic activity relationships of aromatic nitrogen compounds relevant to synfuels. Mutation Research/Environmental Mutagenesis Related Subjects 85(5):335-345.

Holder JW. 1999. Nitrobenzene carcinogenicity in animals and human hazard evaluation. Toxicology & Industrial Health 15(5):445-457.

Huang Q, Wang L, Han S. 1995. The genotoxicity of substituted nitrobenzenes and the quantitative structure-activity relationship studies. Chemosphere 30(5):915-923.

Huang QG, Kong LR, Liu YB, et al. 1996. Relationships between molecular structure and chromosomal aberrations in in vitro human lymphocytes induced by substituted nitrobenzenes. Bull Environ Contam Toxicol 57(3):349-353.

Hughes TJ, Sparacino C, Frazier S. 1984. Validation of chemical and biological techniques for evaluation of vapors in ambient air/mutagenicity testing of twelve(12) vapor-phase compounds. U.S. Environmental Protection Agency, Research Triangle Park, NC EPA 600/l-684-005.

Hurley R, Testa AC. 1966. Photochemical n→ π* Excitation of Nitrobenzene. J Am Chem Soc 88(19):4330-4332.

Hurley R, Testa AC. 1967. Nitrobenzene photochemistry. II. Protonation in the excited state. J Am Chem Soc 89(26):6917-6919.

IARC. 1996. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Printing Processes and Printing Inks, Carbon Black and Some Nitro Compounds. Lyon, France: International Agency for Research on Cancer.

IARC. 2019. Agents classified by the IARC monographs. Volumes 1–123 Lyon, France: International Agency for Research on Cancer. https://monographs.iarc.fr/wp-content/uploads/2019/02/List_of_Classifications.pdf.

Iida S, Misaka H, Naya M. 1997. A flow cytometric analysis of cytotoxic effects of nitrobenzene on rat spermatogenesis. The Journal Of Toxicological Sciences 22(5):397-407.

Ikeda M, Kita A. 1964. Excretion of p-nitrophenol and p-aminophenol in the urine of a patient exposed to nitrobenzene. Occup Environ Med 21(3):210-213.

IRIS. 2009. Nitrobenzene; CASRN 98-95-3. Washington, DC: U.S. Environmental Protection Agency. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0079tr.pdf.

Jury WA, Spencer WF, Farmer WJ. 1984. Behavior assessment model for trace organics in soil: III. Application of screening model 1. Journal of Environmental Quality 13(4):573-579.

Kao J, Faulkner J, Bridges JW. 1978. Metabolism of aniline in rats, pigs and sheep. Drug Metab Disposition 6(5):549-555.

Kao AS. 1994. Formation and removal reactions of hazardous air pollutants. J Air Waste Manage Assoc 44(5):683-696. 10.1080/1073161X.1994.10467272.

Kawaguchi T, Kawachi M, Morikawa M, et al. 2004. Key parameters of sperm motion in relation to male fertility in rats given alpha-chlorohydrin or nitrobenzene. The Journal Of Toxicological Sciences 29(3):217-231.

Kawashima K, Usami M, Sakemi K, et al. 1995. Studies on the establishment of appropriate spermatogenic endpoints for male fertility disturbance in rodent induced by drugs and chemicals. I. Nitrobenzene. The Journal of Toxicological Sciences 20(1):15-22.

Kiese M. 1966. The biochemical production of ferrihemoglobinforming derivatives from aromatic amines, and mechanisms of ferrhemoglobin formation. Pharmacol Rev 18(3):1091-1161.

Kincannon DF, Stover EL, Nichols V, et al. 1983. Removal mechanisms for toxic priority pollutants. J Water Pollit Control Fed (55):157-163.

Kincannon DF, Lin YS. 1985. Microbial degradation of hazardous wastes by land treatment. Proc Indust Waste Conf 40607-619.

Kissel JC, Curl CL, Kedan G, et al. 2005. Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington State.

Journal of Exposure Science & Environmental Epidemiology 15(2):164-171. 10.1038/sj.jea.7500384.

Kligerman AD, Erexson GL, Wilmer JL, et al. 1983. Analysis of cytogenetic damage in rat lymphocytes following in vivo exposure to nitrobenzene. J Toxicology letters 18(3):219-226.

Kopfler FC, Melton RG, Mullaney JL, et al. 1977. Human exposure to water pollutants. In:ed. Fate of pollutants in the air and water environments; part 2: Chemical and biological fate of pollutants in environment. J. Wiley, 419-433.

Korte F, Klein W. 1982. Degradation of benzene in the environment. Ecotoxicol. Environ. Saf. 6(4).

Kudale S, Sethi SK, Dhaliwal M, et al. 2014. Methemoglobinemia due to quinine causing severe acute kidney injury in a child. Indian J Nephrol 24(6):394-396. 10.4103/0971-4065.134681.

Kumar A, Chawla R, Ahuja S, et al. 1990. Nitrobenzene poisoning and spurious pulse oximetry. Anaesthesia 45(11):949-951.

Kumar A, Bhavsar C, Aggarwal P, et al. 2017. Toxic brain injury with nitrobenzene poisoning. Int J Appl Basic Med Res 7(3):207-209. 10.4103/ijabmr.IJABMR_271_16.

LaRegina J, Bozzelli JW, Harkov R, et al. 1986. Volatile organic compounds at hazardous waste sites and a sanitary landfill in New Jersey. J Environmental progress 5(1):18-27.

Leader SD. 1932. Nitrobenzene poisoning (report of an unusual case in a child). J Arch Pediat 49245.

Lee CH, Kim SH, Kwon DH, et al. 2013. Two cases of methemoglobinemia induced by the exposure to nitrobenzene and aniline. Annals Of Occupational and Environmental Medicine 25(1):31-31.

Lei B, Huang S, Qiao M, et al. 2008. Prediction of the environmental fate and aquatic ecological impact of nitrobenzene in the Songhua River using the modified AQUATOX model. J Environ Sci 20769-777.

Levin AA, Dent JG. 1982. Comparison of the metabolism of nitrobenzene by hepatic microsomes and cecal microflora from Fischer-344 rats in vitro and the relative importance of each in vivo. J Drug Metabolism Disposition 10(5):450-454.

Levin AA, Dent JG. 1982a. Nitrobenzene metabolism in vitro: Comparison of cecal microflora and hepatic microsomes from Fischer-344 rats [Abstract]. Proceedings of the 5th CIIT Conference of Toxicology: Toxicity of Nitroaromatic Compounds, January 28-29, Raleigh, NC.

Levin AA, Bosakowski T, Earle LL, et al. 1988. The reversibility of nitrobenzene-induced testicular toxicity: continuous monitoring of sperm output from vasocystotomized rats. Toxicology 53(2-3):219-230.

Levins PL. 1981. Sources of toxic pollutants found in influents to sewage treatment plants: AD Little, Inc., Boston, MA.

Li H, Cheng Y, Wang H, et al. 2003. Inhibition of nitrobenzene-induced DNA and hemoglobin adductions by dietary constituents, 291.

Li H, Wang H, Sun H, et al. 2003. Binding of nitrobenzene to hepatic DNA and hemoglobin at low doses in mice, 25.

Li P, Yin W, Li P, et al. 2010. Distribution and migration of nitrobenzene in water following a simulated spill. J Hazard Mater 182(1-3):787-791.

Lide DR, ed. 2005. CRC Handbook of Chemistry and Physics 85th edition (Lide DR Ed.). Boca Raton, FL: CRC Press.

Lin Y, Han X, Lu H, et al. 2013. Study of archaea community structure during the biodegradation process of nitrobenzene wastewater in an anaerobic baffled reactor. Int Biodeterior Biodegrad 85:499-505.

Linch AL. 1974. Biological monitoring for industrial exposure to cyanogenic aromatic nitro and amino compounds. J American Industrial Hygiene Association Journal 35(7):426-432.

Linder RE, Strader LF, Slott VL, et al. 1992. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 6(6):491-505.

Lioy PJ, Daisey JM, Atherholt T, et al. 1983. The New Jersey project on airborne toxic elements and organic substances (ATEOS): a summary of the 1981 summer and 1982 winter studies. Journal of the Air Pollution Control Association 33(7):649-657.

Lu P-Y, Metcalf RL. 1975. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. Environ Health Perspect 10269-284.

Ludlow JT, Wilkerson RG, Nappe TM. 2021. Methemoglobinemia. [Updated 2021 Sep 2]. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537317/.

Lutin PA, Cibulka JJ, Malaney GW. 1965. Oxidation of selected carcinogenic compounds by activated sludge. Purdue University Engineering Extension Series 131-145.

Mabey WR, Smith JH, Podoll RT, et al. 1982. Aquatic fate process data for organic priority pollutants. US Environmental Protection Agency, Office of Water Regulations Standards, Washington, DC EPA 4404-81.

Mahmood N, Khan MU, Haq IUL, et al. 2019. A case of dapsone induced methemoglobinemia. J Pharm Policy Pract 12:22. 10.1186/s40545-019-0185-y.

Mallouh AA, Sarette WO. 1993. Methemoglobinemia induced by topical hair oil. Annals of Saudi medicine 13(1).

Maples KR, Eyer P, Mason RP. 1990. Aniline-, phenylhydroxylamine-, nitrosobenzene-, and nitrobenzene-induced hemoglobin thiyl free radical formation in vivo and in vitro. Mol Pharmacol 37(2):311-318.

Marion CV, Malaney GW. 1963. Ability of activated sludge microorganisms to oxidize aromatic organic compounds. J Purdue UniV. Eng. Ext. Ser 115297-308.

Marshall JB, Ecklund RE. 1980. Methemoglobinemia from overdose of nitroglycerin. JAMA: The Journal of the American Medical Association 244(4):330. 10.1001/jama.1980.03310040014005.

Martínez MA, Ballesteros S, Almarza E, et al. 2003. Acute nitrobenzene poisoning with severe associated methemoglobinemia: Identification in whole blood by GC—FID and GC-MS. J Anal Toxicol 27(4):221-225. 10.1093/jat/27.4.221.

Mason RP, Holtzman JL. 1975a. Mechanism of microsomal and mitochondrial nitroreductase. Electron spin resonance evidence for nitroaromatic free radical intermediates. Biochemistry 14(8):1626-1632. 10.1021/bi00679a013.

Mason RP, Holtzman JL. 1975b. The role of catalytic superoxide formation in the O2 inhibition of nitroreductase. Biochem Biophys Res Commun 67(4):1267-1274. https://doi.org/10.1016/0006-291X(75)90163-1.

Mason RP. 1982. Chapter 6 - Free-Radical Intermediates in the Metabolism of Toxic Chemicals. In: Pryor WA, ed. Free Radicals in Biology. Academic Press, 161-222.

Matsumara H, Yoshida T. 1959. Experimental studies of nitrobenzol poisoning. Kyushu J. Med. Sci 10259.

Mattioli F, Martelli A, Gosmar M, et al. 2006. DNA fragmentation and DNA repair synthesis induced in rat and human thyroid cells by chemicals carcinogenic to the rat thyroid. Mutat Res 609(2):146-153.

McCann KG, Moomey CM, Runkle KD, et al. 2002. Chicago area methyl parathion response. Environ Health Perspect 110 Suppl 6(Suppl 6):1075-1078. 10.1289/ehp.02110s61075.

McCarthy DJ, Waud WR, Struck RF, et al. 1985. Disposition and metabolism of aniline in Fischer 344 rats and C57BL/6 × C3H F₁ mice. Cancer Res 45(1):174-180.

McFarlane C, Nolt C, McDowell M, et al. 1987. The uptake, distribution and metabolism of four organic chemicals by soybean plants and barley roots. J Environ Toxicol 6(11):847-856.

McFarlane JC, Pfleeger T, Fletcher J. 1987. Transpiration effect on the uptake and distribution of bromacil, nitrobenzene, and phenol in soybean plants 1. Journal Of Environmental Quality 16(4):372-376.

McLaren TT, Foster PM, Sharpe RM. 1993a. Effect of age on seminiferous tubule protein secretion and the adverse effects of testicular toxicants in the rat. Int J Androl 16(6):370-379.

McLaren TT, Foster PM, Sharpe RM. 1993b. Identification of stage-specific changes in protein secretion by isolated seminiferous tubules from the rat following exposure to either m-dinitrobenzene or nitrobenzene. Fundam Appl Toxicol 21(3):384-392.

Medinsky MA, Irons RD. 1985. Sex, Strain, and Species Differences in the Response. In: Ricker D, ed. Toxicity of Nitroaromatic Compounds. New York: Hemispher Publishing Corporation, 35-51.

Mestankova H, Parker AM, Bramaz N, et al. 2016. Transformation of Contaminant Candidate List (CCL3) compounds during ozonation and advanced oxidation processes in drinking water: Assessment of biological effects. Water Res 93110-120.

Miller JA. 1970. Carcinogenesis by chemicals: An overview—G. H. A. Clowes memorial lecture. Cancer Res 30(3):559-576.

Mirsalis JC, Tyson CK, Butterworth BE. 1982. Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay. Environmental mutagenesis 4(5):553-562.

Mitsumori K, Kodama Y, Uchida O, et al. 1994. Confirmation study, using nitrobenzene, of the Combined Repeat Dose and Reproductive/Developmental Toxicity Test protocol proposed by the Organization for Economic Cooperation and Development (OECD). J Toxicol Sci 19(3):141-149.

Morgan KT, Gross EA, Lyght O, et al. 1985. Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. Neurotoxicology 6(1):105-116.

Myslak Z, Piotrowski JK, Musialowicz E. 1971. Acute nitrobenzene poisoning. A case report with data on urinary excretion of p-nitrophenol and p-aminophenol. J Archiv fur Toxikologie 28(3):208-213.

Nappe TM, Pacelli AM, Katz K. 2015. An atypical case of methemoglobinemia due to self-administered benzocaine. Case Rep Emerg Med 2015:670979. 10.1155/2015/670979.

Nelson CR, Hites RA. 1980. Aromatic amines in and near the Buffalo River. Environ Sci Technol 14(9):1147-1149.

Neumann H-G. 1988. Biomonitoring of aromatic amines and alkylating agents by measuring hemoglobin adducts. Int Arch Occup Environ Health 60(3):151-155. 10.1007/bf00378690.

Newton JF, Kuo CH, Gemborys MW, et al. 1982. Nephrotoxicity of p-aminophenol, a metabolite of acetaminophen, in the Fischer 344 rat. Toxicol Appl Pharmacol 65(2):336-344. https://doi.org/10.1016/0041-008X(82)90017-5.

NIOSH. 1977. Nitrobenzene-method S217. In: NIOSH manual of analytical methods. Vol 3. Cincinnati, OH; National Institute for Occupational Safety and Health S217-l-S217-219.

NIOSH. 1984. Nitrobenzene-method 2005. In: NIOSH manual of analytical methods. Cincinnati, OH: National Institute for Occupational Safety and Health 2005-l-2005-2005.

NIOSH. 1988. National occupational exposure survey. Cincinnati, OH: National Institute of Occupational Safety and Health.

NIOSH. 2018. NIOSH pocket guide to chemical hazards. https://www.cdc.gov/niosh/npg/npgd0271.html.

Nojima K, Kanno S. 1977. Studies on photochemistry of aromatic hydrocarbons. IV- Mechanism of formation of nitrophenols by the photochemical reaction of benzene and toluene with nitrogen oxides in air. J Chemosphere 6(6):371-376.

Nolt CL. 1988. Uptake and translocation of six organic chemicals in a newly-designed plant exposure system and evaluation of plant uptake aspects of the prebiologic screen for ecotoxicologic effects: Cornell University, Aug.

NTP. 1982. Repeated dose dermal toxicity test of nitrobenzene in fischer 344 rats and B6C3F1 mice. Research Triangle Park, NC: National Toxicology Program, National Institute of Environmental Health Services, Public Health Service, U.S. Department of Health and Human Services MRI-NTP 17-82-28.

NTP. 1983a. Report on subchronic toxicity via gavage of nitrobenzene (C60082) in Fischer 344 rats and B6C3F1 mice. Worcester, MA: EG&G Mason Research Institute.

NTP. 1983b. Report on the subchronic dermal toxicity of nitrobenzene (C60082) in Fischer 344 rats and B6C3F1 mice Research Triangle Park. National Toxicology Program, National Institute of Environmental Health Services, Public Health Service, U.S. Department of Health and Human Services, MRI-NTP 06-83-13.

NTP. 2016. Report on carcinogens. Research Triangle Park, NC: U.S. Department of Health and Human Services, Office of Health Assessment and Translation. https://ntp.niehs.nih.gov/ntp/roc/content/profiles/nitrobenzene.pdf.

Ohkuma Y, Kawanishi S. 1999. Oxidative DNA damage by a metabolite of carcinogenic and reproductive toxic nitrobenzene in the presence of NADH and Cu(II). Biochem Biophys Res Commun 257(2):555-560.

Olsson AO, Nguyen JV, Sadowski MA, et al. 2003. A liquid chromatography/electrospray ionization–tandem mass spectrometry method for quantification of specific organophosphorus pesticide biomarkers in human urine. Anal Bioanal Chem 376(6):808-815. 10.1007/s00216-003-1978-y.

Parke DV. 1956. Studies in detoxication. 68. The metabolism of [14C] nitrobenzene in the rabbit and guinea pig. Biochem J 62(2):339.

Patel A, Dewan A, Upadhyay K, et al. 2008. Chemically induced methemoglobinemia from acute nitrobenzene poisoning. The Internet Journal of Laboratory Medicine 3(2).

Patel G, Misra A. 2011. Oral Delivery of Proteins and Peptides: Concepts and Applications. In: Misra A, ed. Challenges in Delivery of Therapeutic Genomics and Proteomics. London, England: Elsevier 481-529. https://doi.org/10.1016/B978-0-12-384964-9.00010-4.

Patil SS, Shinde VM. 1988. Biodegradation studies of aniline and nitrobenzene in aniline plant wastewater by gas chromatography. Environ Sci Technol 22(10):1160-1165.

Pellizzari ED. 1978b. Quantification of chlorinated hydrocarbons in previously collected air samples: US Environmental Protection Agency, Office of Air, Noise, and Radiation ….

Perera M, Shihana F, Kularathne K, et al. 2009. Acute methaemoglobinaemia after massive nitrobenzene ingestion. BMJ case reports 2009bcr07.2008.0515. 10.1136/bcr.07.2008.0515.

Perry DL, Chuang CC, Jungclaus GA, et al. 1979. Identification of organic compounds in industrial effluent discharges. Athens, GA: Environmental Protection Agency.

Piotrowski JK. 1967. Further investigations on the evaluation of exposure to nitrobenzene. Occup Environ Med 24(1):60-65.

Piotrowski JK. 1977. Exposure tests for organic compounds in industrial toxicology (DHEW): National Institute for Occupational Safety and Health.

Pitter P. 1976. Determination of biological degradability of organic substances. Water Res 10(3):231-235.

Piwoni MD, Wilson JT, Walters DM, et al. 1986. Behavior of organic pollutants during rapid-infiltration of wastewater into soil: I. Processes, definition, and characterization using a microcosm. Haz Waste Haz Mater 3(1):43-55.

PubChem. 2021. Compound summary. Nitrobenzene. National Library of Medicine, National Center for Biotechnology Information. https://pubchem.ncbi.nlm.nih.gov/compound/Nitrobenzene. November 11, 2021.

Reddy BG, Pohl LR, Krishna G. 1976. The requirement of the gut flora in nitrobenzene-induced methemoglobinemia in rats. Biochem Pharmacol 25(9):1119-1122.

Richburg JH, Nañez A. 2003. Fas- or FasL-deficient mice display an increased sensitivity to nitrobenzene-induced testicular germ cell apoptosis. Toxicol Lett 139(1):1-10.

Rickert DE, Bond JA, Long RM, et al. 1983. Metabolism and excretion of nitrobenzene by rats and mice. Toxicol Appl Pharmacol 67(2):206-214.

Rickert DE. 1987. Metabolism of nitroaromatic compounds. J Drug metabolism reviews 18(1):23-53.

Robbiano L, Baroni D, Carrozzino R, et al. 2004. DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney. Toxicology 204(2-3):187-195.

Robinson D, Smith JN, Williams RT. 1951. Studies in detoxication. 40. The metabolism of nitrobenzene in the rabbit. o-, m-and p-Nitrophenols, o-, m-and p-aminophenols and 4-nitrocatechol as metabolites of nitrobenzene. Biochem J 50(2):228.

Roth CM, Goss K-U, Schwarzenbach RP. 2004. Sorption of diverse organic vapors to snow. Environ Sci Technol 38(15): 4078-4084. https://doi.org/10.1021/es0350684.

Rubin C, Esteban E, Kieszak S, et al. 2002. Assessment of human exposure and human health effects after indoor application of methyl parathion in Lorain County, Ohio, 1995-1996. Environ Health Perspect 110 Suppl 6(Suppl 6):1047-1051. 10.1289/ehp.02110s61047.

Ruth JH. 1986. Odor thresholds and irritation levels of several chemical substances: a review. American Industrial Hygiene Association Journal 47(3):A-142-A-151.

Salmowa J, Piotrowski J, Neuhorn U. 1963. Evaluation of exposure to nitrobenzene: Absorption of nitrobenzene vapour through lungs and excretion of p-nitrophenol in urine. Occup Environ Med 20(1):41-46.

Saxena H, Prakash Saxena A. 2010. Acute methaemoglobinaemia due to ingestion of nitrobenzene (paint solvent). Indian J Anaesth 54(2):160-162. 10.4103/0019-5049.63635.

Sealy RC, Swartz HM, Olive PL. 1978. Electron spin resonance-spin trapping. Detection of superoxide formation during aerobic microsomal reduction of nitro-compounds. Biochem Biophys Res Commun 82(2):680-684. https://doi.org/10.1016/0006-291X(78)90928-2.

Seip HM, Alstad J, Carlberg GE, et al. 1986. Measurement of mobility of organic compounds in soils. Sci Total Environ 5087-101. https://doi.org/10.1016/0048-9697(86)90353-0.

Shackelford WM, Cline DM, Faas L, et al. 1983. An evaluation of automated spectrum matching for survey identification of wastewater components by gas chromatography—mass spectrometry. Anal Chim Acta 14615-27.

Shimkin MB. 1939. Acute toxicity of mononitrobenzene in mice. Proceedings of the Society for Experimental Biology Medicine 42(3):844-846.

Shinoda K, Mitsumori K, Yasuhara K, et al. 1998. Involvement of apoptosis in the rat germ cell degeneration induced by nitrobenzene. Arch Toxicol 72(5):296-302.

Smith RP, Alkaitis AA, Shafer PR. 1967. Chemically induced methemoglobinemias in the mouse. Biochem Pharmacol 16(2):317-328.

Smyth Jr. HF, Weil CS, West JS, et al. 1969. An exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol Appl Pharmacol 14(2):340-347.

Staples CA, Werner AF, Hoogheem TJ. 1985. Assessment of priority pollutant concentrations in the United States using STORET database. Environ Toxicol Chem 4(2):131-142.

Stover EL, Kincannon DF. 1983. Biological treatability of specific organic compounds found in chemical industry wastewaters. Water Pollut Control Fed 5797-109.

Suganya S, Sophia D, Raj CA, et al. 2011. Amelioration of nitrobenzene-induced nephrotoxicity by the ethanol extract of the herb Euphorbia hirta. Pharmacognosy research 3(3):201-207. 10.4103/0974-8490.85009.

Suzuki J, Koyama T, Suzuki S. 1983. Mutagenicities of mono-nitrobenzene derivatives in the presence of norharman. Mutat Res 120(2-3):105-110. 10.1016/0165-7992(83)90150-1.

Suzuki J, Takahashi N, Kobayashi Y, et al. 1987. Dependence on Salmonella typhimurium enzymes of mutagenicities of nitrobenzene and its derivatives in the presence of rat-liver S9 and norharman. Mutat Res 178(2):187-193. 10.1016/0027-5107(87)90268-5.

Tabak HH, Quave SA, Mashni CI, et al. 1981. Biodegradability studies with organic priority pollutant compounds. J Water Pollut Control Fed 1503-1518.

Toker I, Yesilaras M, Tur FC, et al. 2016. Methemoglobinemia caused by dapsone overdose: Which treatment is best? Turkish Journal of Emergency Medicine 15(4):182-184. 10.1016/j.tjem.2014.09.002.

Trabalka JR, Garten Jr. CT. 1982. Development of predictive models for xenobiotic bioaccumulation in terrestrial ecosystems Oak Ridge National Lab., TN (USA).

TRI17. 2019. Toxic Chemical Release Inventory. National Toxicology Information Program: Bethesda, MD

Tyl RW, France KA, Fisher LC, et al. 1987. Developmental toxicity evaluation of inhaled nitrobenzene in CD rats. Appl Toxicol 8(4):482-492.

Vance WA, Levin DE. 1984. Structural features of nitroaromatics that determine mutagenic activity in Salmonella typhimurium. Environ Mutagen 6(6):797-811.

Veith GD, DeFoe DL, Bergstedt BV. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. Journal of the Fisheries Board of Canada 36(9):1040-1048.

Verna L, Whysner J, Williams GM. 1996. 2-Acetylaminofluorene mechanistic data and risk assessment: DNA reactivity, enhanced cell proliferation and tumor initiation. Pharmacol Ther 71(1):83-105. https://doi.org/10.1016/0163-7258(96)00063-0.

Verschueren K. 1985. Handbook of environmental data on organic chemicals. In (2 ed.): LWW.

Von Oettingen WF. 1941. The aromatic amino and nitro compounds: Their toxicity and potential dangers: US Government Printing Office.

Wang A, Hu C, Qu J, et al. 2008. Phototransformation of nitrobenzene in the Songhua River: kinetics and photoproduct analysis. J Environ Sci (China) 20(7):787-795.

Warner HP et al. 1987. Determination of Henry's Law Constants of Selected Priority Pollutants. USEPA/600/D-87/229; NTIS PB87-212684.

Weisburger JH, Weisburger EK. 1973. Biochemical formation and pharmacological, toxicological, and pathological properties of hydroxylamines and hydroxamic acids. Pharmacol Rev 25(1):1-66.

WHO. 2003. Environmental Health Criteria 230. Nitrobenzene. Geneva, Switzerland: World Health Organization.

WHO. 2009. Nitrobenzene in drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality. Geneva, Switzerland: World Health Organization. https://www.who.int/water_sanitation_health/water-quality/guidelines/chemicals/nitrobenzene-background.pdf.

WHO. 2017. Guidelines for drinking-water quality: fourth edition incorporating the first addendum. Geneva, Switzerland: World Health Organization. https://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/.

Wilson JT, Enfield CG, Dunlap WJ, et al. 1981. Transport and fate of selected organic pollutants in a sandy soil 1. J Environ Qual 10(4):501-506.

Windholz M, Budavari S, Blumetti RF, et al. 1983. Merck index: an encyclopedia of chemicals, drugs, and biologicals: Merck.

Wirtschafter ZT, Wolpaw R. 1944. A case of nitrobenzene poisoning. Ann Intern Med 21.

Wolfe NL. 1992. Abiotic transformations of pesticides in natural waters and sediments. In: Schnoor JR, ed. Fate of pesticides and chemicals in the environment. Chicester: John Wiley and Sons

Young DR, Gossett RW, Baird RB, et al. 1983. Wastewater inputs and marine bioaccumulation of priority pollutant organics off Southern California. Water chlorination: Environmental impact health effects 4.

Yu YS, Bailey GW. 1992. Reduction of nitrobenzene by four sulfide minerals: Kinetics, products, and solubility. J Environ Qual 21(1):86-94. 10.2134/jeq1992.00472425002100010014x.

Zasada K, Karbownik-Lewinska M. 2015. Comparison of potential protective effects of melatonin and propylthiouracil against lipid peroxidation caused by nitrobenzene in the thyroid gland. Toxicol Ind Health 31(12):1195-1201.

Zepp RG, Schlotzhauer PF. 1983. Influence of algae on photolysis rates of chemicals in water. Environ Sci Technol 17(8):462-468.

Zepp RG, Braun AM, Hoigne J, et al. 1987a. Photoproduction of hydrated electrons from natural organic solutes in aquatic environments. Environmental science technology 21(5):485-490.

Zepp RG, Hoigne J, Bader H. 1987b. Nitrate-induced photooxidation of trace organic chemicals in water. Environ Sci Technol 21(5):443-450.

Zhang H-K, Liang S-X, Liu S-J. 2007. Determination of nitrobenzene by differential pulse voltammetry and its application in wastewater analysis. Anal Bioanal Chem 387(4):1511-1516.

Zhang F, Bartels MJ, Pottenger LH, et al. 2008. Quantitation of methylated hemoglobin adducts in a signature peptide from rat blood by liquid chromatography/negative electrospray ionization

tandem mass spectrometry. Rapid Commun Mass Spectrom 22(10): 1455-1460. https://doi.org/10.1002/rcm.3530.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

MRLs (Minimal Risk Levels) 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 or benchmark dose modeling with applied uncertainty factors. 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 substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide

MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s):	Nitrobenzene
CAS number(s):	98-95-3
Date:	April 2022
Profile status:	Draft for Public Comment
Route:	Inhalation
Duration:	Acute
Provisional MRL	0.04 ppm (0.2 mg/m^3)
Critical Effect:	increased methemoglobin
Reference:	Medinsky and Irons 1985
Point of Departure:	$BMCL_{1SD}$ = 16 ppm ($BMCL_{HEC}$ = 4 ppm)
Uncertainty Factor:	90
LSE Graph Key:	3
Species:	Sprague-Dawley Rat

MRL Summary: A provisional acute-duration inhalation MRL of 0.04 ppm was derived for nitrobenzene based on evidence of increased methemoglobin in female Sprague-Dawley rats following exposure to nitrobenzene via inhalation for 14 days (Medinsky and Irons, 1985). The provisional MRL is based on a $BMCL_{1SD}$ of 16 ppm was divided by 4 for intermittent exposure and converted to a human equivalent concentration ($BMCL_{HEC}$) of 4 ppm for increased methemoglobin and divided by a composite uncertainty factor and modifying factor of 90 (10 for intrahuman variability, 3 for animals to humans after using dosimetric adjustment and 3 for a modifying factor to account for the differences in methemoglobin physiology in rodents and humans).

Selection of the Critical Effect: The major health effects associated with acute nitrobenzene inhalation exposure include adverse effects on the hematologic system, including methemoglobinemia, and adverse male reproductive effects. In addition, other outcomes associated with acute nitrobenzene inhalation exposure include renal, hepatic and neurological effects. Tyl et al. (1987) also evaluated the developmental effects of nitrobenzene exposure. The NOAELs and LOAELs from these studies are presented in Table A-1. The evidence for the acute inhalation toxicity of nitrobenzene comes from a variety of experimental animal studies in New Zealand white rabbits (Biodynamics 1983, 1984), Sprague-Dawley and F344 rats (Medinsky and Irons, 1985), and B6C3F1 mice (Medinsky and Irons, 1985).

Increased methemoglobin levels were observed in all studies which evaluated this endpoint. In considering the results of the rodent studies the differences between human and rodent methemoglobin levels and metabolism should be considered. The amount of methemoglobin in blood is mainly controlled by the level of the enzyme methemoglobin reductase (Smith, 1993). The amount of innate methemoglobin reductase activity in rodents is greater than that in humans. Specifically, it is estimated that the rat has about 2-to 5- times as much methemoglobin reductase activity compared to humans and the mouse about 10-times as much as a human (Smith, 1993). This means that the transformation of methemoglobin to hemoglobin, which is facilitated by methemoglobin reductase, occurs more quickly in rodents than in humans (Bloom and Brandt 2006). Therefore, it is likely that the increases in methemoglobin concentration observed at a given dose of nitrobenzene in a mouse is less than that which would be observed in a rat which is less than that would be observed in a human at the same dose. That is, rats are more sensitive to nitrobenzene toxicity than mice and humans are more sensitive to nitrobenzene toxicity than either rats or mice. Further, although a 1-4% methemoglobin level is

without adverse effects in humans it is not known what level of methemoglobin begins to cause adverse effects in rodents.

In Medinsky and Irons (1985) methemoglobin was observed to increase with concentrations as low as 9.1 ppm nitrobenzene for Sprague-Dawley rats. Additionally, both F344 and Sprague-Dawley rats exhibited concentration-dependent increases in methemoglobin levels with increasing exposures to nitrobenzene (Medinsky and Irons, 1985). In addition, at 9.1 ppm, a concentration-dependent increase in the number of hemosiderin-laden macrophages infiltrating the red pulp, increased extramedullary hematopoiesis and acute sinusoidal congestion at acute (i.e., 3 days-post exposure) sacrifice were observed. These effects did not persist after a 14-day recovery period.

Hepatic effects from acute inhalation exposure included centrilobular necrosis super imposed on a severe centrilobular hydropic in male F344 rats exposed to nitrobenzene via inhalation of 35 ppm for 2 weeks (Medinsky and Irons, 1985). However, similar necrotic lesions were not found in female rats. Degenerative non-necrotic changes in hepatocytes were noted in both sexes of mice exposed to 35 ppm of nitrobenzene via inhalation of 35 ppm nitrobenzene (Medinsky and Irons, 19895). These were similar to necrotic lesions observed in rats and B6C3F1 male mice, but at a less severe degree (Medinsky and Irons 1985). Increases in liver weight were also observed in Biodynamics (1983) in all treatment groups (doses ranged from 10-81 ppm) and in Tyl et al. (1981) at 40 ppm. However, the differences were not statistically significant in either study.

Dose-related increases in kidney weights were observed in Fischer-344 rats (both sexes), but not in Sprague-Dawley rats exposed to nitrobenzene via inhalation at 10 to 125 ppm for 14 days (Medinsky and Irons 1985, Tyl 1981). At 125 ppm, hydropic degeneration of the cortical tubular cells was observed only in Sprague-Dawley rats (20% of males; 90% of females), and hyaline nephrosis only in Fischer-344 rats (100% of males; 20% of females) (Medinsky and Irons, 1985). Renal effects reported in B6C3F1 mice in Medinsky and Irons (1985) included minimal to moderate multifocal degenerative changes in tubular epithelium of males exposed to 35 ppm for 2 weeks. However, neither hydropic degeneration of the cortical tubular cells nor hyaline nephrosis was seen in mice even at the highest exposure level (125 ppm). No renal effects were seen in New Zealand rabbits exposed up to 81 ppm (Biodynamics 1983) after acute inhalation exposure.

In experimental animals, nitrobenzene is a known testicular toxicant and has been used as a positive control in many studies designed to evaluate toxic effects on spermatogenesis effects (Allenby et al. 1990; Allenby et al. 1991; Linder et al. 1992). Evidence of testicular toxicity is seen in acute inhalation studies. For example, in an acute study in Fischer 344 rats a decrease in testicular weight and size was observed in rats exposed to 125 ppm for 2 weeks via inhalation (Medinsky and Irons 1985). Further, testicular lesions were also observed at 125 ppm in Fischer 344 rat and Sprague-Dawley rats, though the most common lesion observed varied between species. For example, 4/10 B6C3F1 mice and 4/10 Sprague-Dawley rats has had multinucleated giant cells where 7/10 of F344 rats had the same lesion. In addition, F344 rats also experienced Sertoli cell hyperplasia (8/10), and severe dysspermiogensis (10/10) and interstitial edema (7/10). After a 2-week recovery period the lesions were still present though the Sertoli cell hyperplasia present, but the increased numbers of multinucleated giant cells were less severe.

Tyl et al. (1987) evaluated skeletal malformations of Sprague-Dawley rats exposed to nitrobenzene in utero when the dam was exposed via inhalation from gestational day 6 through 15. The authors state “There were also no treatment-related effects on the incidence of fetal malformations or variations.” However, the incidence of litters with one or more fetuses with external variations was elevated at 40.0 ppm for ecchymosis (discoloration of the skin due to bleeding underneath) on the trunk (but not on the head or extremities). There was also increasing incidences of these malformations in the fetuses with increasing doses, however these differences were not statistically significant. In addition, there was a

significant increase the number of litters with animals having holes in the parietal skull plate, with 73 percent of litters in the 40 ppm group displaying this anomaly compared to 32 percent in the control group, indicating there may be delayed ossification. In Biodynamics (1984) no dose-related malformations in fetuses were observed with maternal exposures up to 100 ppm on days 7-19 of gestation.

Table A-1 presents a summary of the NOAELs and LOAELs considered in the development of the acute duration MRL.

Table A-1. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of an Acute Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL ($NOAEL_{ADJ}$) (ppm)	LOAEL ($LOAEL_{ADJ}$) (ppm)	Effect	Reference
Hematological Effects					
New Zealand White Rabbits	13 days/ 6 hours/day	104 (26)			Biodynamics 1984
F344 Rats	14 days, 5 days/week, 6 hours/day		9.1 (2.3)	Concentration dependent increase in the number of hemosiderin-laden macrophages infiltrating the red pulp, increased extramedullary hematopoiesis, acute sinusoidal congestion at acute sacrifice. In female rats, methemoglobin increased from 3.6% to 4.8%	Medinsky and Irons (1985)
Sprague-Dawley Rats	14 days, 5 days/week, 6 hours/day	9.1M (2.3)	9.1F (2.3)	Methemoglobin increased to 6.3% from 4.8% in controls; spleen weight increased 44% at acute sacrifice.	Medinsky and Irons (1985)
Sprague-Dawley Rats	10 days, gestational days 6-15, 6 hours/day		9.8 (2.5)	40% increase in relative and absolute spleen weight	Tyl et al. (1987)
B6C3F1 Mice	14 days, 5 days/week, 6 hours/day		9.1 (2.3)	Extramedullary hematopoiesis and acute vascular congestion	Medinsky and Irons (1985)
Renal Effects					
F344 Rat	14 days, 5 days/week, 6 hours/day		9.1 (2.3)	15% increase for males and 6% increase for females in relative kidney weight at acute sacrifice	Medinsky and Irons (1985)
Hepatic Effects					
F344 Rat	14 days, 5 days/week, 6 hours/day		9.1M (2.3)	significant 13% increase in relative liver weight at acute sacrifice	Medinsky and Irons (1985)

[1] Acute duration inhalation studies were adjusted from intermittent daily exposures to the equivalent of 24-hour continuous exposure but were not adjusted to continuous weekly exposure per guidance from ATSDR. Therefore, the duration adjusted values were calculated as:

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

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

Selection of the Point of Departure and Principal Study: Based on the available experimental animal evidence hematological effects and effects on kidney and liver weight are the most sensitive effect due to acute inhalation exposure to nitrobenzene. The hematologic effects include increased methemoglobin, hemosiderin-laden macrophages infiltrating the red pulp, and increased extramedullary hematopoiesis and acute sinusoidal congestion at acute (i.e., 3 days-post exposure) sacrifice. The only renal and hepatic effects were on organ weight, which has uncertain toxicological significance. As methemoglobinemia is one of the most noted outcomes associated with nitrobenzene exposure it was selected as the critical effect. Medinsky and Irons (1985) was selected as the principal study for deriving an MRL as it presents data demonstrating a clear increase in methemoglobin levels with increasing concentrations of nitrobenzene.

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) evaluated the effects of acute inhalation exposure of nitrobenzene to determine species, strain and sex differences in target organs, in Fischer 344 and Sprague-Dawley rats and B6C3F1 mice. 10 male and 10 female F344 rats, Sprague-Dawley rats and B6C3F1 mice in each dose group were exposed to target concentrations of 10, 35 and 125 ppm nitrobenzene vapor (analytically measured concentrations averaged 9.1, 35.8 and 124.5 ppm). Animals were exposed for 6 hours per day, 5 days a week for 2 weeks. At the end of the exposure duration up to five animals were sacrificed from each sex and strain. Blood samples were taken by heart puncture and methemoglobin levels were determined spectrophotometrically. After sacrifice, tissues were prepared for histopathological examination.

Medinsky and Irons (1985) observed differences in the acute toxicity response to nitrobenzene. Specifically, Fischer 344 rats exposed to 125 ppm of nitrobenzene for 2 weeks displayed no adverse clinical signs whereas several mice and several Sprague-Dawley rats in the high dose groups died before the end of the study. The assumed cause of death was perivascular hemorrhage in the cerebellar peduncle.

Blood methemoglobin levels in male and female F344 rats and female Sprague-Dawley rats increased in a concentration dependent manner at all dose levels when observed 3 days after exposure ended. Male Sprague-Dawley rats displayed increased methemoglobin levels at 35 ppm nitrobenzene exposure. In most instances, methemoglobin levels returned to control levels 14 days after the last exposure. Details on the methemoglobin levels are presented in Table A-2.

Table A-2. Percent Methemoglobin in Rats Exposed to Nitrobenzene by Inhalation

	Fischer 344 Rat		Sprague-Dawley Rat	
Exposure Group	Male	Female	Male	Female
Acute Sacrifice (3 days after last exposure)				
Control	0	3.6 ± 2.2 (4.92)	6.9 ± 1.3 (2.91)	4.8 ± 0.7 (1.57)
9.1 ppm	1.9 ± 0.7 (1.57)	4.8 ± 0.8 (1.79)	6.1 ± 0.5 (1.12)	6.3 ± 0.6 (1.34)
35.8 ppm	6.6 ± 0.2 (0.45)	6.6 ± 0.8 (1.79)	8.7 ± 1.0 (2.24)	7.3 ± 1.4 (3.13)
124.5 ppm	11.7 ± 1.2 (2.68)	13.4 ± 2.1 (4.70)	14.0 ± 1.3 (2.91)	31.3 ± 2.5 (5.59)
Recovery Sacrifice (14 days after last exposure)				
Control	4.5 ± 0.3 (0.67)	4.1 ± 0.5 (1.12)	4.6 ± 0.3 (0.67)	5.6 ± 0.6 (1.34)
10 ppm	4.1 ±0.1 (0.22)	3.1 ± 0.3 (0.67)	9.2 ± 1.6 (3.58)	5.2 ± 1.0 (2.24)
35 ppm	5.6 ±2.2 (4.92)	5.1 ± 1.9 (4.24)	5.8 ± 0.9 (2.01)	5.0 ± 0.5 (1.12)
125 ppm	4.8 ± 1.9 (4.25)	4.5 ± 1.5 (3.35)	a	a

Source: Medinsky and Irons (1985)

Numbers are mean ± standard error (standard deviation)
N = 5 except where otherwise indicated
a = No high dose animals survived in this group

Selection of the Point of Departure for the (Provisional) MRL: BMD modeling was conducted on the acute (i.e., 3 days after last exposure) sacrifice methemoglobin data. The data were fit to all continuous models in EPA's Benchmark Dose Software (BMDS, version 3.1.2) using a benchmark response (BMR) of 1 standard deviation. Given the lack of understanding of what magnitude of change in methemoglobin results in adverse effects in rodents, and how this relates to human changes in methemoglobin ATSDR opted to use a benchmark response (BMR) of 1 standard deviation.

The BMDS software makes a recommendation on viable model fit as part of the output. The highest dose group was dropped for both sexes in Sprague-Dawley rats after acute sacrifice given the high mortality rate in these animals. 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, BMCL <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 $BMCL_{1SD}$ was selected as the POD when the difference between the BMCLs estimated from these models was >3 fold; otherwise, the $BMCL_{1SD}$ from the model with the lowest Akaike's Information Criterion (AIC) was chosen.

No suitable models were identified for male and female Fischer 344 rats. Adequate fits to models were obtained for both male and female Sprague-Dawley rats using a BMR of 1 standard deviation. The resulting $BMCL_{1SD}$ for the male rats was 18 ppm and for female rats, 16ppm. Since the $BMDL_{1SD}$ for female rats was lower in female rats than in male rats, the female rat model was selected for deriving the MRL. Results from the modeling for the female Sprague-Dawley rats at acute sacrifice are presented below. The point of departure selected from this modeling exercise was 16 ppm, which is a $BMCL_{1SD}$ based on a linear model. The same $BMCL_{1SD}$ (and associated model fit parameters) was estimated with the polynomial 2 degree and the power model. The polynomial and power models appear linear when their functions are plotted and therefore the simpler linear model was selected. There is no difference in the resulting BMCLs from these three models. Modeling results are presented in Table A-3 and the exposure response curve is presented in Figure A-1.

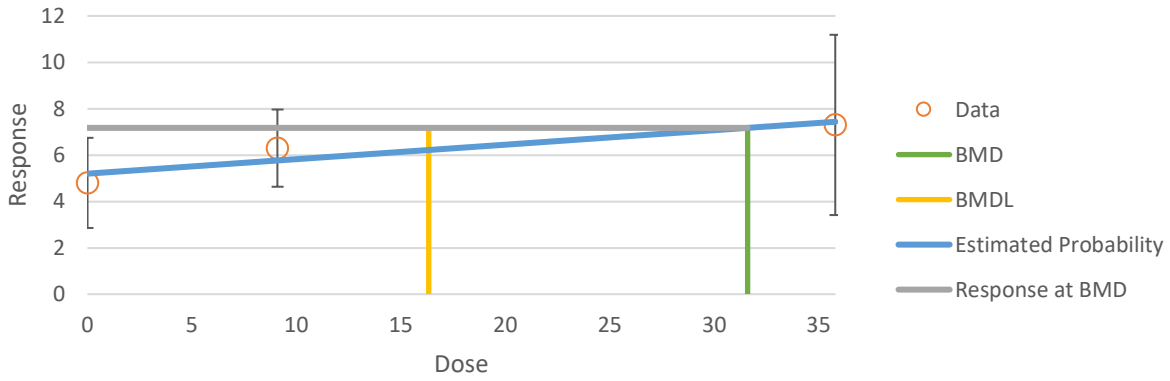
Table A-3. Model Predictions for Increased Methemoglobin in Female F344 Rats Exposed to Nitrobenzene via inhalation for Two Weeks (Medinsky and Irons, 1985)

Model	BMC_{1SD}[a] (ppm)	$BMCL_{1SD}$[a] (ppm)	P Value[a]	AIC	Scaled residuals[b] Dose below BMC	Scaled residuals[b] Dose above BMC
Exponential 2	33.4	19.6	0.40	69.08	-0.12	-0.52
Exponential 3	33.4	19.6	0.40	69.08	-0.12	-0.52
Exponential 4	14.5	1.5	NA	70.38	0.000001	-0.000001
Exponential 5	14.5	1.5	<0.0001	72.38	-0.00004	0.000005
Polynomial Degree 2	31.6	16.3	0.44	68.98	-0.15	-0.45
Power	31.6	16.3	0.44	68.98	-0.15	-0.45
Linear[c]	31.6	16.3	0.44	68.98	-0.15	-0.45

[a]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also, the largest residual at any dose.
[c]Selected model. The Polynomial 2 degree, power and linear models all provided adequate fits to the data and predicted the same BMCL. Therefore, the simple linear model was selected.

AIC = Akaike Information Criterion; BMC = benchmark concentration (maximum likelihood estimate of the dose associated with the selected benchmark response); BMCL10 = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., 1SD = dose associated with 1 standard deviation); DF = degree of freedom

Figure A-1. Fit of Linear Model to Data on Increased Methemoglobin in Female Sprague-Dawley Rats Exposed to Nitrobenzene with BMR of 1 Standard Deviation



Adjustment from Intermittent Exposure: Given the exposure in the Medinsky and Irons (1985) study was only six hours the $BMCL_{1SD}$ for intermittent exposure was extrapolated to account for a continuous exposure scenario:

$$BMCL_{ADJ} = BMCL_{1SD} \times \frac{6\ hr}{24\ hrs} = 16\ ppm \times \frac{6\ hr}{24\ hrs} = 4\ ppm$$

Human Equivalent Concentration: The human equivalent concentration (HEC) was calculated using Formula 4-48 from Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA 1994b). Interspecies extrapolation requires consideration of the nitrobenzene air: blood partition coefficient for humans and rats. However, this data is not available and therefore, as

recommended by EPA (1994b) in the absence of this data unity is assumed. Therefore, the HEC is determined by the following equation:

$$BMCL_{HEC} = BMCL_{ADJ} \times \frac{(HB/g)_A}{(HB/g)_H} = 4 \times 1 = 4\ ppm$$

Where:

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

Uncertainty Factor: The $BMCL_{HEC}$ is divided by a total UF and modifying factor of 90:

- 10 for intrahuman variability
- 3 for animal to human extrapolation after dosimetric adjustment
  3 for a modifying factor to account for the $BMCL_{1SD}$ being based on methemoglobin levels, and that there are several differences in rodent versus human physiology in this endpoint.

This results in the following MRL:

$$MRL = \frac{BMCL_{HEC}}{UF} = \frac{4\ ppm}{90} = 0.04\ ppm\ (0.2\ mg/m^3)$$

Chemical Manager: Malcolm Williams, DVM, PhD

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s):	Nitrobenzene
CAS number(s):	98-95-3
Date:	April 2022
Profile status:	Draft for Public Comment
Route:	Inhalation
Duration:	Intermediate
Provisional MRL	0.003 ppm (0.01 mg/m^3)
Critical Effect:	hemolytic anemia and increased methemoglobin
Reference:	Hamm Jr. et al. 1984
Point of Departure:	LOAEL = 5 ppm ($LOAEL_{HEC}$ = 0.89 ppm)
Uncertainty Factor:	300
LSE Graph Key:	8
Species:	F344 rat

MRL Summary: A provisional intermediate-duration inhalation MRL of 0.003 ppm was derived for nitrobenzene based on hematological health effects. The alterations in biochemical markers are indicative of hemolytic anemia, including increased methemoglobin in male F344 rats following exposure to nitrobenzene via inhalation for 90 days based on Hamm Jr. et al. 1984. The provisional MRL is based on a LOAEL of 5 ppm which was adjusted to continuous duration exposure and converted to a human equivalent concentration ($LOAEL_{HEC}$) of 0.89 ppm for increased methemoglobin. The $LOAEL_{HEC}$ was divided by a total uncertainty factor of 300 (10 for intrahuman 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 nitrobenzene's toxicity due to intermediate duration inhalation exposure (Dodd et al. 1987; Hamm Jr. et al. 1984). Dodd et al. (1987) was a two generation reproduction study with Sprague-Dawley rats. These rats were dosed with 1 to 40 ppm nitrobenzene for 10 weeks over the course of mating, gestation and parturition. Dodd et al., (1987) observed effects which included decreased fertility rates, decreased parturition and gestation indices, atrophy of seminiferous tubules, spermatocyte degeneration and reduced testicular and epididymal weights in the F_0 and F_1 generations and decreased offspring birth weight at 40 ppm.

Hamm et al., (1984) evaluated the toxicological profile of nitrobenzene in F-344 and Sprague-Dawley rats and B6C3F1 mice. Specifically, rodents were exposed to 0, 5, 16 or 50 ppm nitrobenzene for 6 hr/day 5 days/week for 90 days. At the lowest dose tested in the study, increases in methemoglobin were seen in male F344 rats. Female F344 rats and male Sprague-Dawley rats showed increases at 16 ppm and female Sprague-Dawley rats at 50 ppm. Additionally, in all mice and rats evaluated there was a demonstrated increase in spleen weights at 50 ppm and male F334 rats and female Sprague-Dawley rats experienced increased spleen weights at 16 ppm. Additionally, Hamm Jr. et al (1984) noted dose-related hematologic changes in rats that are consistent with hemolytic anemia. This effect was not observed in mice. Additionally, cellular vacuolization of the zona reticularis was seen in the adrenal gland in female mice at 5 ppm.

At higher exposure concentrations, effects observed by Hamm Jr. et al., (1984) included a minimal to slight hyperplasia observed in the bronchial epithelium after 50 ppm exposure for 90 days in male and female rats and mice. In male Sprague-Dawley rats' rhinitis associated with epithelial and goblet cell hyperplasia was observed in nasal turbinates after exposure to 16 and 50 ppm nitrobenzene. This was also observed in female rats exposed to 50 ppm nitrobenzene (Hamm Jr. et al. 1984). Further, increased

liver weight, hepatocyte hyperplasia, and multinucleated hepatocytes were observed in male B6C3F1 mice exposed to 16 ppm nitrobenzene via inhalation for 90 days (Hamm Jr. et al. 1984). Additionally, in both sexes of F-344 rats exposed to 50 ppm nitrobenzene via inhalation demonstrated centrilobular necrosis and disorganization of hepatic cords and Sprague-Dawley rats displayed centrilobular hepatocyte hypertrophy. 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 Jr. et al. 1984). Values considered for the derivation of the MRL are presented in Table A-4.

Table A-4. Summary of Relevant NOAEL and LOAEL (Adjusted for Duration[1]) Values Considered for Derivation of an Intermediate Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL (ppm) ($NOAEL_{ADJ}$)	LOAEL (ppm) ($LOAEL_{ADJ}$)	Effect	Reference
Reproductive Effects					
Sprague-Dawley Rats	10 weeks (2 generations), 5 days/week, 6 hours/day	10 (1.8)			Dodd et al. 1987
F344 Rats	90 days, 5 days / week, 6 hours / day	15.8 (2.8)			Hamm Jr. 1984
Sprague-Dawley Rats	90 days, 5 days / week, 6 hours / day	5 (0.89)	15.8 (2.82)	Slight reduction in mature sperm in 2 animals	Hamm Jr. 1984
B6C3F1 Mouse	90 days, 5 days / week, 6 hours / day	48.7 (8.7)			Hamm Jr. 1984
Hematological Effects					
F344 Rats	90 days, 5 days / week, 6 hours / day		5 (0.89)	M: Significantly increased methemoglobin, increased from 1.2% in controls to 3.0% accompanied by other hematologic changes that were consistent with hemolytic anemia. F: splenic hyperplasia; decreased RBCs, hemoglobin, and hematocrit; increased RBC width, platelet volume, and platelet width.	Hamm Jr. 1984
Sprague-Dawley rat	90 days, 5 days / week, 6 hours / day		48.7 (8.7)	splenic lesions and increased methemoglobin (M: 0.6% to 10.5%; F: 2.1% to 9.6%)	Hamm Jr. 1984

Table A-4. Summary of Relevant NOAEL and LOAEL (Adjusted for Duration[1]) Values Considered for Derivation of an Intermediate Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL (ppm) ($NOAEL_{ADJ}$)	LOAEL (ppm) ($LOAEL_{ADJ}$)	Effect	Reference
B6C3F1 Mice	90 days, 5 days / week, 6 hours / day	15.8 (2.82)	48.7 (8.7)	methemoglobin significantly increased (M: 0.7% to 5.8% and F: 1.3% to 5.1%) extramedullary hematopoiesis	Hamm Jr. 1984
Renal Effects					
F344 Rats	90 days, 5 days / week, 6 hours / day		5M (0.89)	Mild nephrosis	Hamm Jr. 1984
Hepatic					
B6C3F1 Mouse	90 days, 5 days / week, 6 hours / day	5M (0.89)	15.8M (2.82)	hepatocyte hyperplasia in 4/9 mice	Hamm Jr. 1984
Developmental Effects					
Sprague-Dawley Rat	10 weeks (2 generations), 5 days/week, 6 hours/day	10 (1.8)	40 (7.1)	12% decrease in mean body weight of F1 pups	Dodd et al. 1987
Endocrine Effects					
B6C3F1 Mouse	90 days, 5 days / week, 6 hours / day		5 (0.89)	adrenal lesions consisting of cellular vacuolization	Hamm Jr. 1984

[1] Intermediate and Chronic duration inhalation studies were adjusted from intermittent daily exposures to the equivalent of 24 hours a day 7 days a week continuous exposure. Therefore, 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); LOAEL = lowest-observed-adverse-effect level; $LOAEL_{ADJ}$ = LOAEL adjusted to continuous exposure; M = male(s); NOAEL = no-observed-adverse-effect-level

Selection of the Critical Effect and Principal Study:

The reproductive effects observed by Dodd et al., (1987) occurred with 40 ppm nitrobenzene. At 5 ppm Hamm Jr. et al. (1984) observed impacts to the hematological, renal and endocrine system. Therefore Hamm Jr. et al. (1984) was selected as the principal study for intermediate inhalation MRL derivation.

Summary of the Principal Study:

Hamm Jr. TE, Phelps M, Raynor TH, et al. 1984. Ninety day inhalation toxicity study of nitrobenzene in F-344 rats, and Sprague-Dawley rats and B6C3F1 mice. Research Triangle Park, NC: Chemical Industry Institute of Toxicology.

Hamm Jr. et al. (1984) evaluated the toxicological profile of nitrobenzene in F344 and Sprague-Dawley rats and B6C3F1 mice. Specifically, rodents were exposed to 0, 5, 16 or 50 ppm (analytically measured concentrations = 5.0, 15.8 and 48.7 ppm) nitrobenzene for 6hr/day 5 days/week for 90 day. Animals were observed for clinical signs twice a day and were weighed once per week. At the end of the 90 day exposure period ten animals of each species, sex and treatment group were sacrificed for histopathology assessments. F344 rats were determined to be the most sensitive species to the hematological effects. The data on the methemoglobin effects from these animals are summarized in Table A-5.

Table A-5. Data from Hamm Jr. et al. (1984) on Percent Methemoglobin with Nitrobenzene Exposure by inhalation in F344 Rats

Sex of F344 rat	0 ppm	5ppm	16 ppm	49 ppm
Male	1.2 ± 0.4	3.0 ± 1.0*	4.4 ± 1.3*	10.1± 1.2*
Female	1.6 ± 0.8	3.2± 0.9	3.9 ± 1.3*	10.5±1.2*

* significantly higher than control.

Selection of the Point of Departure for the (provisional) MRL: The only data which were amenable to potential dose-response modeling were those for the percent methemoglobin. Although Hamm Jr. (1984) refers to appendices with additional data that may inform dose-response modeling, we have not been able to locate them at this time. However, given that methemoglobinemia is a hallmark outcome of nitrobenzene exposure and is a commonly indicated outcome in humans exposed to nitrobenzene (Ikeda and Kita 1964; Lee et al. 2013; 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) it is justifiable to concentrate on this data when considering dose-response modeling.

BMD modeling was conducted on the methemoglobin data for F344 rats. Given the lack of understanding of what magnitude of change in methemoglobin results in adverse effects in rodents, and how this relates to human changes in methemoglobin ATSDR opted to use a benchmark response (BMR) of 1 standard deviation.

The data were fit to all continuous models in EPA's Benchmark Dose Software (BMDS, version 3.1.2) using a benchmark response (BMR) of 1 standard 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. No adequate model fits were obtained. Subsequently, we defaulted to identifying the appropriate NOAEL or LOAEL to protect against the effects observed in Hamm Jr. et al. (1984).

Although there is uncertainty about the percent change in methemoglobin levels in experimental animals that may result in an adverse effect which would be analogous for estimating human response,

it is clear from the Hamm Jr. et al. (1984) study that additional hematologic effects are occurring with nitrobenzene exposure. That is, the small increases in methemoglobin were accompanied by changes in the blood that were consistent with hemolytic anemia. Therefore 5 ppm was categorized as a LOAEL for hematologic effects and was selected as a LOAEL and the point of departure for the nitrobenzene intermediate MRL. 5 ppm is also the LOAEL associated with the lesions in the adrenal gland and mild nephrosis in the kidney as observed in Hamm Jr. (1987), which may be considered co-critical effects.

Adjustment for Intermittent Exposure: Given the exposure in the Hamm Jr. et al. (1984) was intermittent the LOAEL for intermittent exposure was extrapolated to a continuous LOAEL through the following equation:

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

Human Equivalent Concentration: The human equivalent concentration (HEC) was calculated using Formula 4-48 from Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA 1994b). Interspecies extrapolation requires consideration of the nitrobenzene air: blood partition coefficient for humans and rats. However, this data is not available and therefore, as recommended by EPA (1994b) in the absence of this data unity is assumed. Therefore, the HEC is determined 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 UF of 300:

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

This results in the following MRL:

- $MRL = \frac{LOAEL_{HEC}}{UFs} = \frac{0.89\ ppm}{300} = 0.003\ ppm$ (0.01 mg/m^3)

Chemical Manager: Malcolm Williams, DVM, PhD

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s):	Nitrobenzene
CAS number(s):	98-95-3
Date:	April 2022
Profile status:	Draft for Public Comment
Route:	Inhalation
Duration:	Chronic
MRL	0.001 ppm (0.006 mg/m^3)
Critical Effect:	Degeneration of the olfactory epithelium and bronchiolization of the alveoli
Reference:	Cattley et al. 1994
Point of Departure:	$BMCL_{10}$= 0.93 ppm ($BMCL_{HEC}$= 0.04 ppm)
Uncertainty Factor:	30
LSE Graph Key:	13
Species:	B6C3F1 Mouse

MRL Summary: A provisional chronic-duration inhalation MRL of 0.001 ppm was derived for nitrobenzene based on evidence of degeneration of the olfactory epithelium in B6C3F1 mice following exposure to nitrobenzene via inhalation for two years, as observed in Cattley et al. (1994). The provisional MRL is based on a $BMCL_{10}$ of 0.93 ppm which was adjusted to continuous duration exposure and converted to a human $BMCL_{HEC}$ of 0.04 ppm for olfactory degeneration. The $BMCL_{HEC}$ was divided by a total uncertainty factor of 30 (10 for intrahuman variability and 3 for animal to human extrapolation after applying dosimetric adjustment).

Selection of the Critical Effect: The major health effects associated with nitrobenzene exposure include adverse effects on the hematologic, male reproductive, renal, hepatic, neurological and respiratory systems. Only one chronic inhalation study was located in the development of this profile which was Cattley et al., 1994. In Cattley et al. (1994) male and female B6C3F1 mice, male and female F344 rats and male Sprague-Dawley rats were evaluated in a 2 year chronic cancer bioassay study and a full toxicological assessment was conducted on the animals, providing data from which to assess potential MRLs. In this study rats were exposed to 0, 1, 5 and 25 ppm nitrobenzene, and mice were exposed to 0, 5, 25 and 50 ppm nitrobenzene.

The most striking effect observed as a result of nitrobenzene exposure was that on the respiratory system. Specifically, at 1 ppm exposure to nitrobenzene male and female F344 rats displayed a significant increase in pigment deposition in the olfactory epithelium. Pigment deposition in the nasal cavity may be indicative of the chemical depositing in the epithelium, it could be secondary to inflammation, degeneration, or hemorrhage. The presence of the pigment deposition increased with increasing doses. Male Sprague-Dawley rats displayed the same effect starting at 5 ppm. There was also an increase in the incidence of inflammation, suppurative exudate (25-ppm exposure group), and squamous epithelial hyperplasia (all exposure groups) in the anterior nose in male and female F344 mice (Cattley et al. 1994).

Further, with 5 ppm exposure to nitrobenzene 58/67 male mice and 55/60 female mice displayed bronchiolization of the alveoli and at 50 ppm, the highest dose in the study, 62/66 male and 62/62 female mice displayed this effect. No control mice of either sex exhibited this lesion (Cattley et al. 1994). In addition, mice also experienced an increase in degeneration of the olfactory epithelium. Specifically, both male and female mice had an increased incidence of degeneration in the 25 and 50 ppm exposure groups. Females also experienced degeneration at 5 ppm (Cattley et al. 1994).

Hematological effects occurred in Cattley et al. (1994) with 25 ppm exposure of nitrobenzene in male and female rats. There was also a statistically significant increase in hypercellularity in femur bone marrow in mice exposed to 50 ppm nitrobenzene (Cattley et al. 1994; CIIT 1993). There was also a noted increase in extramedullary hematopoiesis with 1 ppm exposure to nitrobenzene, though there was a high incidence of this outcome in controls. Further, centrilobular hepatocytomegaly and multinucleated hepatocytes were observed in male mice at all concentrations, with the lowest dose for mice being 5 ppm (Cattley et al. 1994). The same effects were seen at the 50 ppm exposure level in female mice. In rats, centrilobular hepatocytomegaly occurred at 25 ppm in male F344 rats and at 5 and 25 ppm in Sprague-Dawley rats. Further, the incidence of F344 rats with eosinophilic foci increased in the 5 and 25 ppm groups in males and 25 ppm group in females. The occurrence of spongiosis hepatis (cystic degeneration of liver cells) was also increased in 25-ppm nitrobenzene-exposed Sprague-Dawley rats. In addition, 5 and 25 ppm nitrobenzene exposure resulted in an increased incidence of centrilobular hepatocytomegaly in Sprague-Dawley rats. The incidence of rats with Kupffer cell pigmentation was increased at all nitrobenzene exposure concentrations, with the lowest dose being 1 ppm in Sprague-Dawley rats (Cattley et al. 1994).

In the renal system, significant increase in cysts on the kidney with 50 ppm exposure in B6C3F1 mice and F344 rats was observed. The severity of nephropathy increased with increasing doses of nitrobenzene exposure in male and female F344 rats. Additionally, the effect of chronic nitrobenzene inhalation resulted in increased thyroid follicular cell hyperplasia in male mice at 25 and 50 ppm nitrobenzene (Cattley et al., 1994).

Reproductive effects were also seen in this study. For example, in male mice exposed to nitrobenzene for two years at 50 ppm there was an increase in epididymal hypospermia. The same effect was observed in Sprague-Dawley male rats exposed to 25 ppm nitrobenzene including an increased atrophy of the testes (Cattley et al. 1994). An overview of the NOAEL and LOAEL values considered for MRL development are presented in Table A-6.

Table A-6. Summary of Relevant NOAEL and LOAEL Values (Duration Adjusted[1]) Considered for Derivation of a Chronic Inhalation MRL for Nitrobenzene

Species	Duration/Route	NOAEL (ppm) (NOAEL $_{ADJ}$)	LOAEL (ppm) (LOAEL $_{ADJ}$)	Effect	Reference
Respiratory Effects					
B6C3F1 Mice	505 days over 2 years, 5 days / week, 6 hours / day		5 (0.89)	87% increase in incidence in males and 92% increase in incidence in females of bronchiolization of the alveoli, females also had 32% increase in incidence of degeneration and loss of the olfactory epithelium	Cattley et al. 1994
Sprague-Dawley Rats	505 days over 2 years, 5 days / week, 6 hours / day	5M (0.89)	24.8M (4.4)	increased incidence of inflammation, suppurative exudate and squamous epithelial hyperplasia - not otherwise described	Cattley et al. 1994
Hematological Effects					
F344 rats	505 days over 2 years, 5 days / week, 6 hours / day		1 (0.2)	90% incidence of extramedullary hematopoiesis of the spleen versus 77% in control, 91% incidence of pigmentation of the spleen versus 80% in control	Cattley et al. 1994
Hepatic Effects					
F344 rats	505 days over 2 years, 5 days / week, 6 hours / day		24.8 (4.4)	increase in eosinophilic foci (9/66 in control; 16/70 in 25 ppm); increase in spongiosis hepatitis (0/70 in control; 6/70 in 25 ppm)	Cattley et al. 1994
Sprague-Dawley Rats	505 days over 2 years, 5 days / week, 6 hours / day		5 (0.89)	15% increase in the incidence of centrilobular hepatocytomegaly; 19% increase in multinucleated hepatocytes	Cattley et al. 1994
B6C3F1 Mice	505 days over 2 years, 5 days / week, 6 hours / day		5M (0.89)	22% increase in incidence of centrilobular hepatocytomegaly, 19% increase in incidence of multinucleated hepatocytes	Cattley et al. 1994
Renal Effects					
F344 rats	505 days over 2 years, 5 days / week, 6 hours / day	5M (0.89)	24.8M (4.4)	16% increase in incidence of tubular hyperplasia of the kidney	Cattley et al. 1994
Sprague-Dawley Rats	505 days over 2 years, 5 days / week, 6 hours / day	24.8M (4.4)			Cattley et al. 1994

Table A-6. Summary of Relevant NOAEL and LOAEL Values (Duration Adjusted[1]) Considered for Derivation of a Chronic Inhalation MRL for Nitrobenzene

Species	Duration/Route	NOAEL (ppm) ($NOAEL_{ADJ}$)	LOAEL (ppm) ($LOAEL_{ADJ}$)	Effect	Reference
B6C3F1 Mice	505 days over 2 years, 5 days / week, 6 hours / day		49.1M (8.72)	15% increase in incidence of kidney cysts	Cattley et al. 1994
Reproductive Effects					
Sprague-Dawley Rats	505 days over 2 years, 5 days / week, 6 hours / day	5M (0.89)			Cattley et al. 1994
F344 rats	505 days over 2 years, 5 days / week, 6 hours / day	24.8M (4.4)			Cattley et al. 1994

[1] Intermediate and Chronic duration inhalation studies were adjusted from intermittent daily exposures to the equivalent of 24 hours a day 7 days a week continuous exposure. Therefore, 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; M = male(s); NOAEL = no-observed-adverse-effect level

Selection of the Critical Effect and Point of Departure:

Cattley et al. (1994) is the only study located which evaluated adverse health outcomes associated with chronic nitrobenzene inhalation exposure. This is a full toxicological evaluation of nitrobenzene toxicity and is therefore appropriate to be used as the basis of the MRL. Given the significant increase in respiratory effects, olfactory degeneration and alveolar bronchiolization were evaluated as critical effects.

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 Sprague-Dawley rats. Fundam Appl Toxicol*,* 22(3): 328-340.

Cattley et al. (1994) refers to Sprague-Dawley rats as CD rats, but this report calls them Sprague-Dawley rats for consistency. Cattley et al. (1994) evaluated the toxicity and carcinogenicity of inhaled nitrobenzene in F344 male and female rats, male Sprague-Dawley rats, and B6C3F1 mice following chronic exposure. All animals were exposed to nitrobenzene for 6 hours/day, 5 days/week for 2 years in an inhalation chamber. All rats were exposed to target concentrations of 0, 1, 5 or 25 ppm nitrobenzene, and all mice were exposed to 0, 5, 25 or 50 ppm nitrobenzene (analytically measured concentrations for 0, 1, 5, 25, and 50 ppm were 0, 1, 4.96, 24.8, and 49.1 ppm, respectively). Hematological evaluations and post-mortem examinations were conducted on all animals, and tissues examined included the thyroid, parathyroid, spleen, lungs, femur, sternum, nose and liver as target tissues, and various non-target tissues including the kidney and ear cancel. Treatment related effects in all animals included significant decreases in hematological parameters (red blood cell, hematocrit, and hemoglobin count), and increased incidence of neoplastic and non-neoplastic lesions. In male and female mice, incidence of degenerative and inflammatory lesions in the nose increased, and mammary gland neoplasms increased in female mice. In male mice liver, centrilobular hepatocytomegaly and multinucleated hepatocyte lesions were significantly increased at all exposure levels, and only at 50 ppm for female mice. For rats in all exposure concentrations, the incidence of increased pigment deposition was significant. In all species of male rats, hepatocellular neoplasms increased, and additionally renal neoplasms in male F344 rats only, while female rats had significant increases in endometrial stromal neoplasms. Cattley et al. (1994) found increased incidence of neoplasia in all animals examined and toxicity manifestation was attributed to hematological changes, and adaptive and/or degenerative changes in the nose, liver and testis of all animals examined.

Selection of the Point of Departure for the (Provisional) MRL: From the effects seen in Cattley et al. (1994) it is clear a number of effects occur around 5 ppm of exposure including significant increases in the incidence of bronchiolization of the alveolar wall in male and female mice along with olfactory degeneration, pigment deposition in male Sprague-Dawley rats, centrilobular hepatocytomegaly and eosinophilic foci in the livers of male F344 rats. At 1 ppm increased pigment deposition and extramedullary hematopoiesis were observed. However, given that pigment deposition can be caused by a number of factors (age, chemical deposition, hemorrhage, inflammation, etc.) the toxicological significance of this endpoint is not specific enough to select it as a candidate critical effect. Further, extramedullary hematopoiesis had a very high background rate in controls (77%) and therefore it was not selected as the critical effect.

Given the significant increase in bronchiolization of the alveolar wall and the increased degeneration of the olfactory epithelium the respiratory effects were evaluated for potential to inform the MRL for nitrobenzene. The data used in BMD modeling is summarized in Table A-7.

Table A-7. Incidence of Bronchiolization of the Alveoli and Degeneration of the Olfactory Epithelium and B6C3F1 Mice (Cattley et al. 1994)

	Males				Females			
Endpoint	0 ppm	4.96 ppm	24.8 ppm	49.1 ppm	0 ppm	4.96 ppm	24.8 ppm	49.1 ppm
Bronchiolization	0/68 (0%)	58/67 (87%)	58/65 (89%)	62/66 (94%)	0/53 (0%)	55/60 (92%)	63/64 (98%)	62/62 (100%)
Degeneration /Loss of Olfactory Epithelium	1/67 (1%)	1/66 (2%)	32/65 (49%)	41/66 (62%)	0/52 (0%)	19/60 (32%)	47/63 (75%)	42/61 (69%)

BMD modeling was conducted for male and female data for both alveolar bronchiolization and olfactory degeneration. The data were fit to all dichotomous models in EPA's Benchmark Dose Software (BMDS, version 3.1.2) using a benchmark response (BMR) 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 Akaike's Information Criterion (AIC) was chosen.

The BMDS software makes a recommendation on viable model fit as part of the output. When using the data for all dose groups the only group which had a viable model fit was female mice when evaluating olfactory degeneration. Additional model fits were available if the high dose group was dropped, specifically for the male and female mice for olfactory degeneration. Given that the majority of animals survived in the highest dose group there is not a good biologic justification for excluding the highest dose group and therefore opted to not further pursue those models.

The results of the BMD modeling for olfactory degeneration are provided in Table A-8 with the exposure-response curve for the selected model in Figure A-2.

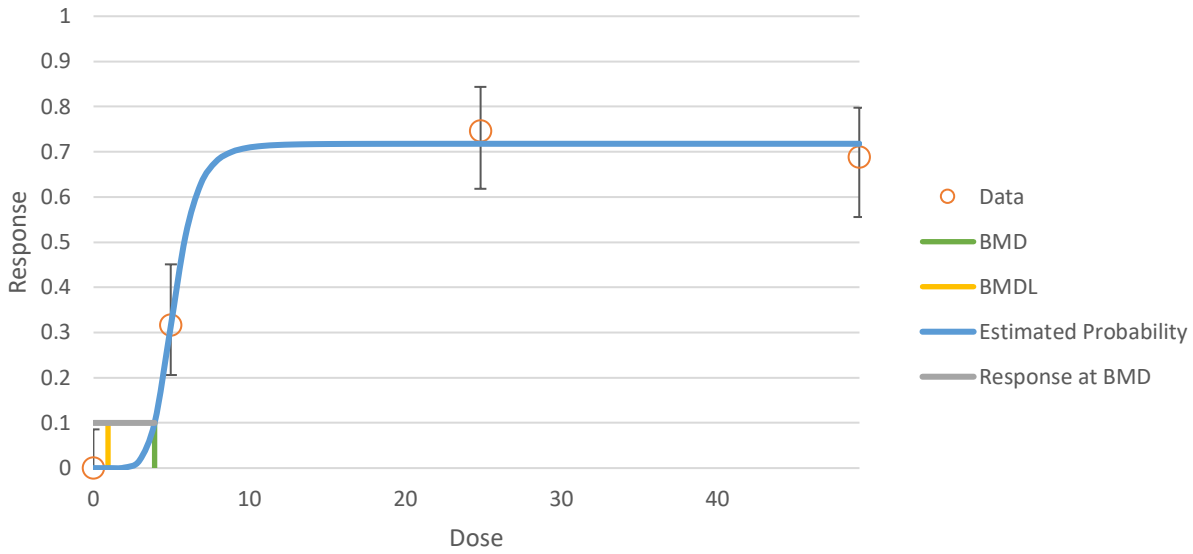
Table A-8. Results from BMC Analysis of Incidences of Female B6C3F1 Mice with Olfactory Degeneration at Chronic Sacrifice During Inhalation Exposure to Nitrobenzene Using a 10% Relative Deviation

Model	BMC_{10}[a] (ppm)	$BMCL_{10}$[a] (ppm)	P Value[b]	AIC	Scaled residuals[c]	
					Dose below BMD	Dose above BMD
Dichotomous Hill[d]	**3.92**	**0.93**	**0.48**	**228.50**	**-0.00003**	**-0.0009**
Gamma	2.72	2.31	<0.0001	247.96	2.90	-0.0009
Log-Logistic	1.37	1.04	0.08	230.23	-0.0009	-0.0009
Multistage Degree 2	2.72	2.31	<0.0001	245.96	2.90	-0.0009
Multistage Degree 1	2.72	2.31	<0.0001	245.96	2.90	-0.0009
Weibull	2.72	2.31	<0.0001	247.96	2.90	-0.0009
Logistic	7.54	6.37	<0.0001	247.96	2.90	-0.009
Log-Probit			<0.0001	270.21	1.13	-3.64
Probit	7.45	6.40	0.10	230.67	-0.001	-0.0009

[a] BMDLs <10 times the lowest non-zero dose and their corresponding BMDs 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; also, the largest residual at any dose.
[d]Selected model. The Dichotomous Hill was the only model that provided adequate fit to the data.

AIC = Akaike Information Criterion; BMC = benchmark concentration; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., 10 – dose associated with 10% risk); BMD = benchmark dose (maximum likelihood estimate of the dose associated with the selected benchmark response); BMDL10 = 95% lower confidence limit on the BMD (subscripts denote benchmark response); DF = degree of freedom

Figure A-2. Fit of Dichotomous Hill Model to Data on Nitrobenzene, Olfactory Degeneration in Female Mice



The viable model as dictated by the BMDS software in accordance with the criteria above as the Dichotomous Hill model which has an associated $BMCL_{10}$ of 0.93 ppm. Although there are 4 parameters in this model, one is bounded, allowing the use of this model for derivation of the MRL. Therefore, we selected the $BMCL_{10}$ of 0.93 ppm for olfactory degeneration as the POD. This POD also provides protection against alveolar bronchiolization.

Adjustment for Intermittent Exposure: Given the exposure in the Cattley et al. (1994) study was intermittent the $BMCL_{10}$ for intermittent exposure was extrapolated to a continuous $BMCL_{10}$ through the following equation:

$$BMCL_{ADJ} = BMCL_{10} \times \frac{6\ hr}{24\ hrs} \times \frac{5\ days}{7\ days} = 0.93\ ppm \times \frac{6\ hr}{24\ hrs} \times \frac{5\ days}{7\ days} = 0.166\ ppm$$

Human Equivalent Concentration: Given the critical effect occurs in the respiratory system and is not a systemic effect a regional gas dose ratio (RGDR) [1] is needed (as opposed to the blood:air partition coefficient) to estimate a $BMCL_{HEC}$. The $BMCL_{ADJ}$ was converted to a $BMCL_{HEC}$ using the regional gas dose ratio (RGDR)[2]. Further the effects are due to the distribution of nitrobenzene through the extra thoracic region (as opposed to the pulmonary region or a systemic effect). Following EPA's guidance (U.S. EPA 1994) we used the following equation:

[1] Minute Volume data from: U.S. EPA (1988) Recommendations For and Documentation of Biological Values for Use in Risk Assessment. EPA/600/6-87/008
Surface Area volume data from: U.S. EPA (1994) Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F

[2] Minute Volume data from: U.S. EPA (1988) Recommendations For and Documentation of Biological Values for Use in Risk Assessment. EPA/600/6-87/008
Surface Area volume data from: U.S. EPA (1994) Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F

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

Where:

MV_a= minute volume for mice = 0.06 m^3 per day
SA_a = ET surface area for mice = 3.0 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 0.2. The HEC then becomes:

$$BMCL_{HEC} = BMCL_{ADJ} \times RGDR = 0.166\ ppm \times 0.2 = 0.033\ ppm\ (0.02\ mg/m^3)$$

Uncertainty Factor: The $BMCL_{HEC}$ was divided by a composite UF of 30:

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

This results in the following MRL:

$$MRL = \frac{BMCL_{HEC}}{UFs} = \frac{0.033\ ppm}{30} = 0.001\ ppm\ (0.006\ mg/m^3)$$

Chemical Manager: Malcolm Williams, DVM, PhD

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Nitrobenzene
CAS number(s): 98-95-3
Date: April 2022
Profile status: Draft for Public Comment
Route: Oral
Duration: Acute
Provisional MRL: 0.05 mg/kg/day
Critical Effect: Changes in the bone marrow resulting in hematological effects
Reference: Burns et al. 1994
Point of Departure: $BMDL_{1SD}$ of 4.7 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 12
Species: B6C3F1 mouse

MRL Summary: A provisional acute-duration oral MRL of 0.05 mg/kg/day was derived for nitrobenzene based on changes in hematological health effects. The evidence of changes in femur bone marrow, as indicated by increased DNA synthesis in female B6C3F1 mice administered nitrobenzene via gavage in corn oil for 14 days (Burns et al. 1994). The provisional MRL is based on a $BMDL_{1SD}$. This was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability).

Selection of the Critical Effect: Burns et al. (1994) provides a comprehensive toxicological evaluation of acute oral exposure to nitrobenzene. In this study the main toxicological effects observed by study authors occur in the bone marrow, spleen, liver and blood. Burns et al. (1994) observed results consistent with nitrobenzene's hematological toxicity. In this study the most sensitive effect observed were the changes in the bone marrow where the number of cells in the femur bone marrow, DNA synthesis, and the number of colony forming units for granulocyte monocyte progenitor cells were increased in a statistically significant manner following a dose-response trend, starting at 30 mg/kg/day. The immature erythrocytes (reticulocyte) formed caused an increase in mean corpuscular volume and mean corpuscular hemoglobin starting at doses of 100 mg/kg. In addition, the percentage of reticulocytes increased dose-dependently. Hepatomegaly and splenomegaly were observed by the researchers as were pathological observations consistent with extramedullary hematopoiesis. In addition, Burns et al. (1994) observed immunotoxicity with nitrobenzene exposure including decreases in IgM response in the spleen to T-dependent antigens, alterations in phagocytic activity of macrophages and decreased activity of natural killer cells.

Other acute duration oral studies evaluating the toxicological effects of nitrobenzene were mainly single dose exposure studies where nitrobenzene was used to study the adverse reproductive outcomes in male rodents (Levin et al., 1988; Iida et al., 1997; Kawaguchi et al., 2004; Kawashima et al., 1995; Linder et al., 1992; McLaren et al., 1993, Shinoda et al., 1998). In these studies, the lowest dose utilized was 60 mg/kg/day, which resulted in decreased relative weight of testes in Sprague-Dawley Rats (Iida et al., 1997), decreased testicular and epididymis weights, decreased sperm count and decreased sperm motility (Kawashima et al., 1995). In Kawaguchi et al. (2004) no effects were seen in male Sprague-Dawley rats treated for 3 days with 60 mg/kg nitrobenzene. However, it is possible that Kawaguchi et al. (2004) may not have allowed for enough time to observe the adverse reproductive effect caused by exposure to 60 mg Nitrobenzene/kg since the study did not observe the adverse effects until after day 7 of their study.

In addition, one study observed methemoglobinemia resulting from a single dose of nitrobenzene in F-344 rats (Goldstein et al., 1984). Goldstein et al., (1984) observed >20% methemoglobin after a single dose of 200 mg/kg/day in male F344 rats. This study reported a NOAEL of 150 mg/kg/day based on a single dose.

In a summary of the NTP (1983a)[3] study there is reference to a 14-day repeated dose study where doses ranged from 37.5 to 600 mg/kg. All rats and mice died or were sacrificed moribund at the 600 mg/kg dose level. One mouse and all rats at the 300 mg/kg dose levels died or were sacrificed moribund prior to the end of treatment; only one mouse was sacrificed moribund at the 300 mg/kg dose level. This study observed significant changes in hematologic parameters including increases in reticulocyte counts and methemoglobin measurements in the rats. In male mice, significant increases were seen in reticulocyte counts at all doses except at the 37.5 mg/kg dose level. Methemoglobin was significantly increased at all doses except at the 37.5 mg/kg dose level in female mice and the 75 mg/kg dose level in the male mice and 37.5 mg/kg dose level in female mice. However, the NTP (1983a) report does not include the dose response details from the 14-day exposure.

NOAEL and LOAEL values evaluated considered for MRL derivation are presented in Table A-9.

Table A-9. Summary of Relevant NOAEL and LOAEL Values Evaluated for Derivation of an Acute Oral MRL for Nitrobenzene

Species	Duration	NOAEL (mg/kg)	LOAEL (mg/kg)	Effect	Reference
Hematological Effects					
B6C3F1 Mice	once/day for 14 days (GO)		30	Dose-dependent increase in number of cells in the bone marrow, DNA synthesis, and the number of granulocyte-monocyte progenitor cells per femur	Burns et al. 1994
Hepatic Effects					
B6C3F1 Mice	once/day for 14 days (GO)	30	100	12% increase in relative liver weight	Burns et al. 1994
Immune Effects					
B6C3F1 Mice	once/day for 14 days (GO)	30	100	50% decrease in IgM AFC in the spleen cells; increase in Natural Killer Cell activity at 30:1 and 100:1 effector: target ratios	Burns et al. 1994

G = gavage; GD = gestation day; GO = gavage in oil vehicle; GW = gavage in water vehicle; LOAEL = lowest observed adverse effect level; NOAEL = no-observed-adverse-effect level; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; AFC= Antibody-forming cell

[3] All the available information from NTP (1983) is reported. It does not appear likely that more details from this study are provided elsewhere.

Selection of the Principal Study and Point of Departure:

Burns et al. (1994) is the only oral exposure acute-duration (14 day) study that had reliable data to inform an MRL derivation for the acute exposure duration. The B6C3F1 female mice received doses of 0, 30, 100 or 300 mg/kg/day nitrobenzene in corn oil. Standard organ weights, hematological effects, tissue histopathology, and levels of biomarkers for tissue damage in the liver, spleen and femur bone marrow were evaluated in addition to several parameters related to immunological defenses (IGM, IGG). The 30 mg/kg/day dose was the NOAEL for most of the parameters evaluated. However, there was also a clear and significant impact on bone marrow at 30 mg/kg that is linked to the hematological effects observed at the 100 mg/kg/day dose. These effects included increased cell counts, DNA synthesis and colony forming units that the authors considered to be an early response leading to red blood cell replacement associated with the significant increase in reticulocytes at the 100 mg/kg/day dose (p. 313, Burns et al., 1994).

The hematological toxicity of nitrobenzene is well-established for other exposure routes and durations (Medinsky and Irons, 1985; Hamm Jr., et al., 1984; NTP 1983a). Burns et al. (1994) demonstrated a decrease in blood erythrocytes as well as increased femur bone marrow DNA synthesis. Given that the effects observed for the hematological endpoints were the most sensitive (specifically those within the bone marrow), they were selected as the critical effect.

Summary of the Principal Study:

Burns et al. 1994. Immunotoxicity of Nitrobenzene in Female B6C3F1 Mice. Drug and Chemical Toxicology. 17(3): 271-315.

Burns et al. (1994) is a 14-day acute duration study that evaluated general toxicity and immunotoxicity in female B6C3F1 mice. Doses ranged from 30 to 300 mg/kg/day and a single dose per day was administered via gavage with corn oil. At dose initiation female mice were approximately 8 to 10 weeks old. Eight mice were assigned to one of four dose groups within one of two studies, there was both a standard toxicology component and an immunologic component to the study. In both studies Group 1 animals were the control group (only corn oil), Group 2 animals received 30 mg/kg nitrobenzene, Group 3 animals received 100 mg/kg nitrobenzene and Group 4 animals received 300 mg/kg. All animals were treated for 14 consecutive days. The immunologic and toxicologic assessments were conducted on day 15, one day after exposure ended. As described above, the main effects observed in this study were in the erythrocytes, liver, spleen, and bone marrow. Specifically, the authors concluded that the erythrocyte is a main target of nitrobenzene toxicity. Erythrocyte levels decreased significantly at the 300 mg/kg/day dose leading to a subsequent dose-related compensatory increase in the percent reticulocytes (see A-10) with statistically significant differences at 100 mg/kg/day and 300 mg/kg/day when compared to control animals.

Table A-10. Reticulocyte Counts[1] in Female B6C3F1 Mice Orally Exposed to Nitrobenzene for Fourteen Days

Dose (mg/kg/day)	N	Mean % Reticulocytes (±SE)
0	8	1.03 (±0.09)
30	7	1.09 (±0.17)
100	8	3.51 (±0.43)*
300	7	4.57 (±0.48)*

Source: Burns et al. (1994)

[1]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 0.5 to 1.5%.

N = number of animals; SE = standard error
* p<0.01

In addition, there was a significant impact of nitrobenzene on bone marrow cell numbers, DNA synthesis in the bone marrow and colony forming units (CFU) for granulocyte monocyte progenitor cells. The data demonstrating the dose-response trend for each of the parameters is shown in Table A-11.

Table A-11. Femur Bone Marrow Analysis Results in Female B6C3F1 Mice Orally Exposed to Nitrobenzene for Fourteen Days

	Dose of Nitrobenzene (mg/kg)			
Parameter	0	30	100	300
N	8	8	7	7
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 (x 10^4)	0.58 (0.06)	0.88 (0.06)*	0.82 (0.05)*	1.01 (0.06)*

Values are mean (SE)
* p< 0.01
CFU-GM = cell forming units – granulocyte monocyte
Source: Table 7 from Burns et al. (1994)

Selection of the Point of Departure for the (provisional) MRL: BMD modeling was conducted on the Table A-10 and Table A-11 data after converting the presented standard errors to standard deviations. Software (BMDS, version 3.1.2) was utilized to determine the point of departure for the MRL using a benchmark response (BMR) of 1 standard deviation. The BMDS software makes a recommendation on viable model fit as part of its output. No suitable models were identified for reticulocyte counts or CFU-GM/femur. The constant variance model for reticulocytes indicated the data did not pass the assumptions for constant variance. Therefore, BMDS modeling was also conducted assuming non-constant variance for the reticulocyte data. This data also had no viable model fits.

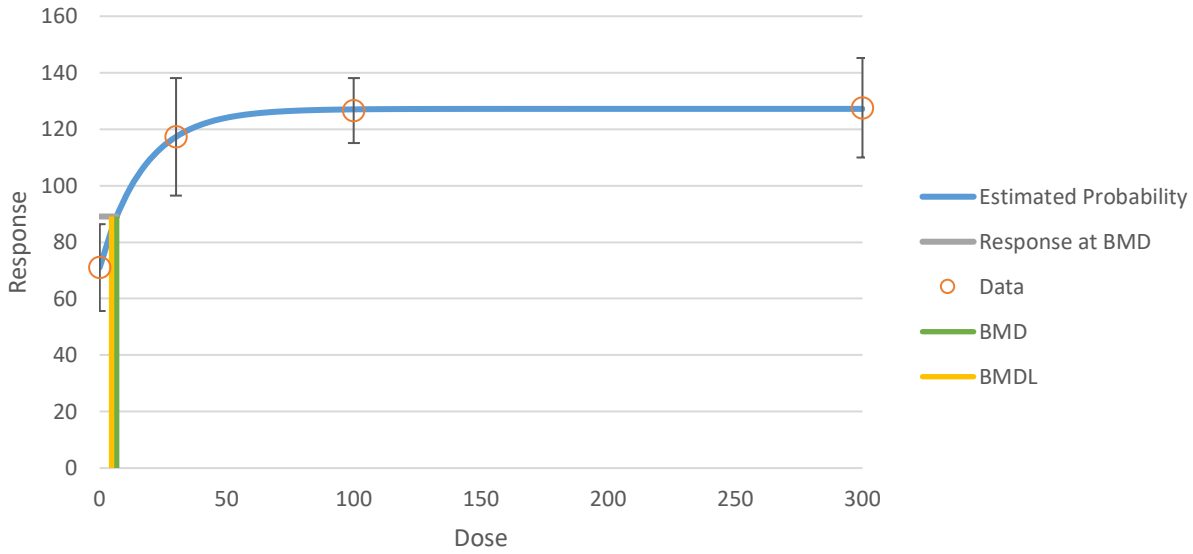
Both cells/femur and DNA synthesis fit the data best with an exponential 4 model. However, the DNA synthesis analysis had the lowest BMD_{1SD} of 6.7 mg/kg compared to 10.6 mg/kg for cells/femur. The data for DNA synthesis demonstrated a good fit of the data after visual examination. Therefore, its corresponding $BMDL_{1SD}$ of 4.7 mg/kg was selected as the POD for the MRL. The $BMDL_{1SD}$ is based on the restricted, frequentist exponential 4 model with constant variance. The output from the BMDS modeling for DNA synthesis is presented in Table A-12 and the fit of the data to the selected model is presented in Figure A-3.

Table A-12. Results from BMD Analysis for DNA Synthesis Status in Femur Bone Marrow of Female B6C3F1 Mice after 14-days of Oral Exposure to Nitrobenzene

Model	BMD	BMDL	BMDU	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group
Exponential 2 (CV - normal)	225.28	153.68	489.54	286.57	-0.64	-2.92
Exponential 3 (CV - normal)	225.27	153.68	489.54	286.57	-0.64	-2.92
Exponential 4 (CV - normal)	6.72	4.69	15.31	266.84	0.00	0.00
Exponential 5 (CV - normal)	19.85	4.69	28.88	268.84	0.00	0.00
Hill (CV - normal)	9.73	0.00	26.70	268.83	0.00	0.00
Polynomial Degree 3 (CV - normal)	197.96	127.02	446.18	285.87	1.78	-2.80
Polynomial Degree 2 (CV - normal)	197.97	127.02	446.19	285.87	1.78	-2.80
Power (CV - normal)	197.97	127.02	446.17	285.87	1.78	-2.80
Linear (CV - normal)	197.95	127.02	446.17	285.87	1.78	-2.80

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = 95% lower confidence limit on the BMD; BMDU = benchmark dose upper confidence level; CV = constant variance

Figure A-3. Frequentist Exponential Degree 4 Model with BMR of 1 Std. Dev. for the BMD and 0.95 Lower Confidence Limit for the BMDL for DNA Synthesis in B6C3F1 Mice with 14 days of Oral Exposure to Nitrobenzene (Burns et al. 1994)



Using this POD an MRL was derived with the following steps.

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

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

This results in the following MRL:

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

Agency Contacts (Chemical Manager): Malcolm Williams, DVM

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Nitrobenzene
CAS number(s): 98-95-3
Date: April 2022
Profile status: Draft for Public Comment
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: 17
Species: F344 Rats

MRL Summary: A provisional intermediate-duration (15-365 days) 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 for 90 days (NTP 1983a). The provisional 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 several single dose-group studies which evaluated the adverse effects of nitrobenzene on the male reproductive system for more than 14 but less than 365 days (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 on adverse effects to sperm including reduced count or concentration, decreased motility and decreased viability.

There are two additional oral intermediate duration toxicological studies which aimed to profile the toxicity of nitrobenzene, Mitsumori et al. (1994) and NTP (1983a). In Mitsumori et al. (1994), several effects occurred at 20 mg/kg/day in Sprague-Dawley rats (the lowest dose group tested). Specifically, at 20 mg/kg/day, decreased red blood cells (RBCs), hemoglobin and hematocrit were observed alongside increased methemoglobin levels in male rats. Further, there was an increase in the absolute and relative liver weights, centrilobular swelling of hepatocytes and extramedullary hematopoiesis. Brown pigmentation in Kupffer cells and in proximal tubules was also observed at 20 mg/kg/day as was decreased body weights in male pups at day 4 post-gestation.

NTP (1983a) dosed B6C3F1 mice and F344 rats of both sexes with doses ranging from 9.375 to 150 mg/kg for rats and 18.75 to 300 mg/kg for mice. Doses were administered via gavage with corn oil. NTP (1983a) observed effects in on the hematological, hepatic, renal, and respiratory systems. Specifically, in male and female F344 rats there were increases in methemoglobin levels and reticulocytes at 9.375 mg/kg. In both sexes these effects were accompanied by decreased levels of RBCs, hematocrit, and hemoglobin. At the 18.75 mg/kg dose level, male and female rats exhibited elevated methemoglobin, reticulocytes (total and absolute) and slight anisocytosis accompanied by decreased hematocrit and hemoglobin. B6C3F1 mice also displayed dose related increase in reticulocytes (beginning at 37.5 mg/kg in males and 18.7 mg/kg in females) and methemoglobin (at 18.75 mg/kg in males and females).

NTP (1983a) also observed, in male and female rats, liver weights that increased in a dose-dependent manner, being different from control with 9.375 mg/kg nitrobenzene, the lowest dose in the study. In

male mice a significant increase in liver weight occurred at 150 mg/kg whereas in females it occurred at all dose levels. Further, NTP observed congested livers with 150 mg/kg of nitrobenzene in rats. F344 rats also experiences a loss of cytoplasmic basophilia and a reduction of centrilobular hepatocytes at the same dose. In B6C3F1 mice, hepatocytes in the centrilobular zone were enlarged and nuclei had coarse or stippled chromatin and a large nucleolus with 300 mg/kg exposure (NTP 1983a). In addition to the increasing liver weights NTP (1983a) also observed consistent increases in kidney weight at all dose levels in F344 rats. Additionally, pale green hyaline globules were observed in rat cortical tubular cells in the 75mg/kg and 150mg/kg dose groups. In mice, kidney weights were increased at 75 and 300 mg/kg nitrobenzene. Further, lung weight ratios were significantly increased in the 18.75, 75 and 150 mg/kg dose groups in rats (NTP 1983a). Similar effects were not observed in mice, indicating likely species differences in the toxicity of orally administered nitrobenzene in the lungs.

NTP (1983a) also observed neurological effects of nitrobenzene exposure in male and female rats and mice which included ataxia, head tilt, trembling, moribundity, immobility, circling and lethargy. These effects were seen at 150 mg/kg and 300 mg/kg in rats and mice, respectively. In 7/20 rats (both male and female) in the 150 mg/kg exposure group hemorrhage and/or vacuolization were observed in the brain or brainstem. Other rats exposed to 150 mg/kg had various degenerative effects observed in in the pons, dentate nucleus, and cerebellar peduncle/olivary nucleus, which varied in severity and were characterized by spongy degeneration, necrosis, karyorrhexis, neutrophils, lymphocytes, plasma cells, and fusiform cells. The same effects were not seen in any dose group in mice, which were dosed up to 300 mg/kg. Indicating a species difference on the potential neurological effects from nitrobenzene exposure

In addition, NTP also observed reproductive effects on male rats and mice were, specifically atrophied testes. 1/10 rats displayed atrophy in the 37.5 mg/kg dose group and 9/10 displayed atrophy in the 75 mg/kg dose group. At least one mouse displayed atrophy in the testes in all dose groups with the exception of 75 mg/kg. At 150 mg/kg and 300 mg/kg 5/10 mice displayed atrophy in the testes. In addition, both male animal species experienced hypospermatogenseis, with the rats displaying a greater sensitivity to this effect compared to mice. Specifically, 10/10 rats experienced this effect at 75 mg/kg whereas no mice displayed hypospermatogensis at this dose and 4/10 mice displayed hypospermatogensis at 300 mg/kg. Values considered for MRL derivation are presented in Table A-13.

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					
B6C3F1 Mice	once/day for 90 days (GO)		300M	Decrease in right testes weight and its ratio with body weight	NTP 1983a
Sprague-Dawley Rat	Once/day for 54 days (GO)		60[a]	approximately 60% decrease in absolute and relative testes weight, approximately 20% decrease in absolute epididymis weight and approximately 18% decrease in relative epididymis weight, atrophy of seminiferous tubules, Leydig cell hyperplasia, loss of intraluminal sperm or cell debris in epididymis	Mitsumori et al. 1994
Hematological Effects					
F344 Rat	Once / day, 54 days (GO)		20	approximately 13% decrease in red blood cells; approximately 11% decrease in hemoglobin, methemoglobin levels rose from 0.7% to 3.64%, increase of hematopoiesis in bone marrow, increased absolute and relative spleen weight, increase in extramedullary hematopoiesis in spleen and brown pigmentation in the spleen.	Mitsumori et al. 1994
F344 Rat	once/day for 90 days (GO)		9.375	Increased methemoglobin (2.4-fold of controls) and absolute reticulocyte count (16% of controls and decreased hemoglobin (3% of controls)	NTP 1983a
B6C3F1 Mice	once/day for 90 days (GO)		18.75	Increase in methemoglobin in males 2-fold and in females 27% of controls	NTP 1983a
Hepatic Effects					
F344 Rat	once/day for 90 days (GO)		9.375	Liver weight, ratio of liver to body, and ratio of liver to brain weight significantly	NTP 1983a
B6C3F1 Mice	once/day for 90 days (GO)		18.75	8% increase in absolute liver weight	NTP 1983a
Renal Effects					
F344 Rat	once/day for 90 days (GO)		9.375	Ratio of kidney with final body weight were consistently increased and significant.	NTP 1983a

G = gavage; GD = gestation day; GO = gavage in oil vehicle; LOAEL = lowest observed adverse effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NTP = National Toxicology Program

Selection of the Principal Study and Point of Departure:

Both Mitsumori et al. (1994) and NTP (1983a) are strong studies demonstrating an array of toxicological effects of nitrobenzene exposure. However, NTP (1983a) provides data with lower doses in multiple species allowing for an understanding of the lower dose effects of nitrobenzene and it is therefore selected as the Principal Study. In this study the most sensitive effects are on the hematologic, renal and hepatic systems. The main effect seen at the lowest dose levels with the renal and hepatic system are increases in organ weight, which demonstrates an effect on these organs but the toxicological significance of this effect on humans is not as clear. In rats given 9.375 mg/kg doses of nitrobenzene a significant increase in methemoglobin levels were observed along with decreased hemoglobin (16.24 g/dl in control to 15.73 g/dl in 9.375 mg/kg dose group), decreased MCV and HCH in the males and decreased hematocrit in the females. Slight polychromasia was also observed in male and female rats.

The hematological data for both males and females appears to follow a dose-response trend. Additionally, methemoglobinemia is a well-recognized adverse effect associated with nitrobenzene exposure. Therefore, increases in methemoglobin were selected as the critical effect.

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: EG&G Mason Research Institute.

NTP (1983a) is a 90-day intermediate duration study that evaluating the toxicological profile of nitrobenzene in B6C3F1 mice and F344 rats. Specifically, in this study doses ranged from 9.375 to 150 mg/kg for rats and 18.75 to 300 mg/kg for mice. Doses were administered with a single dose per day via gavage with corn oil (NTP 1983a). At dose initiation male and female rats and mice were approximately 7 weeks old. 10 male and 10 female rats and mice were in each dose group and were housed in temperature-controlled cages with water available ad libitum. All animals in the study were observed for clinical effects twice a day, seven days a week. After 90-days of treatment gross anatomical and histopathological observations were made. As described above, the main effects observed in this study were on the hematological, renal, hepatic, respiratory and reproductive systems. In regard to the hematologic effects both male and female mice and rats displayed increased methemoglobin levels. Given that the rats were given lower doses of nitrobenzene we have summarize the data from the study in Table A-14.

Table A-14. Percent of Methemoglobin Observed in NTP (1983a) for F344 Rats

		Male F344 Rats	Female F344 Rats
Dose (mg/kg/day)	N	Mean (±SD)	Mean (±SD)
0	10	1.131 (0.579)	0.941 (0.031)
9.375	10	2.752 (0.583)	2.059 (0.449)
18.75	10	4.22 (1.45)	3.623 (1.088)
37.5	10	5.624 (0.85)	5.269 (0.757)
75	10	7.307 (1.438)	6.851 (2.25)
150	1M/7F	12.220 (0.000)	12.771 (1.825)

F = female(s); M = male(s); SD = standard deviation

Selection of the Point of Departure for the (provisional) MRL: BMD modeling was conducted on the methemoglobin data for both sexes of rat. Individual data were provided in the NTP report and were subsequently used. The data were fit to all continuous models in EPA's Benchmark Dose Software (BMDS, version 3.1.2) using a benchmark response (BMR) of 1 standard deviation. The BMDS software makes a recommendation on viable model fit as part of the output. The highest dose group was dropped for males as a result of a high mortality rate. No suitable models were identified for the female rats. The initial runs for male rats using a constant variance did not provide any suitable model fits. However, running the modeling using a nonconstant variance (NCV) did provide suitable model fits and tests on the assumption of NCV confirmed the assumption of NCV. Specifically, Test 2 in the BMDS output, which tests the null hypothesis that variances are homogenous was significant (P =0.012), indicating that non-constant variance is appropriate. Further, Test 3, which tests the null hypothesis that the variances are adequately modeled (A3 vs A2). Had a p-value > 0.01 inferring that the variances have been modeled appropriately.

The results of the modeling are presented in Table A-15. In accordance with the above guidance, we selected the model with the lowest AIC for the male rat data, this was the Exponential 4- NCV model. The exposure-response curve for this model is displayed in Figure A-4. The $BMDL_{1SD}$ from this model is 1.8 mg/kg/day.

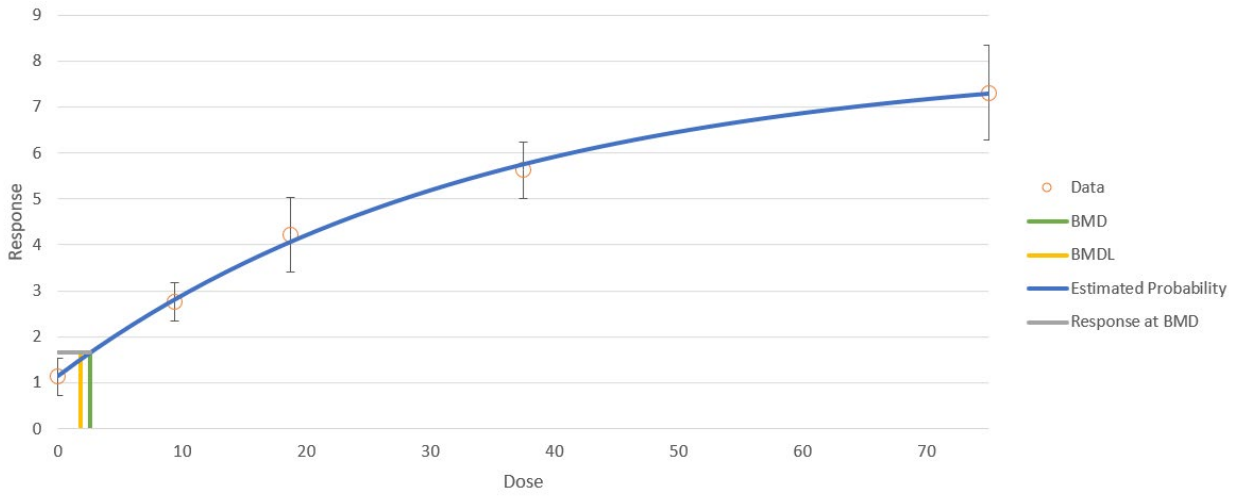
Table A-15. Model Predictions for Increased Methemoglobin in Male F344 Rats Exposed to Nitrobenzene for BMR of 1SD Using Individual Data, Non-Constant Variance Assumed

Model	BMD_{1SD} (ppm)	$BMDL_{1SD}$ (ppm)	P Value[a]	AIC	Scaled residuals[b] Dose below BMD	Scaled residuals[b] Dose above BMD
Exponential 2	26.64	20.32	<0.0001	177.02	2.04	-3.66
Exponential 3	26.64	20.32	<0.0001	177.02	2.04	-3.66
Exponential 4[c]	**2.62**	**1.81**	**0.65**	**136.42**	**-0.07**	**-0.07**
Exponential 5	2.59	1.81	0.65	136.42	-0.03	-0.03
Hill	3.03	1.65	0.42	138.21	-0.08	-0.08
Polynomial Degree 3	7.97	4.70	<0.0001	157.95	0.84	-2.33
Polynomial Degree 2	7.97	4.70	<0.0001	157.95	0.84	-2.33
Power	7.97	4.70	<0.0001	157.95	0.84	-2.33
Linear	7.97	4.70	<0.0001	157.95	0.84	-2.33

[a]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also, the largest residual at any dose.
[c]Selected model. The exponential 4, exponential 5 and Hill models provided adequate fit to the data. The Exponential 4 was selected as it had the lowest AIC.

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the dose associated with the selected benchmark response); $BMDL_{1SD}$ = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., SD = dose associated with one standard deviation extra risk); DF = degree of freedom

Figure A-4. Fit of Exponential 4 Model to Individual Data on Increased Methemoglobin in Male F344 Rats Exposed to Nitrobenzene



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

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

This results in the following MRL:

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

Agency Contacts (Chemical Manager): Malcolm Williams, PhD

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Nitrobenzene
CAS number(s): 98-95-3
Date: April 2022
Profile status: Draft for Public Comment
Route: Oral
Duration: Chronic

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

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

Agency Contacts (Chemical Manager): Malcolm Williams, DVM, PhD

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
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models

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

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

The current literature search was intended to update the existing toxicological profile for nitrobenzene (ATSDR 1990); thus, the literature search was restricted to studies published between 1988 and 2019. The following main databases were searched in May 2019 and June 2019:

- PubMed
- Medline
- Toxline
- Science Direct

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.

Table B-2. Database Query Strings

Database search date	Query string	Number of Hits
Medline (May 21, 2019)	((MH ("Nitrobenzenes") OR RN (98-95-3) OR AB ("C.I. solvent black 6" OR "Essense of Mirbane" OR "Mirbane oil" OR Mononitrobenzene OR "Mononitrol benzol" OR "NCI-C60082" OR "Nigrosine spirit soluble B" OR "Nitrobenzeen" OR "Nitrobenzen" OR Nitrobenzol OR "Oil of mirbane" OR "Oil of myrbane" OR "Solvent Black 6" OR "Pesticide Code: 056501")) NOT AB (dinitrobenzene OR chloronitrobenzene OR trinitrobenzene OR chloramphenicol OR florfenicol OR minnows OR zebra OR conjunctivitis)) Limiters: Date of Publication: 19880101-20191231 Publication Type: Clinical Trial, Clinical Trial, Phase I, Clinical Trial, Phase II, Clinical Trial, Phase III, Clinical Trial, Phase IV, Commentary, Comparative Study, Controlled Clinical Trial, Corrected and Republished Article, Duplicate Publication, Evaluation Studies, Guideline, Historical Article, Introductory Journal Article, Journal Article, Meta-Analysis, Multicenter Study, Practice Guideline, Published Erratum, Randomized Controlled Trial, Report, Research, Scientific Integrity Review, Systematic Review, Academic dissertation, Adaptive clinical trial, Case reports, Clinical conference, Clinical study, Clinical trial protocol, Clinical trial, veterinary, Comment, Consensus Development conference, Consensus development, NIH, Equivalence trial, Expression of concern, All Formulary types, Observational study, Pharmacopoeia, Pharmacopoeia,homoepathic, Pragmatic clinical trial, Preprint, Retraction of publication, Statistics, Unpublished work	2,739
Medline (June 28, 2019)	AB (Nitrobenzene*) Limiters: Date of Publication: 19880101-20191231 Publication Type: Clinical Trial, Clinical Trial, Phase I, Clinical Trial, Phase II, Clinical Trial, Phase III, Clinical Trial, Phase IV, Commentary, Comparative Study, Controlled Clinical Trial, Corrected and Republished Article, Duplicate Publication, Evaluation Studies, Guideline, Historical Article, Introductory Journal Article, Journal Article, Meta-Analysis, Multicenter Study, Practice Guideline, Published Erratum, Randomized Controlled Trial, Report, Research, Scientific Integrity Review, Systematic Review, Academic dissertation, Adaptive clinical trial, Case reports, Clinical conference, Clinical study, Clinical trial protocol, Clinical trial, veterinary, Comment, Consensus Development conference, Consensus development, NIH, Equivalence trial, Expression of concern, All Formulary types, Observational study, Pharmacopoeia,	1,777

Table B-2. Database Query Strings

	Pharmacopoeia,homoepathic, Pragmatic clinical trial, Preprint, Retraction of publication, Statistics, Unpublished work	
PubMed (May 21, 2019)	((("nitrobenzene"[Supplementary Concept] OR "nitrobenzenes"[MeSH Terms]) OR 98-95-3[EC/RN Number]) OR ("C.I. solvent black 6"[Title/Abstract] OR "Essense of Mirbane"[Title/Abstract] OR "Mirbane oil"[Title/Abstract] OR Mononitrobenzene[Title/Abstract] OR "Mononitrol benzol"[Title/Abstract] OR "NCI-C60082"[Title/Abstract] OR "Nigrosine spirit soluble B"[Title/Abstract] OR "Nitrobenzeen "[Title/Abstract] OR "Nitrobenzen "[Title/Abstract] OR Nitrobenzol[Title/Abstract] OR "Oil of mirbane"[Title/Abstract] OR "Oil of myrbane"[Title/Abstract] OR "Solvent Black 6"[Title/Abstract] OR "Pesticide Code: 056501"[Title/Abstract])) NOT (dinitrobenzene[Title/Abstract] OR chloronitrobenzene[Title/Abstract] OR trinitrobenzene[Title/Abstract] OR chloramphenicol[Title/Abstract] OR florfenicol[Title/Abstract] OR minnows[Title/Abstract] OR zebra[Title/Abstract] OR conjunctivitis[Title/Abstract]) AND (("1988/01/01"[PDAT] : "2019/12/31"[PDAT]) AND English[lang])	2,426
PubMed (June 28, 2019)	nitrobenzene*[Title/Abstract] AND (("1988/01/01"[PDAT] : "2019/12/31""[PDAT]) AND English[lang])	2,481
Toxline (May 21, 2019)	(((nitrobenzene OR "C.I. solvent black 6" OR "Essense of Mirbane" OR "Mirbane oil" OR Mononitrobenzene OR "Mononitrol benzol" OR "NCI-C60082" OR "Nigrosine spirit soluble B" OR "Nitrobenzeen" OR "Nitrobenzen" OR Nitrobenzol OR "Oil of mirbane" OR "Oil of myrbane" OR "Solvent Black 6" OR " Pesticide Code: 056501" OR (98-95-3 [rn])) NOT (dinitrobenzene OR chloronitrobenzene OR trinitrobenzene OR chloramphenicol OR florfenicol OR minnows OR zebra OR conjunctivitis)) AND 1988:2019 [yr] AND NOT PubMed [org] AND NOT pubdart [org]	616
Science Direct (May 21, 2019)	(Nitrobenzene OR 98-95-3 OR Mononitrobenzene OR "Mirbane Oil" OR "essence of mirbane") NOT (dinitrobenzene OR chloronitrobenzene OR trinitrobenzene OR chloramphenicol OR florfenicol OR minnows OR zebra OR conjunctivitis) Limiters: Year: 1988-2019 Article Type: Research Articles, Data Articles, Discussion, Practice Guidelines, case reports	4,802
Total		14, 841

We also searched ChemView and the Office of Pesticide Programs Chemical Search database on May 25th, 2019. One substantial risk report was identified through ChemView. However, the document linked in ChemView only contained cover pages for CIIT's 2-year cancer bioassay on nitrobenzene, a document we identified upon reviewing the 2009 IRIS report for nitrobenzene. Therefore, no additional articles were included from these databases.
To confirm the comprehensiveness of our literature database, we evaluated the references published from 1988 to present cited in seven government sources:[4]

- IARC Monograph (Volume 65)
- 2009 EPA IRIS Assessment for Nitrobenzene
- 2014 NIOSH Skin Notation Profile for Nitrobenzene
- NTP Report on Carcinogens (14th Edition)
- 2018 NIOSH Pocket Guide on Nitrobenzene
- IPCS' 2003 Environmental Health Criteria Document for Nitrobenzene
- 2009 WHO Report on Nitrobenzene in Drinking Water

By searching these documents 12 additional publications were located. The reason these documents were not captured in the initial literature review was because these were either published prior to 1988 and were not included in the last version of the profile, did not include "nitrobenzene" or its synonyms in the title or abstract, so they were not identified in the database searches or were unpublished reports. The articles located through QA were automatically included in full text screen.

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: 11,296
- Number of studies considered relevant and moved to the next step: 139

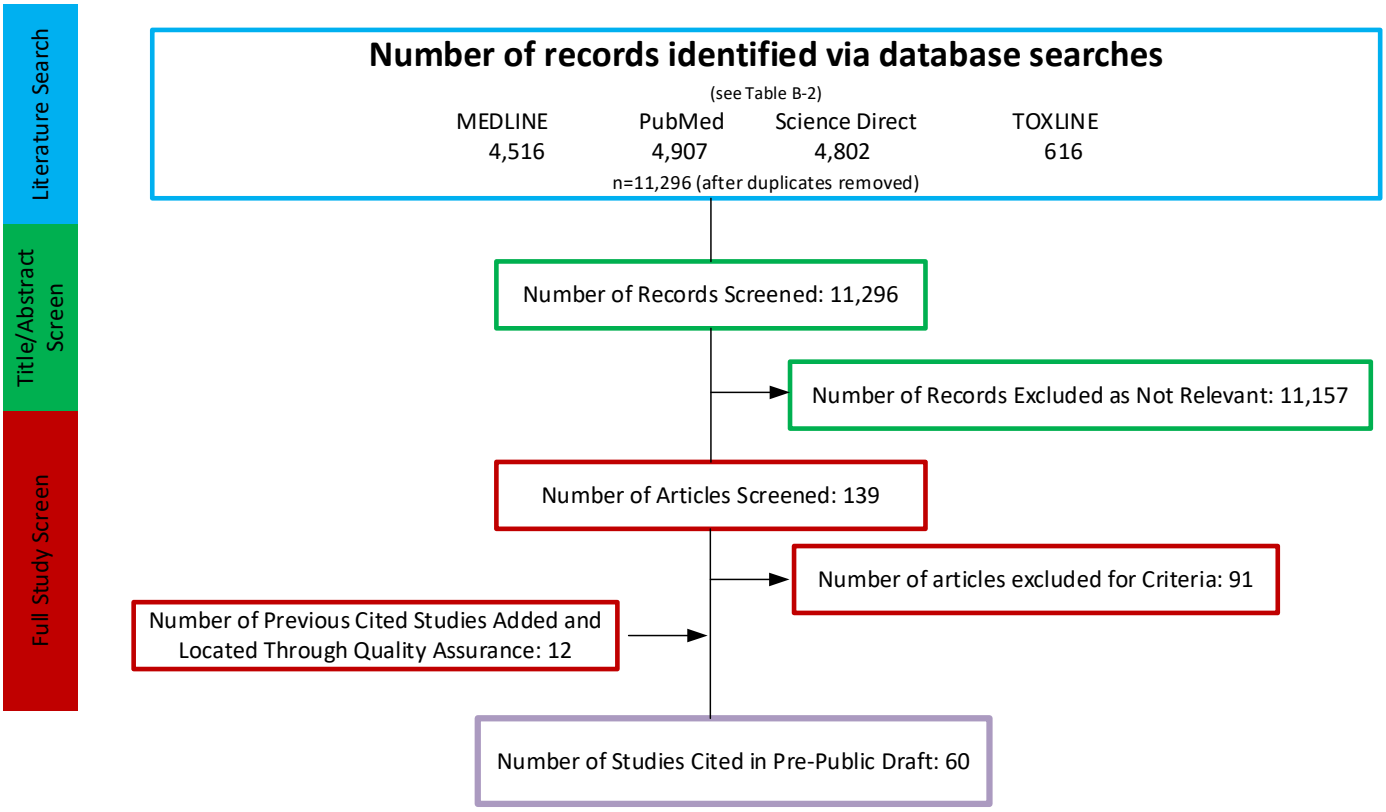
Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 139

[4] ATSDR also searched for relevant documents on the websites of Minnesota Department of Health, the Minnesota Pollution Control Agency, New Jersey Department of Environment, California Environmental Protection Agency, Washington Department of Ecology, and the Maine Department of Environmental Protection. No comprehensive documents useful for QA were identified by searching these sites.

- Number of studies cited in the pre-public draft of the toxicological profile related to health effects: 60

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



APPENDIX C. 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:

Physician Briefs discuss health effects and approaches to patient management in a brief/factsheet style. *Physician Overviews* are narrated PowerPoint presentations with Continuing Education credit available (see https://www.atsdr.cdc.gov/emes/health_professionals/index.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 D. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page 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.

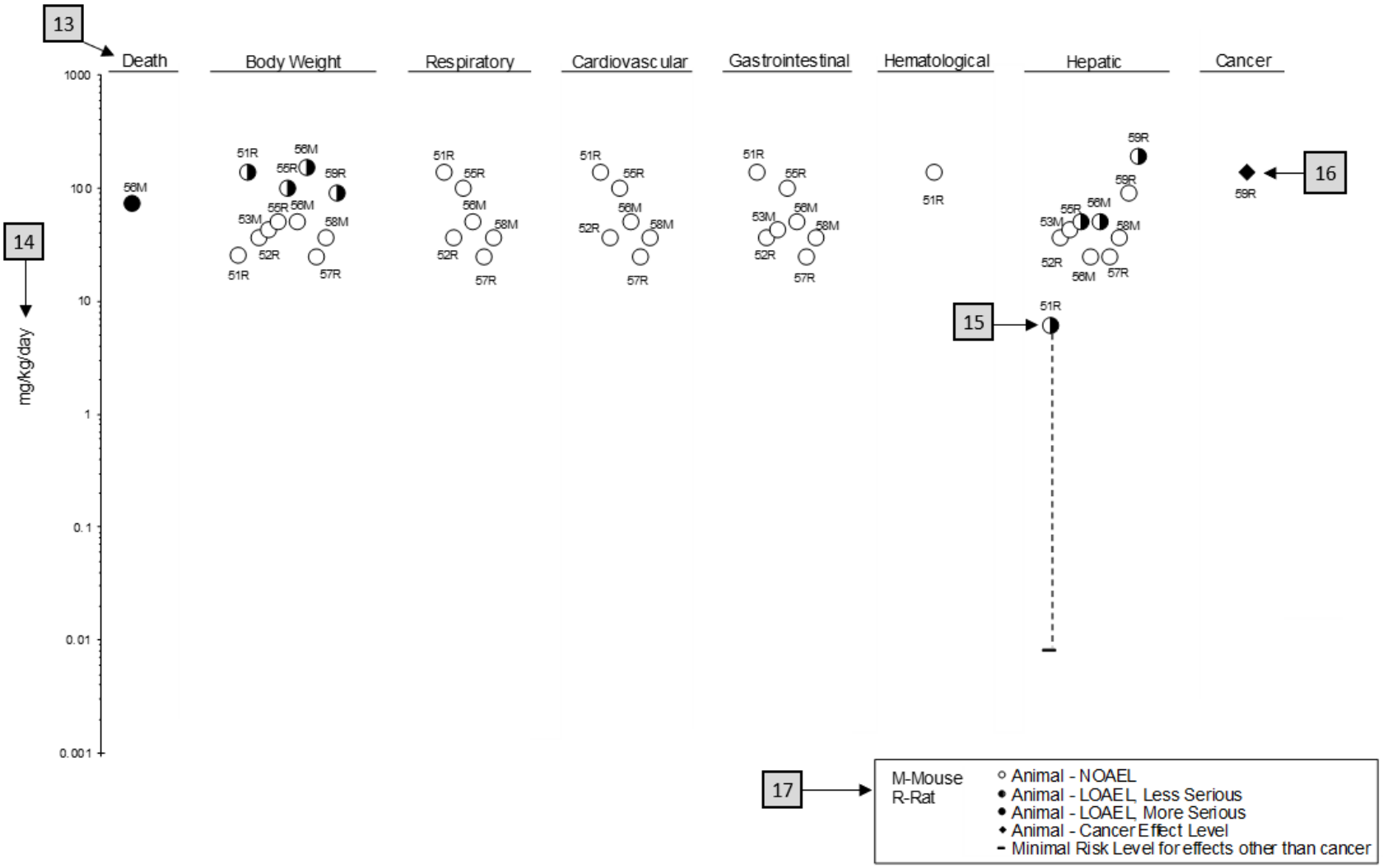
(17) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

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).

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



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 (Koc)—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 BMD10 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/m3 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
BMDX	dose that produces a X% change in response rate of an adverse effect
BMDLX	95% lower confidence limit on the BMDX
BMDS	Benchmark Dose Software
BMR	benchmark response
BSP	bromosulphthalein
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
CREST	Calcinosis, Raynaud's phenomenon, Esophageal dysfunction, Sclerodactyly, and Telangiectasias
CWA	Clean Water Act
DHHS	Department of Health and Human Services
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_i	logarithmic sorption coefficient
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	lactic 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 level/limit
REL-C	recommended exposure level-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
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
q1*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result