CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

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

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

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

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

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

1.2 SUMMARY OF HEALTH EFFECTS

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

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

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

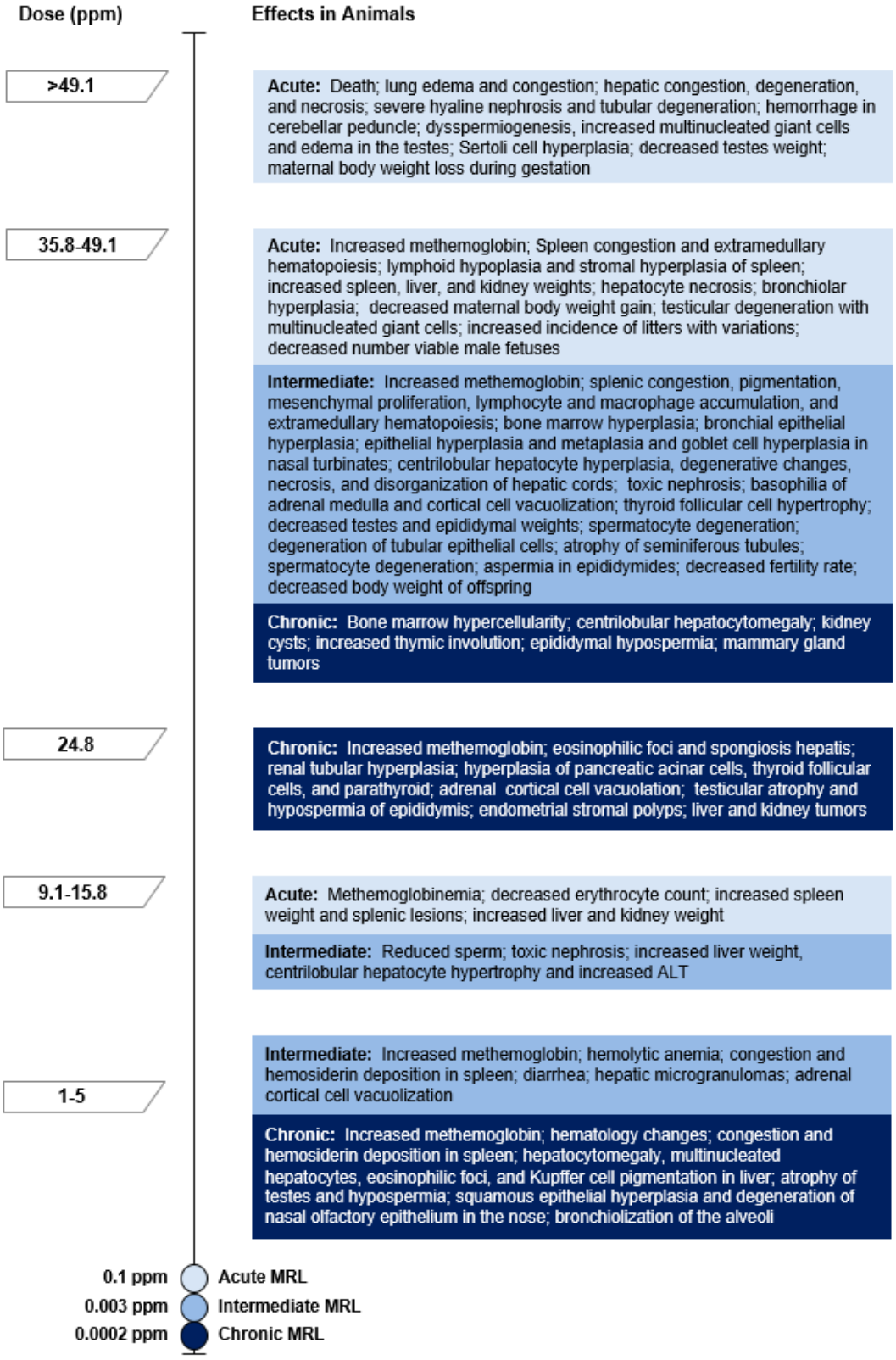
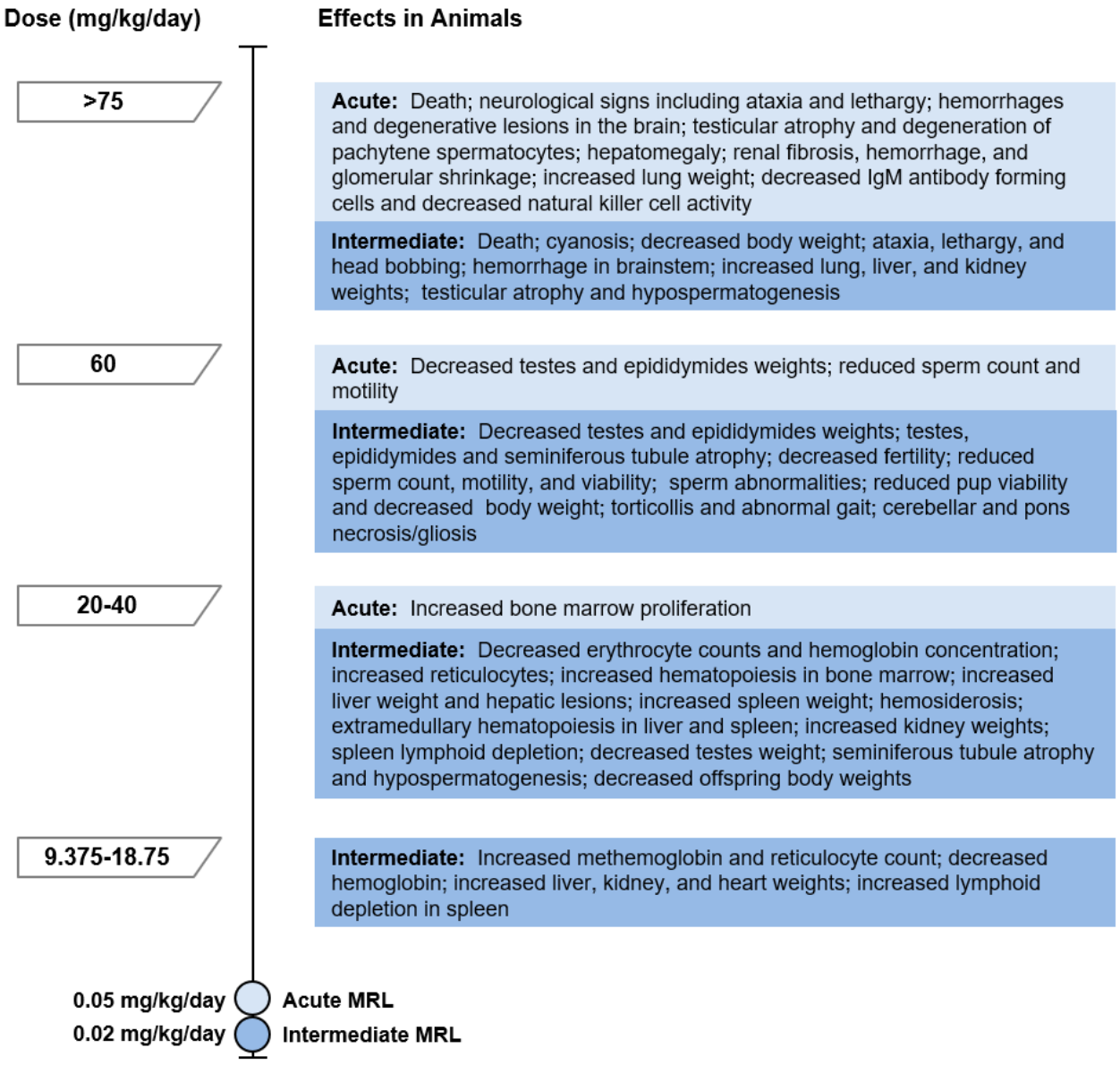


Figure 1-2. Health Effects Found in Animals Following Oral Exposure to Nitrobenzene



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

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

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

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

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

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

1.3 MINIMAL RISK LEVELS (MRLs)

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

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

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

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

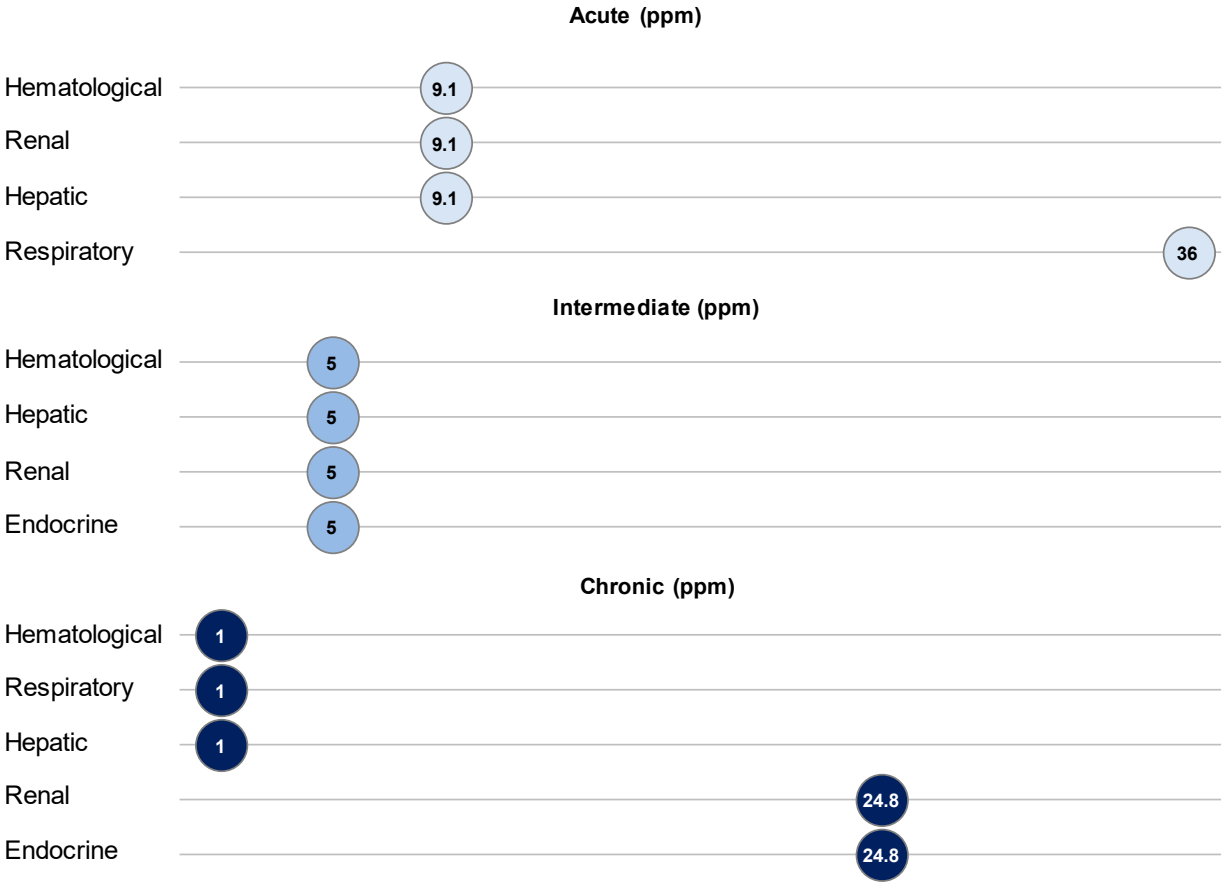


Figure 1-4. Summary of Sensitive Targets of Nitrobenzene – Oral

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

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

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

[a]See Appendix A for additional information.

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