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.

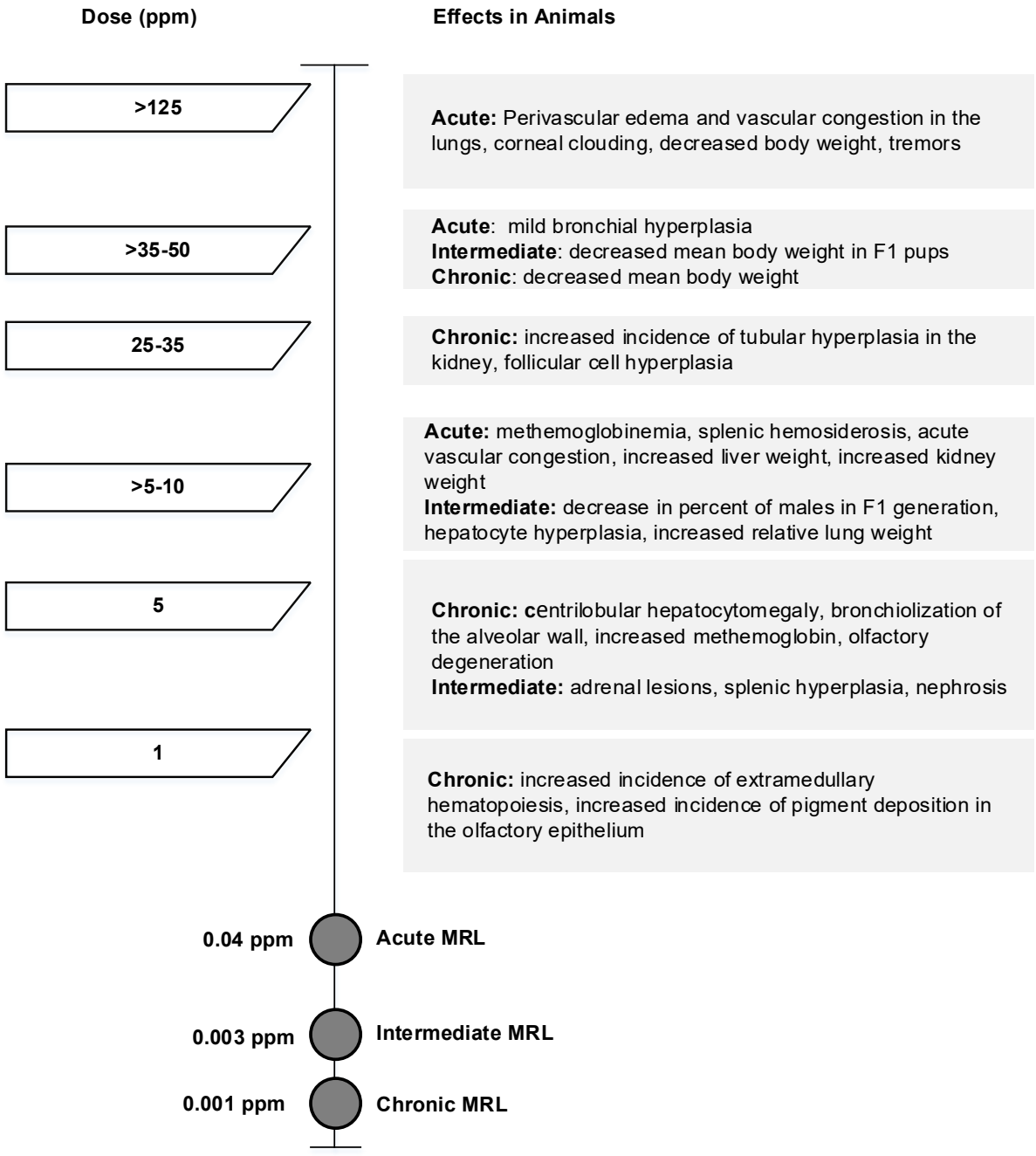
Figure 1-2 demonstrates that the hematological system is also a sensitive target after oral exposure as are the hepatic and renal systems. Figure 1-1 and Figure 1-2 demonstrate that the minimal risk levels (MRLs are established below any doses at which effects have been demonstrated.

- 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).

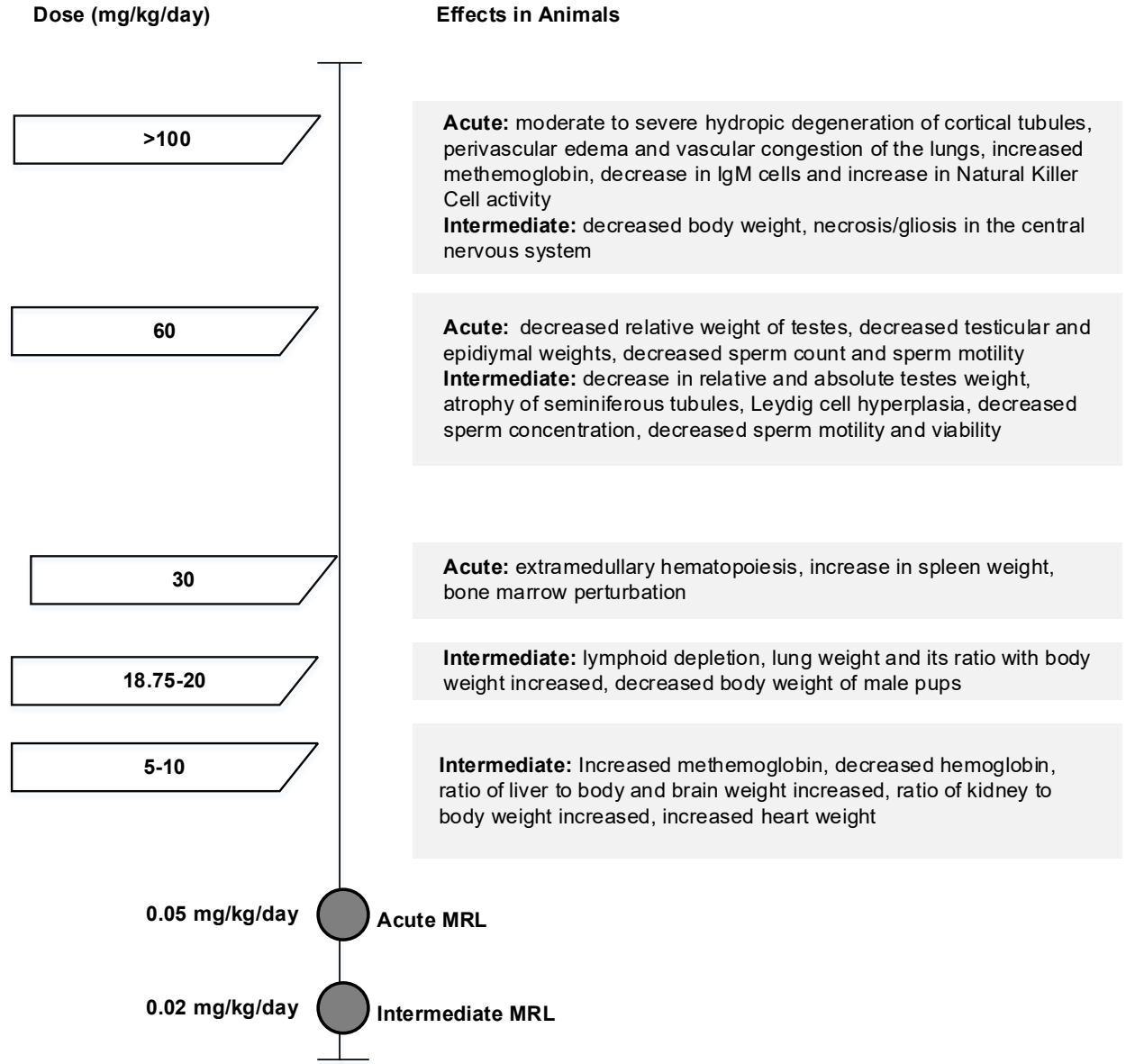
- Renal: The kidney also appears to be a target for nitrobenzene toxicity. However, the evidence for this in humans is limited. Specifically, in one case study, renal tubular necrosis was seen following the death of the subject who ingested nitrobenzene (Gupta et al. 2012). Several experimental animal 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.

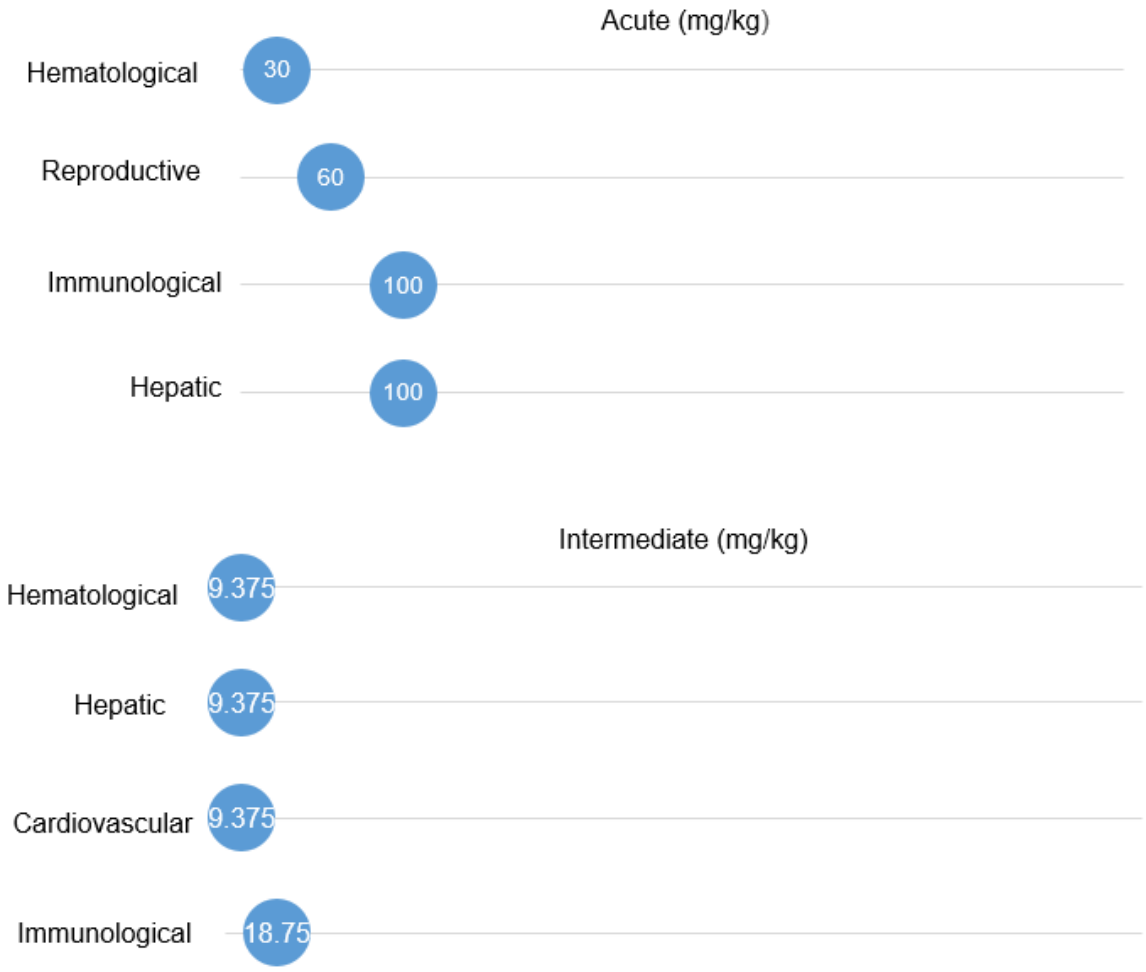


Table 1-1. Provisional Minimal Risk Levels for Nitrobenzene

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