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 cyanide. 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 relevant to specific target organs are discussed along with the health effects data; general mechanisms of action for cyanide (relevant to the entire organism) are discussed in Section 2.21. 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 cyanide, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with oral exposure to cyanide was also conducted; the results of this review are presented in Appendix C.

Human and 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, and human and animal dermal data 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. Effects have been classified into “less serious LOAELs” or “serious LOAELs (SLOAELs).” “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.

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.

This section provides information regarding known health effects of cyanide exposure. Exposure to hydrogen cyanide gas is most common by inhalation. In the discussion below, inhalation exposures are expressed as ppm hydrogen cyanide for a defined period of time. Exposure to cyanide can also occur by inhalation of cyanogen gas, a dimer of cyanide. However, cyanogen breaks down in aqueous solution into cyanide ion (CN^{-1}) and OCN^- ions (Cotton and Wilkinson 1988). The rate of the breakdown depends on pH and is faster in basic media (e.g., hydrogen cyanide is in equilibrium as H^+ and CN^- in blood with a pH of 7.38–7.44) than in acidic media (e.g., hydrogen cyanide is the only species in stomach contents at a pH of 3). The amount of cyanide ion formed within a body tissue or fluid as a result of exposure to cyanogen has been reported; however, the amount varies with type of body tissue and fluid. Thus, it is difficult to estimate cyanide levels in body tissues after cyanogen exposure. Therefore, studies regarding exposure to cyanogen are discussed in the text as ppm cyanogen but are not included in LSE tables or figures.

Oral exposure to cyanide usually results from accidental, homicidal, or suicidal ingestion of cyanide salts. Sodium cyanide and potassium cyanide are the most frequently studied cyanide compounds. Copper cyanide, potassium silver cyanide, silver cyanide, and calcium cyanide are other compounds that humans could encounter through oral or dermal exposure; however, health effects data for cyanide compounds containing copper or silver are omitted from the LSE tables and figures because the toxicological effects

may have been caused by the metal, rather than, or in addition to, CN^-. Toxicological data for ferricyanide compounds are omitted from Chapter 2 because CN^- remains tightly bound to iron and is therefore much less bioavailable than CN^- in soluble cyanide compounds. Cassava roots and certain fruit pits contain natural cyanogenic glycosides (e.g., amygdalin) that can be broken down in the gastrointestinal tract to form cyanide (Lasch and El Shawa 1981; Mlingi et al. 1992, 1993; O'Brien et al. 1992; Swain et al. 1992; Voldrich and Kyzlink 1992). Cassava roots form the staple diet of some populations in Africa, Central and South America, and Asia (WHO 2004). However, it must be noted that cassava roots are notoriously deficient in protein and other nutrients and contain many other compounds, in addition to cyanide, that could be responsible for some of the observed toxic effects (Obidoa and Obasi 1991; WHO 2004). Mycotoxin contamination has also been documented in stored cassava and the most common cassava product in Africa, garri (Olorunnado et al. 2024). Therefore, while discussed in relevant sections of Chapter 2 (based on target organs evaluated) to aid in hazard identification, animal studies administering cassava and other natural cyanogenic glycosides are not useful for dose-response assessment; thus, they are omitted from the LSE tables and figures. Additionally, while discussed in relevant sections of Chapter 2 for hazard identification purposes, studies in dogs are omitted from the LSE tables and figures because they are not considered appropriate animal models for dose-response extrapolation to humans for cyanide toxicity. As discussed in Section 3.1.6, dogs have increased susceptibility to cyanide compared to other mammalian species due to known pharmacokinetic differences. When possible, all oral exposures are expressed as mg CN^-/kg/day throughout the profile.

The health effects of cyanide compounds have been evaluated in 58 human and 87 animal studies. As illustrated in Figure 2-1, most of the health effects data come from inhalation and oral exposure studies in animals and oral studies in humans. For the purposes of Figure 2-1, all occupational human studies were classified as inhalation studies, despite potential for multi-route exposure (e.g., dermal via direct vapor exposure).

For animal data, oral studies are available for all health effect and exposure duration categories and evaluate a complete set of endpoints, although chronic-duration data are limited. Animal inhalation studies include acute- and intermediate-duration studies only and evaluate a limited number of endpoints. The dermal animal database is limited to acute-duration studies evaluating a limited number of endpoints. The most examined endpoints in animal studies were neurological effects, lethality, and hepatic effects. The available human studies include some epidemiological data (including occupational studies and evaluations of populations with high dietary intake of naturally occurring cyanogenic compounds), but

available data are predominantly from case studies and case-series reports. Human studies that are included were predominantly focused on neurological and respiratory effects. As discussed in Section 2.2 and Appendix C, the extensive number of case studies reporting lethal cases of cyanide poisoning are not included in this profile; rather, included studies are lethal case reports focused on estimating lethal exposure levels following inhalation, oral, and dermal routes, relying on reviews when available. In a comprehensive database including all case reports and case-series studies, death is likely the most well-studied endpoint in the human database for cyanide.

As outlined in Chapter 1, the thyroid, neurological system, and male reproductive system appear to be sensitive targets of toxicity following oral exposure to cyanide. A systematic review was conducted on the available human and animal studies for these endpoints following oral exposure. The information in these oral studies indicate the following on the potential targets of cyanide toxicity:

- **Thyroid Endpoints.** Thyroid effects are a presumed health effect associated with cyanide exposure via oral exposure based on a low level of evidence in humans, a moderate level of evidence in animals, and supporting mechanistic data. Reduced serum thyroid hormone levels, increasingly elevated levels of thyroid stimulating hormone, and goiter have been reported in humans with high dietary intake of cassava containing natural sources of cyanide. In animals, adverse thyroid effects (altered serum hormones, enlarged thyroid) have been reported in rats and rabbits following intermediate-duration oral exposure to cyanide compounds. At lower doses, evidence of induction of potential homeostatic mechanisms for thyroid function (dose-related increases in the number of resorption vacuoles in the thyroid gland) in the absence of clear evidence of altered thyroid function has also been observed. Thyroid effects following cyanide exposure can result from the interference of thiocyanate, a metabolite of cyanide, with iodine uptake and utilization in the thyroid gland.

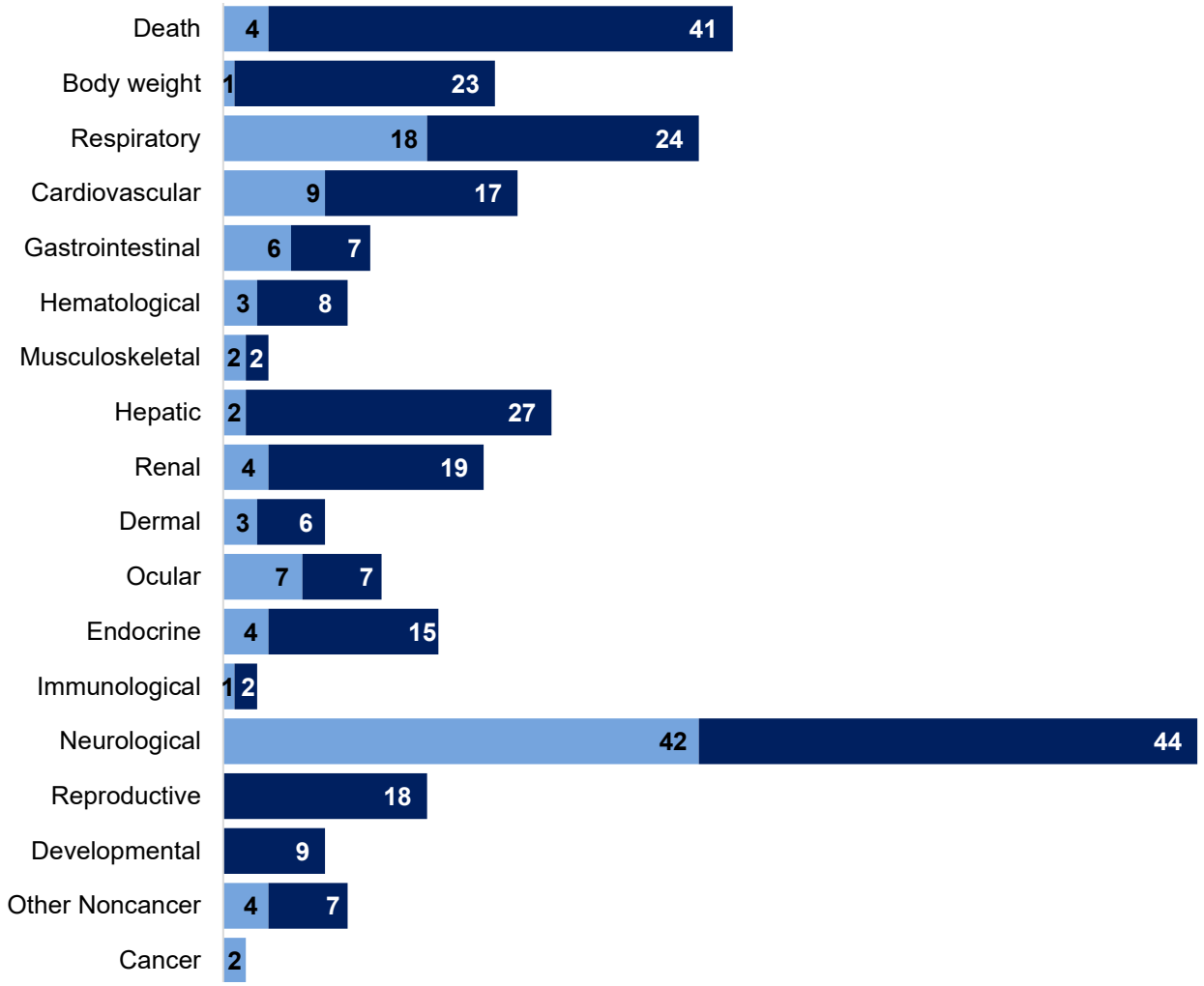
- **Neurological Endpoints.** Neurological effects are a known health effect for humans exposed to cyanide based on a high level of evidence in humans and animals. Regional outbreaks of neurological disease have occurred in African communities reliant on a diet rich in cassava as a carbohydrate source. Numerous case studies provide strong evidence that the CNS is a primary target of acute cyanide poisoning, with permanent and progressive neurological dysfunction occurring after single, high-dose exposures. In animal studies, neurobehavioral changes have been reported at low gavage doses, with overt and severe clinical signs of neurotoxicity prior to death at lethal doses. Damage to the tissues of the CNS have been observed in animal studies following acute- and intermediate-duration exposure to cyanide compounds. The CNS is a primary target of cyanide toxicity via the general mechanism of toxicity (impaired cellular oxygen utilization), which can lead to rapid biochemical changes in the brain.

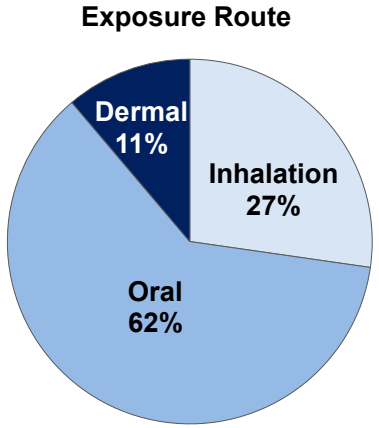
- **Male Reproductive Endpoints.** Male reproductive effects are a suspected health effect associated with cyanide exposure via oral exposure based on no human data and a moderate level of evidence in animals. Data from studies utilizing the most relevant route of exposure (drinking water) are conflicting. In the first study by NTP (1993), adverse effects were reported in male rats (decreases in the caudal epididymal weight, epididymis weight, testis weight, spermatid heads, and spermatid counts) and mice (decreases in the epididymal and caudal epididymal weights); however, a replicate rat study using the same protocol by Tyner and Greeley (2023)

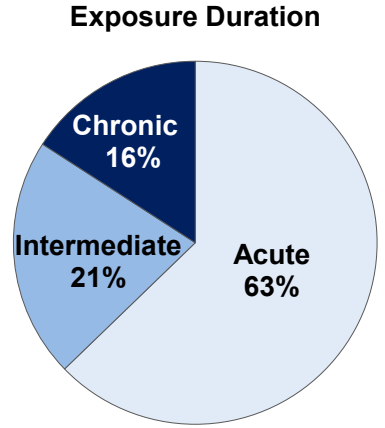
could not reproduce the male reproductive findings. Tyner and Greeley (2023) proposed that male reproductive effects noted in the NTP (1993) study may have been attributable to decreased water consumption in the highest dose group rather than due to direct toxic effects. To control for this, Tyner and Greeley (2023) included a water-restricted control group to match measured water consumption at the highest dose level. No adverse male reproductive effects were observed in exposed rats in the study by Tyner and Greeley (2023), compared to either the water-restricted or the *ad libitum* control group. While drinking water studies are considered more relevant to human exposure, several intermediate-duration gavage studies indicate that bolus administration of cyanide compounds (which may overwhelm detoxification mechanisms) can cause mild adverse effects on the male reproductive system (serum hormone changes, sperm effects, and mild histopathological effects). No studies evaluating male fertility were identified, but NTP (1993) concluded that observed effects in their study were unlikely to impair fertility in rodents.

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

Most studies examined the potential neurological, lethal, and respiratory effects of cyanide
Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 145 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Ballantyne 1983a									**HCN**
1	Rat (NS) 6–10 NS	60 minutes	Not reported	LE	Death			143	60-minute LC_{50}
Fechter et al. 2002									**HCN**
2	Rat (Long-Evans) 6–16 M	3.5 hours	0, 10, 30, 50	HP, NX	Neuro	50			
Higgins et al. 1972									**HCN**
3	Rat (Wistar) 10 NS	5 minutes	283, 657, 368, 497, 583, 690	CS, LE	Death			503	5-minute LC_{50}
Higgins et al. 1972									**HCN**
4	Mouse (ICR) 15 NS	5 minutes	200, 283, 357, 368, 414, 427	CS, LE	Death			323	5-minute LC_{50}
Hume et al. 1995									**HCN**
5	Mouse (ICR) 10 M	3 minutes	400	LE	Death			400	90% lethality
Ma et al. 2021									**HCN**
6	Mouse (CD-1) 16–24 M	40 minutes (N)	0, 327	LE, CS, NX	Death			327	8/24 died
					Resp			327	Gasping, labored breathing
					Neuro			327	Lethargy, loss of righting reflex, convulsions, tremors

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Matijak-Schaper and Alarie 1982									**HCN**
7	Mouse (Swiss-Webster) 4 M	30 minutes	15, 25, 35, 65, 70, 100, 150, 220, 330, 500, 760, 1,000, 1,150	CS, LE	Death			166	30-minute LC_{50}
					Resp			63	Calculated DC_{50} (50% decrease in respiratory rate)
Ballantyne 1983a									**HCN**
8	Rabbit (NS) 6–10 NS	35 minutes	Not reported	LE	Death			188	35-minute LC_{50}
INTERMEDIATE EXPOSURE									
O'Flaherty and Thomas 1982									**HCN**
9	Rat (Long-Evans) 4 M	20 days 4-day intervals between exposures (5 total exposures) 12.5 minutes/day	0, 200	CS, BC, HP	Cardio		200		Increased creatine phosphokinase activity

[a]The number corresponds to entries in 2-2; differences in levels of health effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

BC = serum (blood) chemistry; Cardio = cardiovascular; CS = clinical signs; DC_{50} = concentration associated with 50% depression in respiratory rate; HCN = hydrogen cyanide; HP = histopathology; LC_{50} = concentration associated with 50% lethality; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect-level; NS = not specified; NX = neurological function; Resp = respiratory

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

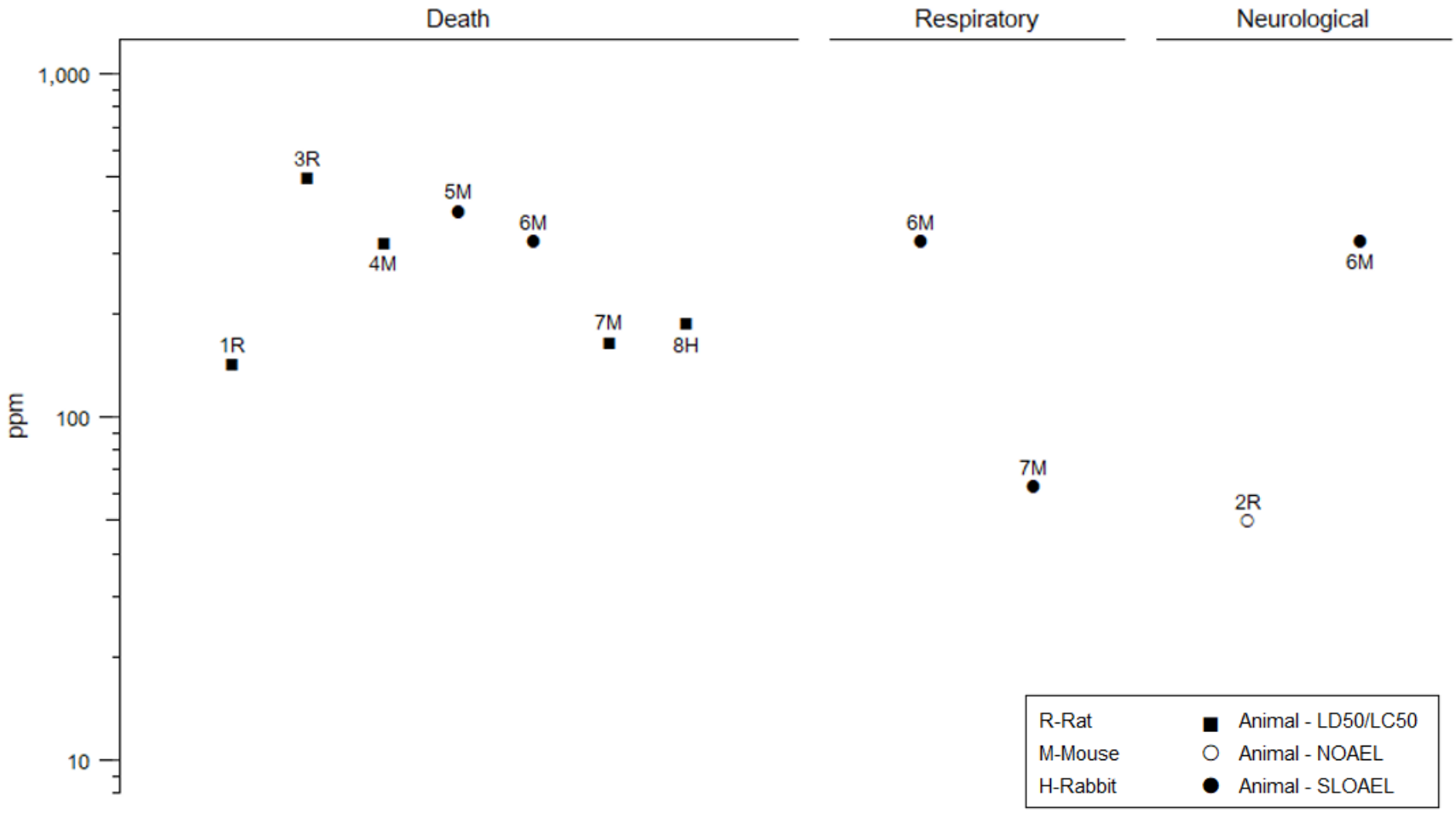


Figure 2-2. Levels of Significant Exposure to Hydrogen Cyanide – Inhalation
Intermediate (15–364 days)

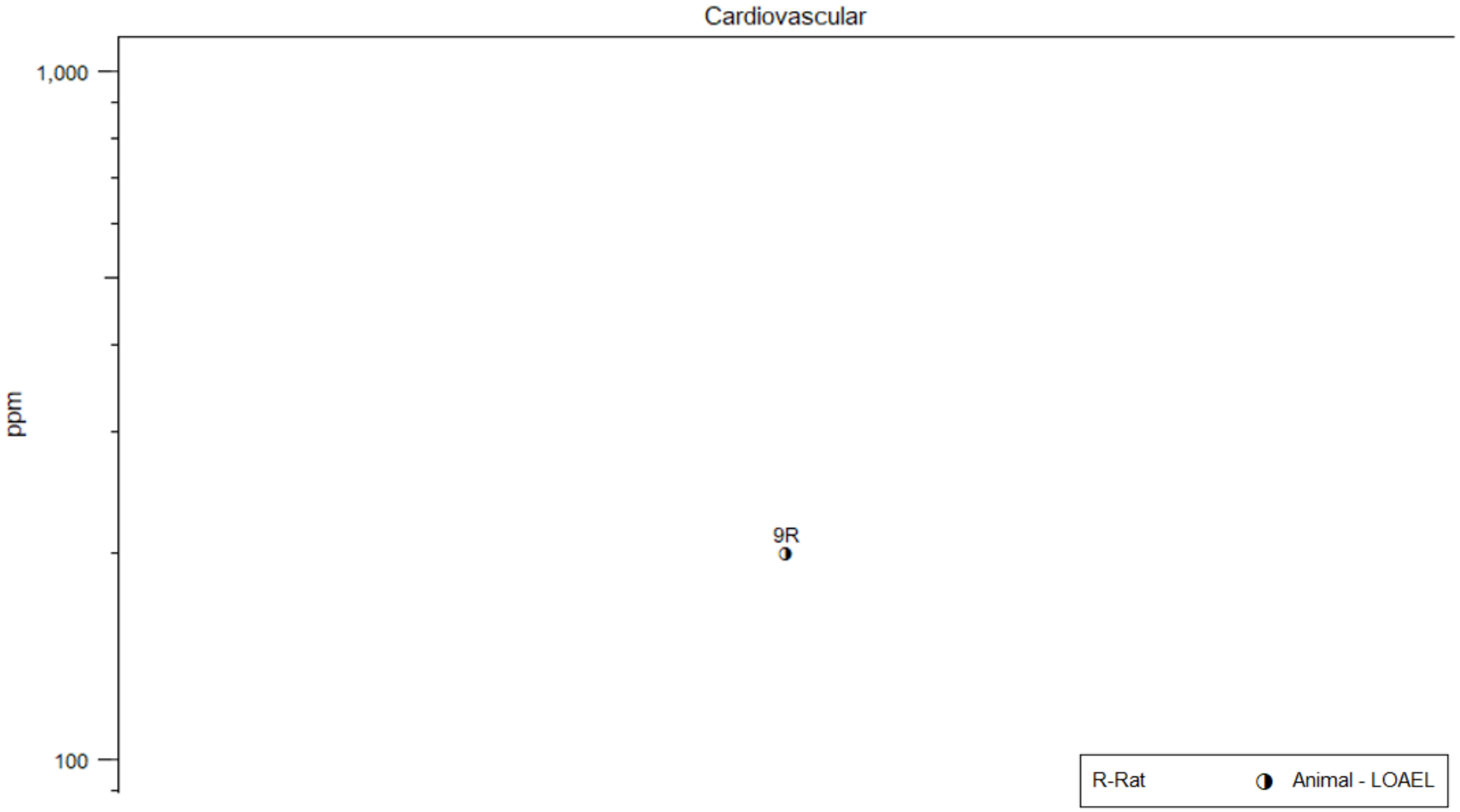


Table 2-2. Levels of Significant Exposure to Cyanide – Oral (mg CN^-/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Ballantyne 1988									**NaCN**
1	Rat (Porton) 10 F	Once (GW)	2.39–3.34	CS, LE	Death			3	LD_{50} in unfasted rats
Ballantyne 1988									**NaCN**
2	Rat (Porton) 10 F	Once (GW)	2.09–4.19	CS, LE	Death			2.7	LD_{50} in fasted rats
de Sousa et al. 2007									**KCN**
3	Rat (Wistar) 10 F	14 days GDs 6–20 (W)	0, 0.4, 1.2, 12	LE, CS, BW, BC, DX, HP	Bd wt	12			
					Resp	12			
					Hepatic		1.2		Mild to moderate hepatocyte vacuolation and congestion
					Renal	12			
					Endocr		12		Moderate pancreas islet cell vacuolation
					Immuno	12			
					Neuro			12	Hemorrhagic areas in the brain and gliosis; mild-to-moderate necrosis, neuronophagia, and CNS congestion
					Develop	12			
					Other noncancer	1.2	12		Elevated serum glucose

Dams were sacrificed on GD 20; NOAELs for histological findings could not be determined

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
de Sousa et al. 2007									**KCN**
4	Rat (Wistar) 10 F	14 days GDs 6–20 (W)	0, 0.4, 1.2, 12	LE, CS, BW, BC, DX, HP	Hepatic		1.2		Mild to moderate hepatic congestion and vacuolation
					Neuro			12	Hemorrhagic areas in the brain and gliosis; mild-to-moderate necrosis, neuronophagia, and CNS congestion
					Develop			12	Effects in PND 22 pups: CNS gliosis, mild-to-moderate neuronophagia, and congestion; mild-to-moderate hepatic congestion and vacuolation; mild bile duct proliferation
Dams and pups sacrificed on PND 22; NOAELs for histological findings could not be determined									
de Sousa et al. 2007									**KSCN**
5	Rat (Wistar) 10 F	14 days GDs 6–20 (W)	0, 0.2, 0.6, 6.4	LE, CS, BW, BC, RX, DX, HP	Bd wt	6.4			
					Resp	6.4			
					Hepatic		6.4		Mild vacuolation of hepatocytes and bile duct proliferation
					Renal	6.4			
					Immuno	6.4			
					Neuro			0.6	Brain gliosis
					Develop	6.4			
					Other noncancer	6.4			
Dams were sacrificed on GD 20; NOAELs for histological findings could not be determined									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
de Sousa et al. 2007									**KSCN**
6	Rat (Wistar) 10 F	14 days GDs 6–20 (W)	0, 0.2, 0.6, 6.4	LE, CS, BW, BC, DX, HP	Hepatic		6.4		Mild vacuolation of hepatocytes and bile duct proliferation
					Neuro			0.6	Brain gliosis
					Develop			6.4	Effects in PND 22 pups: CNS gliosis, mild-to-moderate neuronophagia, and congestion; mild-to-moderate hepatic congestion and vacuolation; mild bile duct proliferation
Dams and pups sacrificed on PND 22; NOAELs for histological findings could not be determined									
Ferguson 1962									**KCN**
7	Rat (Sprague-Dawley) 20 NS	Once (GW)	4	CS, LE	Death			4	19/20 died
Ogundele et al. 2014a									**KCN**
8	Rat (Wistar) 6–12 M	10 days (G)	0, 12	OF	Cardio		12		Arterial wall degeneration (reduction in cell number) and decreased lumen width in middle cerebral artery; increased diameter of common carotid artery
Ogundele et al. 2014b									**KCN**
9	Rat (Wistar) 6–12 M	10 days (G)	0, 12	NX	Neuro			12	Decreased locomotor activity, impaired memory (spatial, object recognition)
Rice et al. 2018									**NaCN**
10	Rat (Sprague-Dawley) 2–7 M	Once (IN)	2, 4, 8.5, 17, 34, 43, 51, 68	LE, CS, BW, FI	Death			34	LD_{50}
					Neuro	4	8.5	17	LOAEL: Lethargy SLOAEL: Convulsions

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Smyth et al. 1969									**Ca(CN)2**
11	Rat (NS) NS	Once (GW)	Not reported	CS, LE	Death			22	LD_{50}
Smyth et al. 1969									**NaCN**
12	Rat (NS) NS	Once (GW)	Not reported	CS, LE	Death			8	LD_{50}
Ferguson 1962									**KCN**
13	Mouse (Swiss-Webster) 20 NS	Once (GW)	6	CS, LE	Death			6	19/20 died
Hawk et al. 2016									**KCN**
14	Mouse (CD-1) 70 M, 70 F	Once (NS)	0, 3.2	LE, BW, OW, HP, NX	Bd wt	3.2			
					Cardio	3.2			
					Hepatic	3.2			
					Renal	3.2			
					Endocr	3.2			
					Neuro			3.2	Findings at 0.5 hours post-exposure: Reduced motor activity and altered gait, decreased sensorimotor reflexes, tremor, and clinical signs of CNS depression
					Repro	3.2 M			
Juvenile rats (10/sex/timepoint) were assessed prior to exposure and 0.5 and 24 hours and 6, 13, 27, and 41 days post-exposure									
Hawk et al. 2016									**KCN**
15	Mouse (CD-1) 70 M, 70 F	Once (NS)	0, 3.2	LE, BW, OW, HP, NX, OF	Bd wt	3.2			
					Cardio		3.2		Decreased systolic, diastolic, and mean arterial blood pressure; reduced heart rate
					Hepatic	3.2			
					Renal	3.2			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	3.2			
					Neuro			3.2	Findings at 0.5 hours post-exposure: Reduced motor activity and altered gait, decreased sensorimotor reflexes, tremor, clinical signs of CNS depression
					Repro	3.2 M			
Adult rats (10/sex/timepoint) were assessed prior to exposure and 0.5 and 24 hours and 6, 13, 27, and 41 days post-exposure									
Ishaku et al. 2018									**KCN**
16	Mouse (Swiss) 7 M	14 days (NS)	0, 0.6	LE, CS, NX	Neuro		0.6		Decreased motor strength and activity
Sabourin et al. 2016									**KCN**
17	Mouse (CD-1) 18 M, 18 F	Once (GW)	0, 2.4, 3.2, 4.16	BW, BC, OW, HP	Bd wt	4.16			
					Cardio	4.16			
					Hepatic	4.16			
					Renal	4.16			
					Endocr	4.16			
					Neuro	4.16			
					Repro	4.16 M			
Juvenile mice were assessed at various timepoints post-exposure for serum biomarkers (8/sex/timepoint) and histopathology (4/sex/timepoint)									
Sabourin et al. 2016									**KCN**
18	Mouse (CD-1) 6–18 M, 6–18 F	Once (GW)	0, 0.8, 1.2, 1.6, 2.4, 3.2, 4.0, 4.4, 4.8, 5.2, 5.6	LE, CS	Death			4.40 F	LD_{50} in adult female mice
								4.75 M	LD_{50} in adult male mice
					Resp	2.4		3.2	Labored and difficult breathing
					Neuro	1.2	1.6	3.2	LOAEL: Decreased activity SLOAEL: Convulsions and tremors

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Sabourin et al. 2016									**KCN**
19	Mouse (CD-1) 6–18 M, 6–18 F	Once (GW)	0, 0.8, 1.6, 2.4, 3.2, 4.0, 4.4, 4.8, 5.2, 5.6	LE, CS	Death			4.0 F	LD_{50} in juvenile female mice
								4.36 M	LD_{50} in juvenile male mice
					Resp	2.4		3.2	Labored and difficult breathing
					Neuro	0.8	1.6	3.2	LOAEL: Decreased activity SLOAEL: Convulsions and tremors
Sabourin et al. 2016									**KCN**
20	Mouse (CD-1) 18 M, 18 F	Once (GW)	0, 2.4, 3.2, 4.60	BW, BC, OW, HP	Bd wt	4.6			
					Cardio	4.6			
					Hepatic	4.6			
					Renal	4.6 F	4.6 M		Minimal-to-mild acute tubular necrosis
					Endocr	4.6			
					Neuro	4.6			
					Repro	4.6 M			
Adult mice were assessed at various timepoints post-exposure for serum biomarkers (8/sex/timepoint) and histopathology (4/sex/timepoint)									
Ballantyne 1983a									**HCN**
21	Rabbit (NS) 6–10 F	Once (GW)	Not reported	LE	Death			2.39	LD_{50}
Ballantyne 1983a									**KCN**
22	Rabbit (NS) 6–10 F	Once (GW)	Not reported	LE	Death			2.34	LD_{50}
Ballantyne 1983a									**NaCN**
23	Rabbit (NS) 6–10 F	Once (GW)	Not reported	LE	Death			2.7	LD_{50}
Ballantyne 1988									**NaCN**
24	Rabbit (New Zealand) 10 F	Once (G)	2.12–3.37	CS, LE	Death			2.7	LD_{50}

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
INTERMEDIATE EXPOSURE									
Mathangi et al. 2011									**KCN**
25	Rat (Wistar) 10 NS	90 days (GW)	0, 0.56	BW, NX, OW, HP	Bd wt	0.56			
					Renal	0.56			
					Neuro		0.56		Decreased motor coordination; increased dopamine in corpus striatum and cerebral cortex
NTP 1993									**NaCN**
26	Rat (Fischer-344) 10 M, 10 F	13 weeks (W)	M: 0, 0.2, 0.5, 1.4, 4.5, 12.5; F: 0, 0.2, 0.5, 1.7, 4.9, 12.5	LE, BW, OW, GN, HP, BC, WI, UR	Bd wt	12.5			
					Resp	12.5			
					Cardio	12.5			
					Gastro	12.5			
					Hemato	12.5			
					Hepatic	12.5			
					Renal	12.5			
					Dermal	12.5			
					Endocr	12.5			
					Immuno	12.5			
					Neuro	12.5			
					Repro	12.5 F			
						4.5 M	12.5 M		Decreased absolute left testes, epididymal, and caudal epididymal weights; decreased number of spermatid heads per testis and total spermatid count
Oyewopo et al. 2021a									**NaCN**
27	Rat (Wistar) 8 M	56 days (G)	0, 0.5, 1	BW, BC, OW	Bd wt			0.5	25% decrease in body weight gain
					Repro		0.5		Decreased serum testosterone, FSH, and LH; increased serum prolactin

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Oyewopo et al. 2021a									**NaCN**
28	Rat (Wistar) 8 M	30 days (NS)	0, 0.5, 1	BW, BC, OW	Bd wt		0.5		15% decrease in body weight gain
					Repro		0.5		Decreased serum testosterone, FSH, and LH; increased serum prolactin
Oyewopo et al. 2021b									**NaCN**
29	Rat (Wistar) 8 M	30 or 56 days (G)	0, 0.5, 1	BW, HP, RX	Repro		0.5		Decreased total sperm count, percent motility, and percent normal sperm; morphological changes in testes (decreased diameter of seminiferous tubules, decreased epithelial cell height; increased Leydig cell area, and decreased nuclear volume of Sertoli cells)
Philbrick et al. 1979									**KCN**
30	Rat (NS) 10 M	11.5 months (F)	0, 53	LE, CS, BW, BC, OW, HP	Bd wt			53	32% decreased weight gain
					Endocr		53		Decreased plasma T4 at 4 months; decreased T4 secretion rate at 4 and 11 months
					Neuro			53	Modest myelin degeneration in spinal cord
Philbrick et al. 1979									**KSCN**
31	Rat (NS) 10 M	11.5 months (F)	0, 47	LE, CS, BW, BC, OW, HP	Bd wt	47			
					Endocr		47		Decreased plasma T4 at 4 and 11 months; decreased T4 secretion rate at 4 months
					Neuro			47	Modest myelin degeneration in spinal cord

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Shivanoor and David 2015									**NaCN**
32	Rat (Wistar) NS M	90 days (GW)	0, 0.34, 0.64, 1.70	LE, BW, RX, HP	Bd wt	1.7			
					Repro	0.34	0.64		Decreased sperm count and motility; decreased serum testosterone and LH; decreased prostate and testes weights; mild atrophy and degeneration of seminiferous tubules; mild vacuolation in the epididymis
Soto-Blanco et al. 2002									**KCN**
33	Rat (Wistar) 6–7 M	3 months 1 time/day (GW)	0, 0.02, 0.12, 0.24	BW, BC, HP	Bd wt	0.24			
					Endocr	0.24			
Tyner and Greeley 2023									**NaCN**
34	Rat (Fischer-344) 20 M	13 weeks (W)	0, 0.12, 0.43, 1.28, 3.96, 11.50	LE, BW, BC, HE, UR, OW, OP, GN, HP, RX	Bd wt	11.5			
					Hemato	11.5			
					Hepatic	11.5			
					Renal	11.5			
					Ocular	11.5			
					Endocr	3.96[b]	11.5		Increased absolute and relative thyroid weights; reduced serum T4
					Repro	11.5			
Ishaku et al. 2018									**KCN**
35	Mouse (Swiss) 7 M	28 days (NS)	0, 0.6	LE, CS, NX	Neuro		0.6		Decreased motor strength and activity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1993									**NaCN**
36	Mouse (B6C3F1) 10 M, 10 F	13 weeks (W)	M: 0, 0.3, 1.0, 2.7, 8.6, 24.3, F: 0, 0.3, 1.1, 3.3, 10.1, 28.8	LE, BW, OW, GN, HP, BC, WI	Bd wt	28.8 F			
						24.3 M			
					Resp	28.8 F			
						24.3 M			
					Cardio	28.8 F			
						24.3 M			
					Gastro	28.8 F			
						24.3 M			
					Hemato	28.8 F			
						24.3 M			
					Hepatic	28.8 F			
						24.3 M			
					Renal	28.8 F			
						24.3 M			
					Dermal	28.8 F			
						24.3 M			
					Endocr	28.8 F			
						24.3 M			
					Immuno	28.8 F			
						24.3 M			
					Neuro	28.8 F			
						24.3 M			
					Repro	28.8 F			
						8.6 M	24.3 M		Decreased epididymal weights

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Avais et al. 2014									**KCN**
37	Rabbit (NS) 6 M	40 days (G)	0, 1.2	BW, FI, HE	Bd wt			1.2	39% decrease in body weight gain
					Hemato		1.2		Decreased RBC count, hemoglobin levels, PCV, and MCV; increased MCHC
Avais et al. 2018									**KCN**
38	Rabbit (NS) 6 M	40 days (G)	0, 1.2	BW, FI, BC, GN	Hepatic		1.2		Increased serum bilirubin, ALT, AST, ALP, and LDH; decreased total serum albumin and protein
					Renal		1.2		Increased serum creatinine, urea, uric acid; decreased albumin and total protein
					Endocr		1.2		Increased serum levels of T3 and T4
Okolie and Iroanya 2003									**NaCN**
39	Rabbit (New Zealand) 6 NS	4 weeks (F)	0, 15	BW, BC, BI, FI	Bd wt			15	22% decrease in body weight gain
					Hepatic		15		Increased serum ALT, ALP, and LDH
Okolie and Osagie 1999, 2000									**KCN**
40	Rabbit (New Zealand) 6 M	40 weeks (F)	0, 20	BC, BW, BI, FI	Bd wt			20	33% decrease in body weight gain
					Cardio	20			
					Hepatic		20		Increased serum SDH, ALT, ALP, and LDH
					Renal		20		Increased serum creatinine and urea
					Endocr	20			
					Other noncancer	20			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ozolua et al. 2007									**KCN**
41	Rabbit NS 4–7 NS	25 days (G)	0, 0.15	LE, BW, HE, BC	Bd wt	0.15			
					Cardio	0.15			
					Hemato	0.15			
					Hepatic	0.15			

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.04 mg CN^-/kg/day; dose was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical changes; Ca(CN)2 = calcium cyanide; Cardio = cardiovascular; CN = cyanide; CNS = central nervous system; CS = clinical signs; DX = developmental toxicity; Endocr = endocrine; F = female(s); (F) = feed; FI = food intake; FSH = follicle-stimulating hormone; (G) = gavage, not specified; Gastro = gastrointestinal; GD = gestational day; GN = gross necropsy; (GW) = gavage, water; HCN = hydrogen cyanide; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; (IN) = ingestion; KCN = potassium cyanide; KSCN = potassium thiocyanate; LD_{50} = dose associated with 50% lethality; LDH = lactate dehydrogenase; LE = lethality; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCHC = Mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MRL = Minimal Risk Level; NaCN = sodium cyanide; Neuro = neurological; NOAEL = no-observed-adverse-effect-level; NS = not specified; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; PND = postnatal day; PCV = packed cell volume; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive function; SDH = sorbitol dehydrogenase; SLOAEL = serious LOAEL; T3 = triiodothyronine; T4 = thyroxine; UR = urinalysis; (W) = water; WI = water intake

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

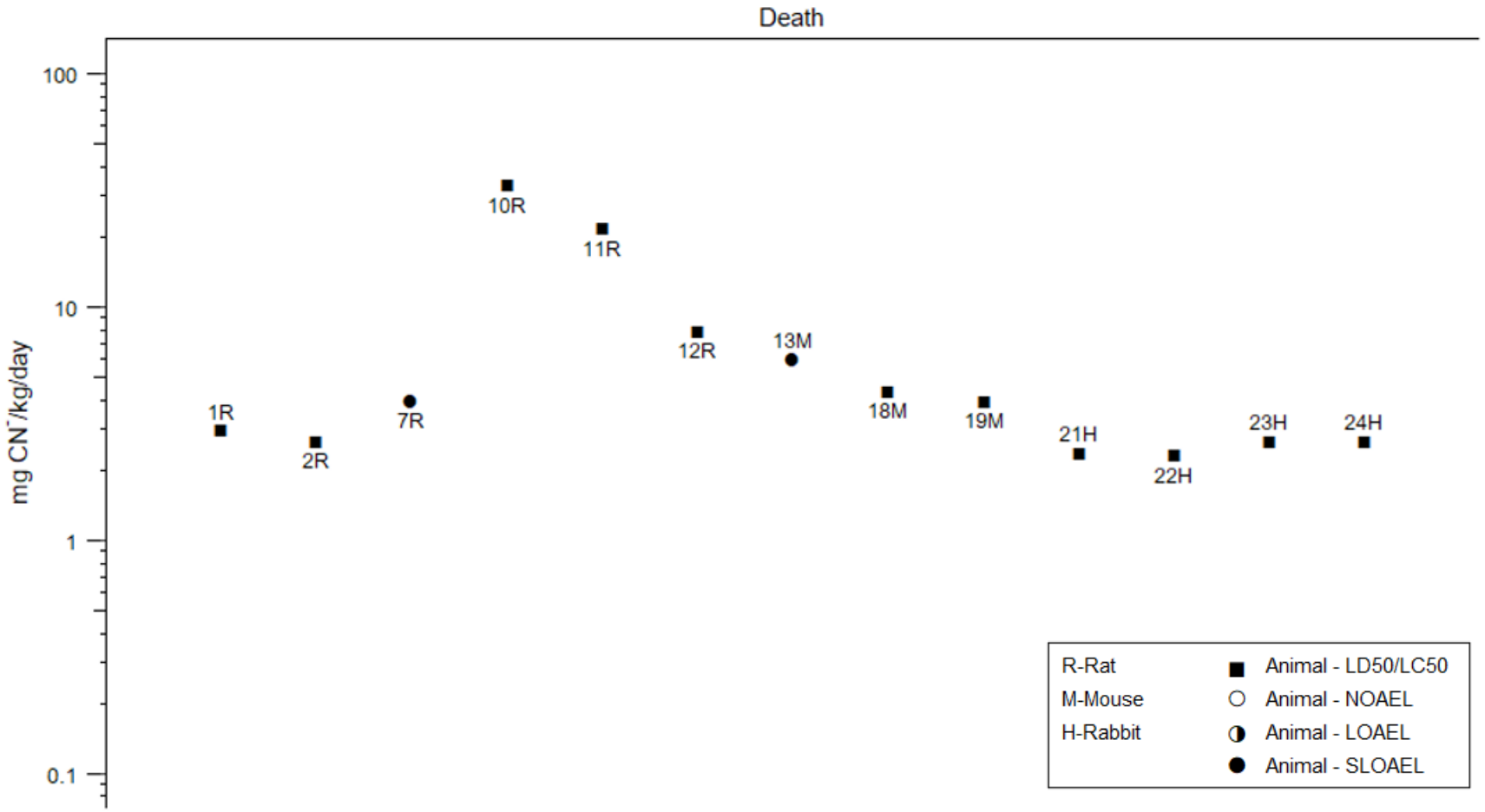


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Acute (≤14 days)

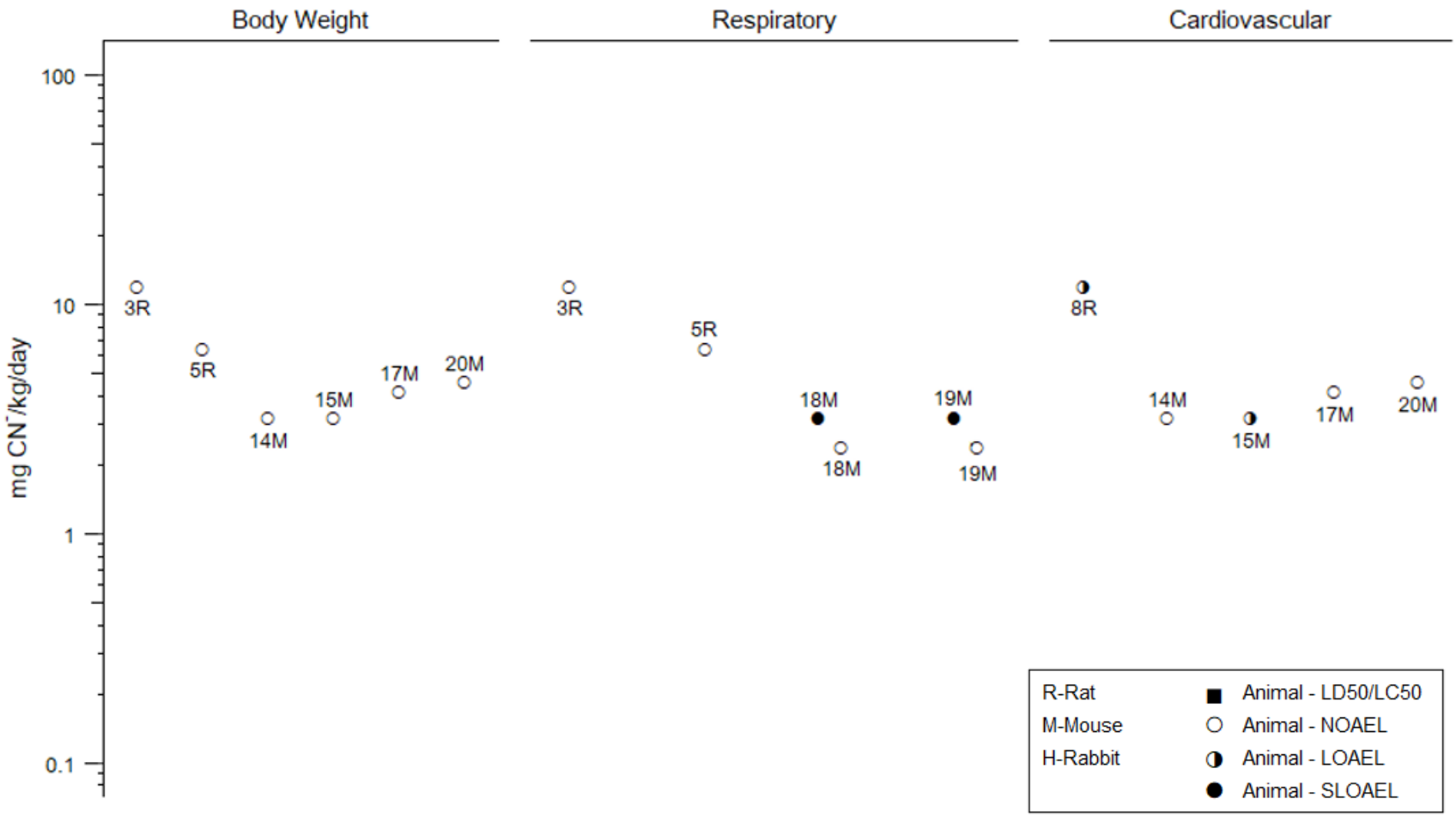


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Acute (≤14 days)

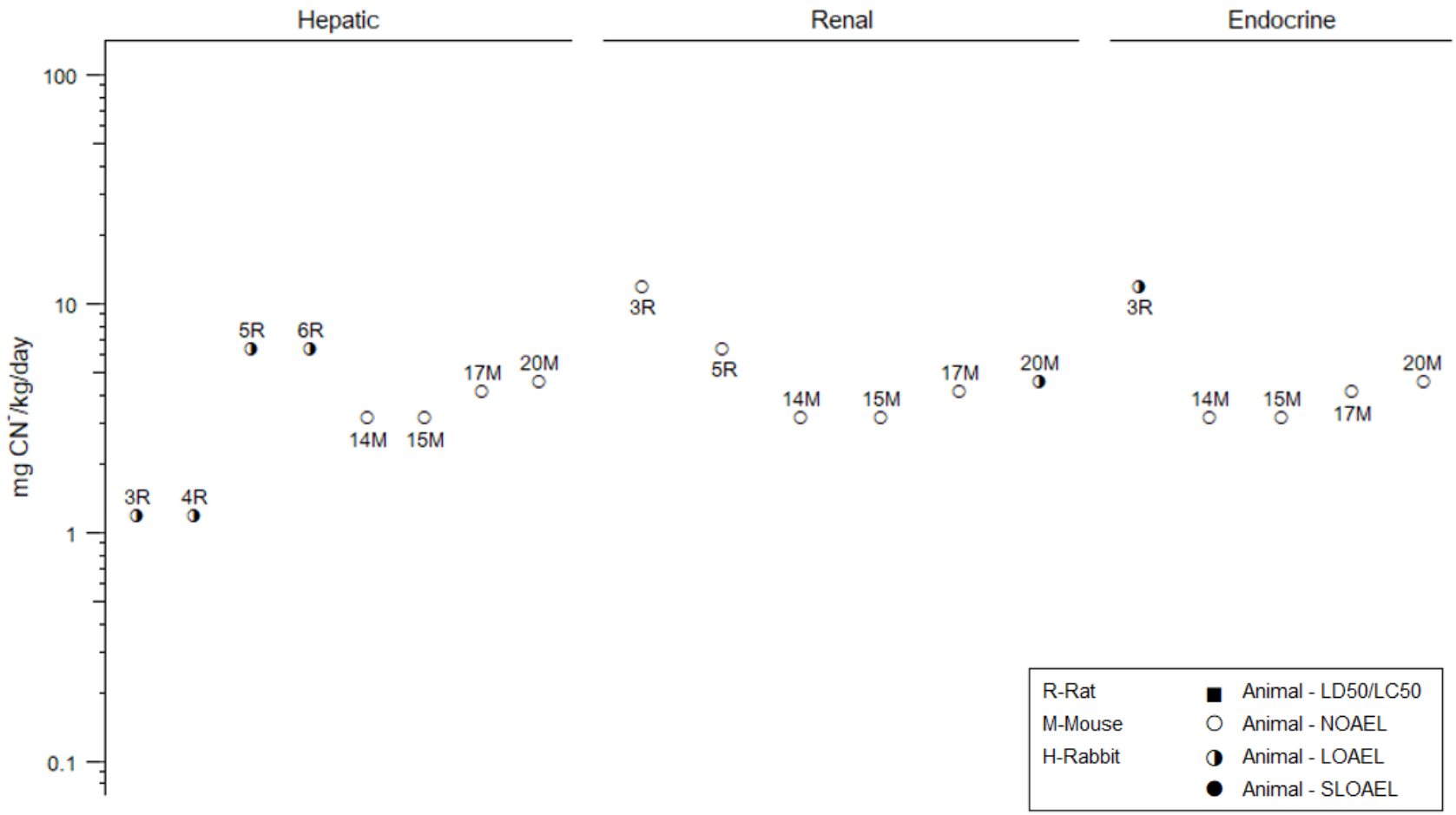


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Acute (≤14 days)

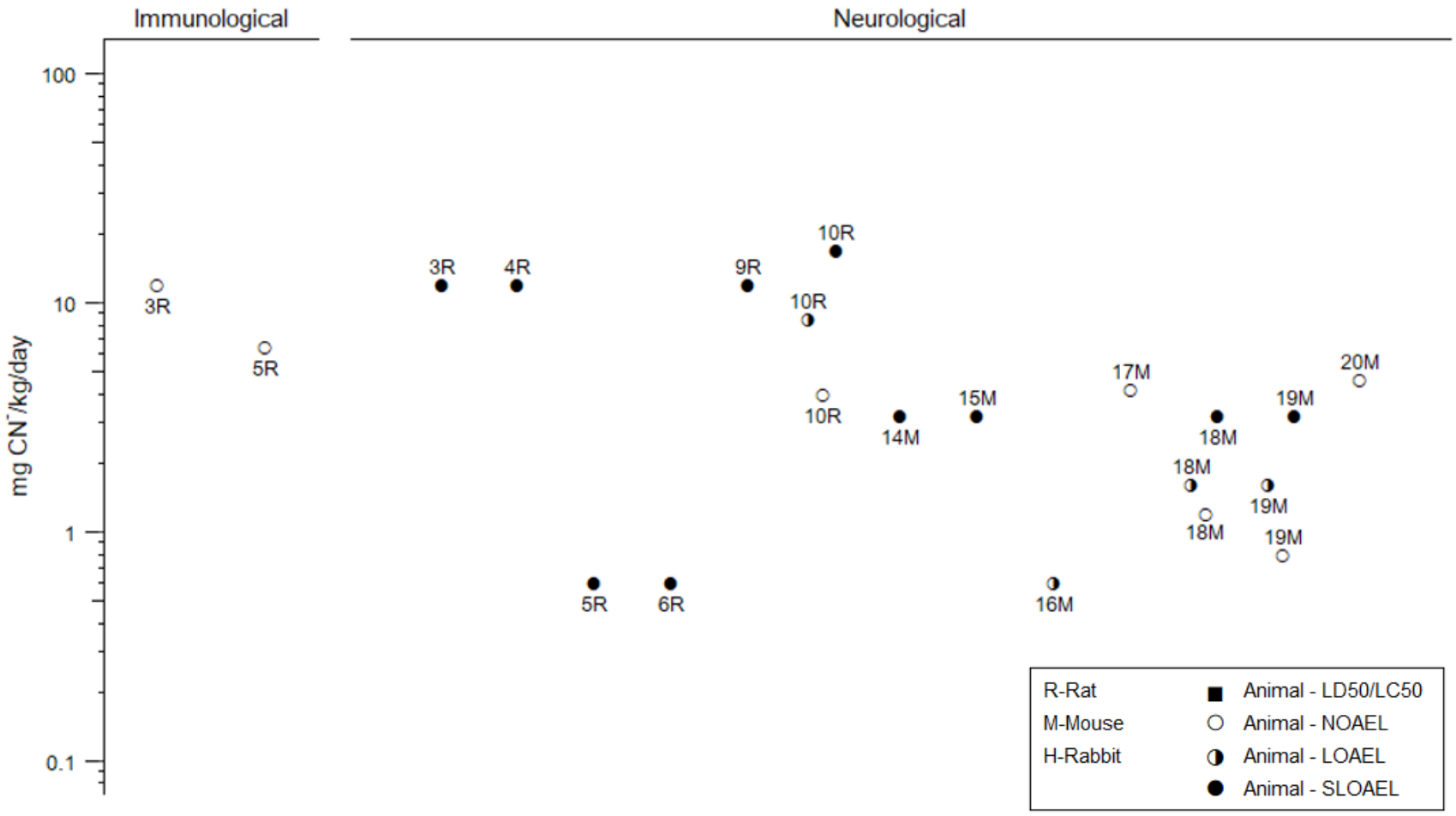


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Acute (≤14 days)

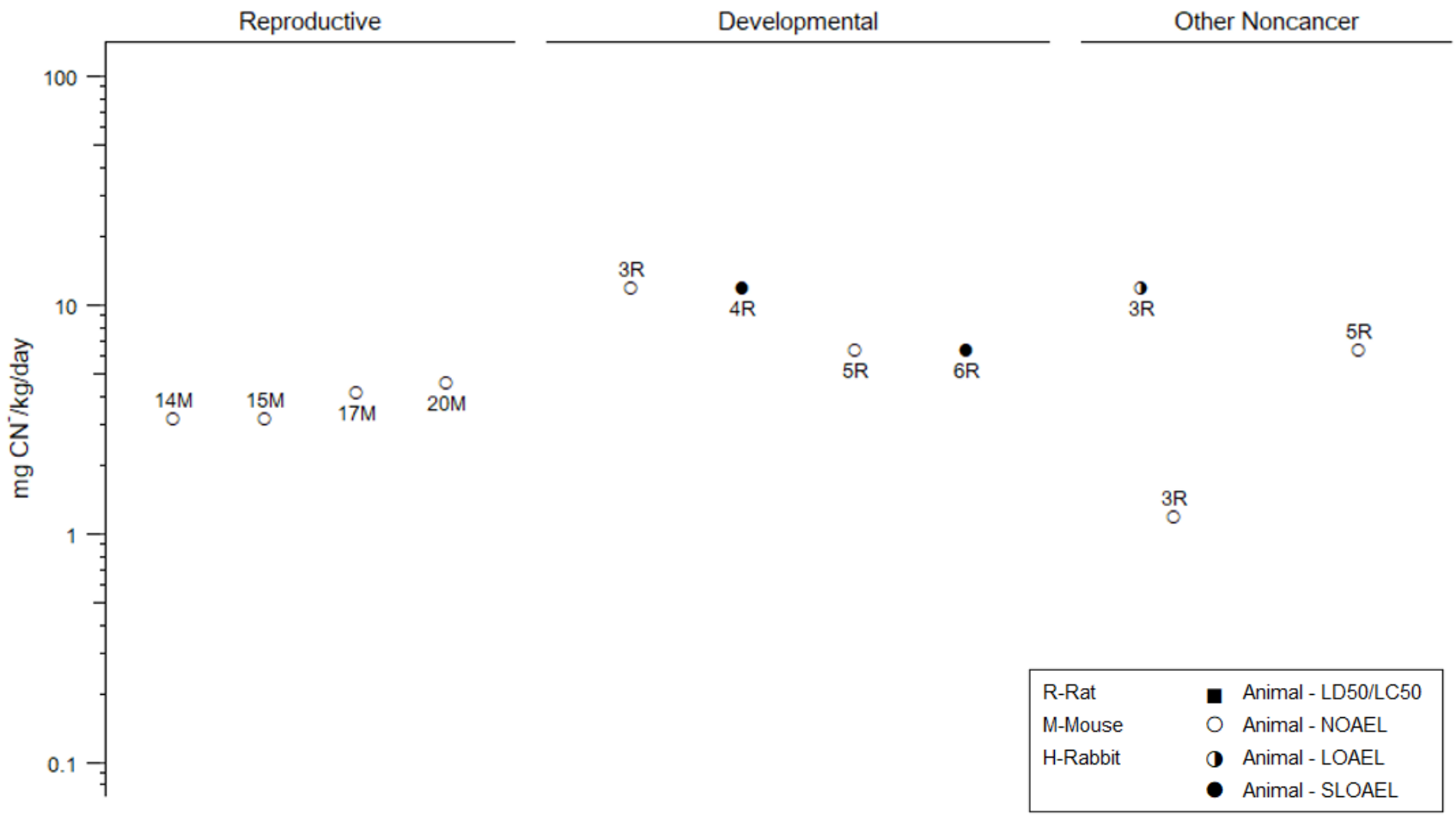


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Intermediate (15–364 days)

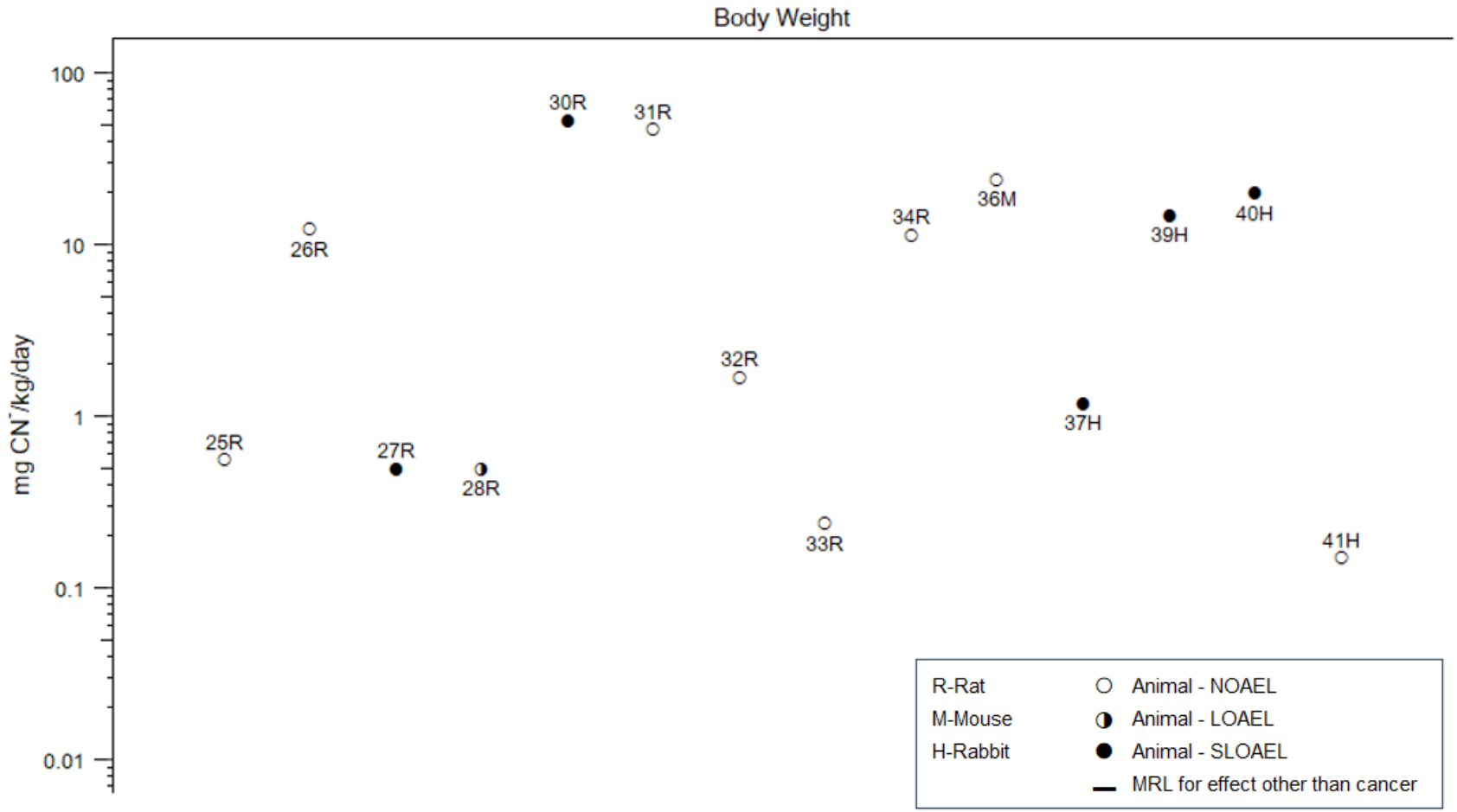


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Intermediate (15–364 days)

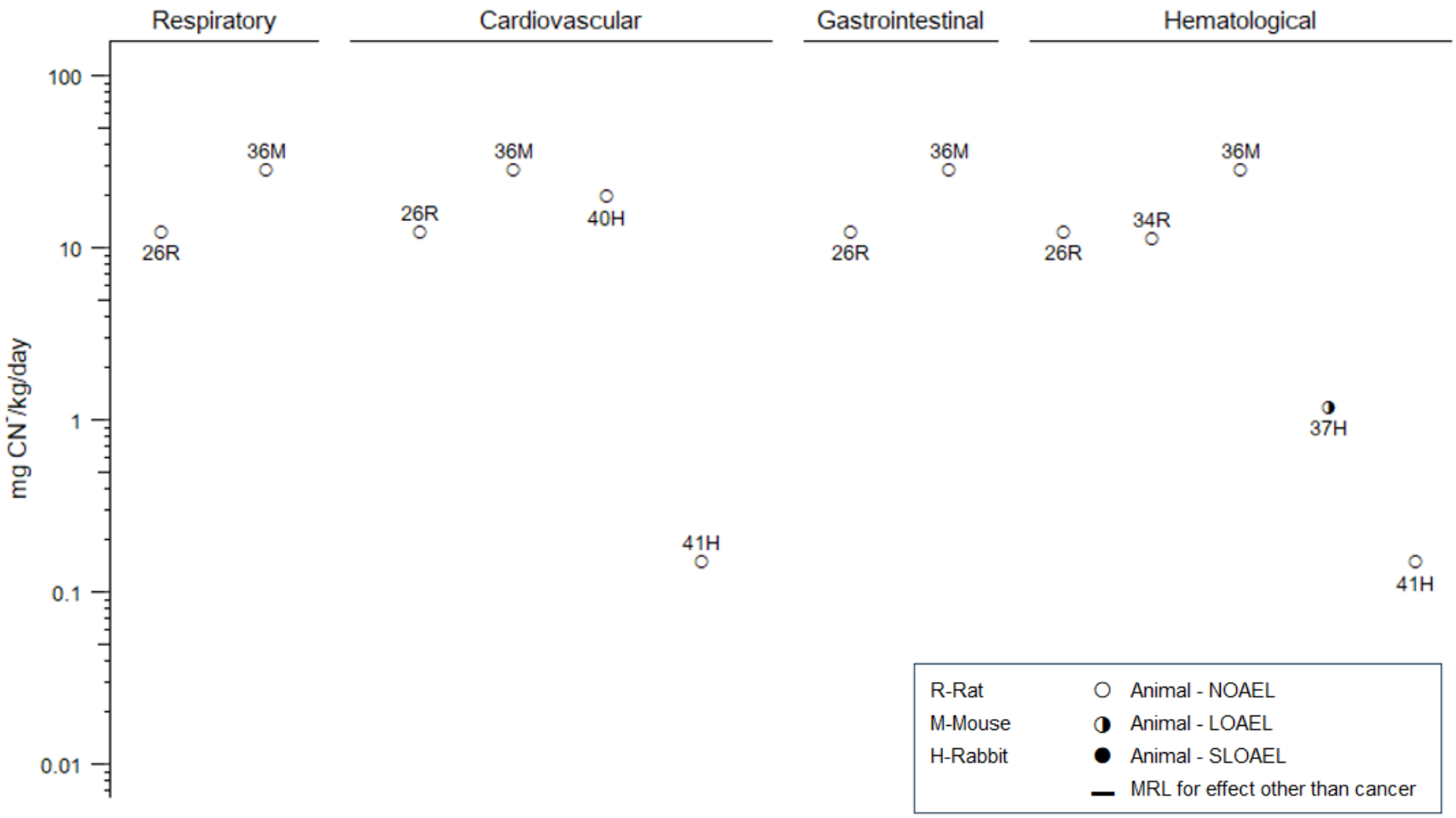


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Intermediate (15–364 days)

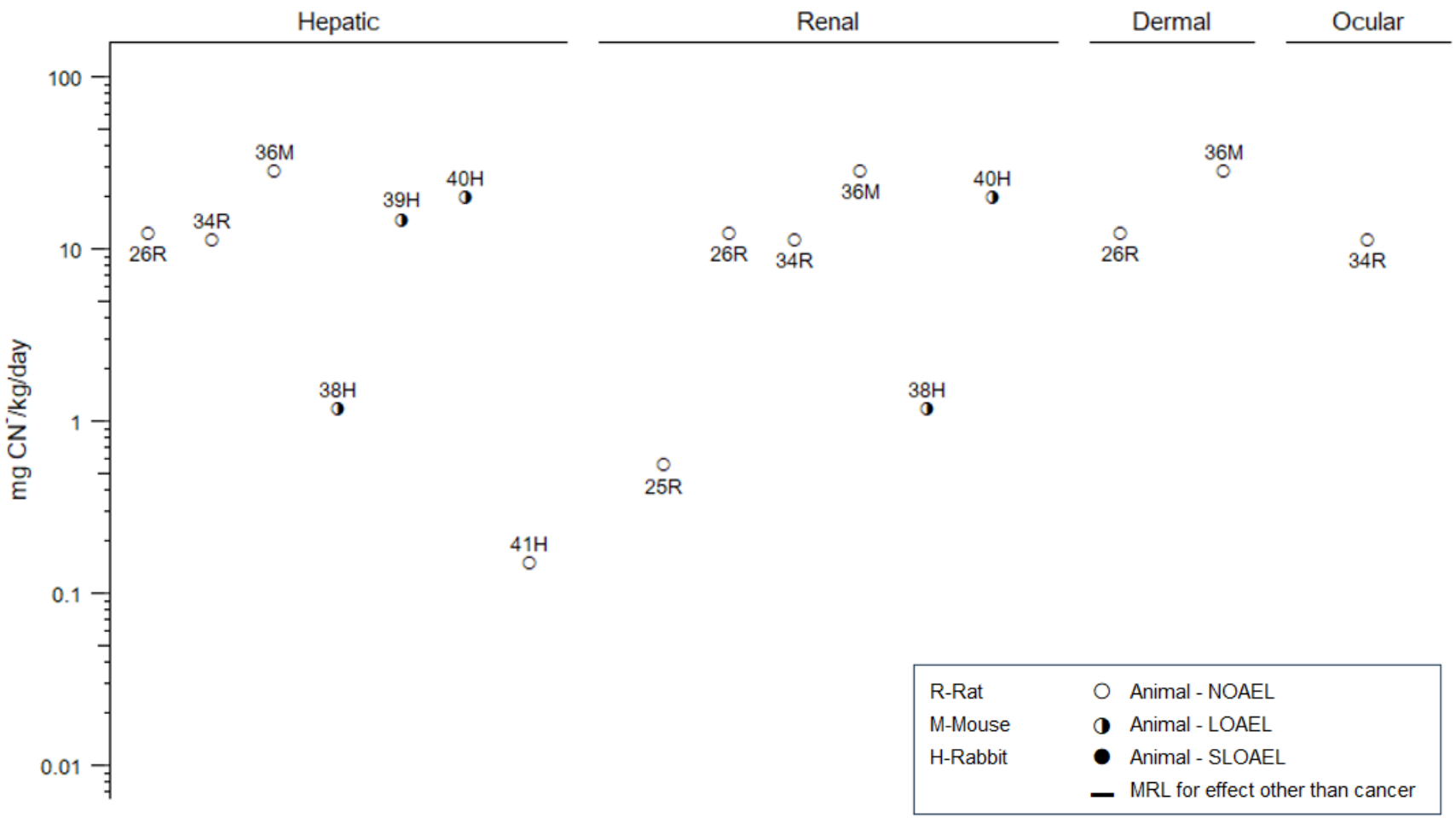


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Intermediate (15–364 days)

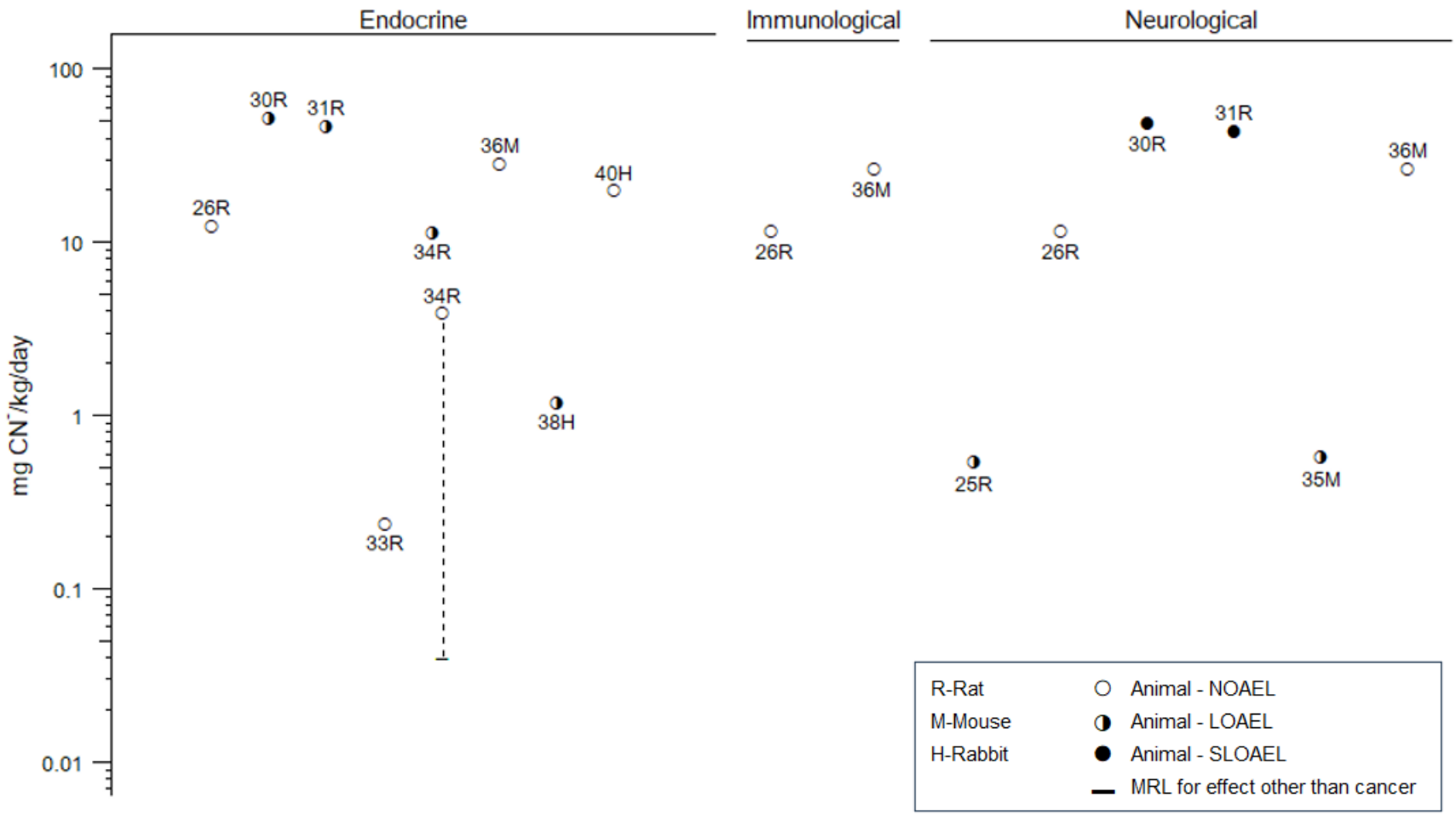


Figure 2-3. Levels of Significant Exposure to Cyanide – Oral
Intermediate (15–364 days)

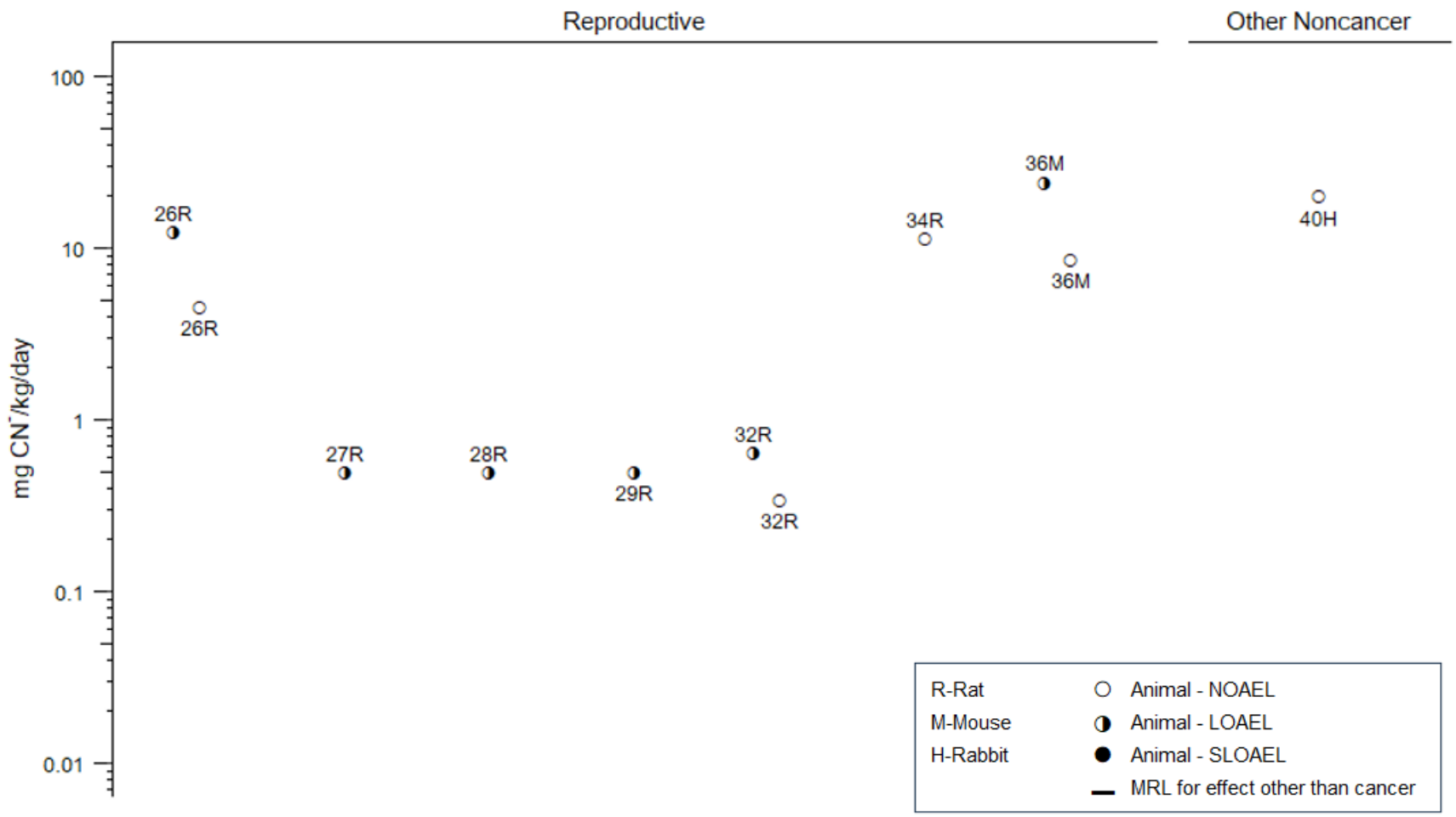


Table 2-3. Levels of Significant Exposure to Cyanide – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Drinker 1932								**HCN**
Human 3 M	8–10 minutes Occupational	20,000 ppm in air	CS	Cardio			20,000	Palpitations
				Neuro			20,000	Dizziness, weakness, headache
Due to use of PPE (respiratory masks), dermal absorption of cyanide was suspected								
Ballantyne 1983a, 1983b								**HCN**
Rabbit (albino) 10 F	Once	0.90–1.14 mg CN^-/kg	LE, CS	Death			1	Transocular LD_{50}
Ballantyne 1983a, 1983b								**KCN**
Rabbit (albino) 10 F	Once	2.5–6.4 mg CN^-/kg	LE, CS	Death			3.2	Transocular LD_{50}
Ballantyne 1983a, 1983b								**NaCN**
Rabbit (albino) 10 F	Once	1.67–3.34 mg CN^-/kg	LE, CS	Death			2.68	Transocular LD_{50}
Ballantyne 1983b								**HCN**
Rabbit (albino) 10 F	Once	0.9–1.14 mg CN^-/kg	LE, CS	Resp		0.9		Rapid breathing
				Ocular			0.9	Corneal opacity, keratitis
				Neuro			0.9	Convulsions and loss of consciousness
Ballantyne 1983b								**KCN**
Rabbit (New Zealand) 4–6 F	Once	2.5–6.4 mg CN^-/kg	LE, CS	Neuro			2.5	Convulsions and loss of consciousness
Ballantyne 1983b								**KCN**
Rabbit (albino) 10 F	Once	2.5–6.4 mg CN^-/kg	LE, CS	Resp		2.5		Rapid breathing
				Ocular			2.5	Corneal opacity, keratitis
Ballantyne 1983b								**NaCN**
Rabbit (albino) 4–6 F	Once	1.7–3.34 mg CN^-/kg	LE, CS	Neuro	1.7		2.1	Convulsions and loss of consciousness

Table 2-3. Levels of Significant Exposure to Cyanide – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ballantyne 1983b								**NaCN**
Rabbit (albino) 10 F	Once	1.69–3.34 mg CN^-/kg	LE, CS	Resp	1.69	2.1		Rapid breathing
				Ocular	1.69		2.1	Corneal opacity, keratitis
Ballantyne 1988								**NaCN**
Rabbit (New Zealand) 10 F	Once	1.69–5.28 mg CN^-/kg	LE, CS	Death			2.4	Transocular LD_{50}
Ballantyne 1994								**HCN**
Rabbit (albino) 9–10 F	Once	2.0–3.2 mg CN^-/kg	LE, CS	Death			2.3	Dermal LD_{50}, abraded skin
Ballantyne 1994								**KCN**
Rabbit (albino) 6–10 F	Once	4.0–16.0 mg CN^-/kg	LE, CS	Death			8.9	Dermal LD_{50}, intact skin
Ballantyne 1994								**NaCN**
Rabbit (albino) 9–19 F	Once	6.7–8.4 mg CN^-/kg	LE, CS	Death			7.7	Dermal LD_{50}, intact skin (NaCN solution)
Ballantyne 1994								**KCN**
Rabbit (albino) 9–10 F	Once	5.0–6.4 mg CN^-/kg	LE, CS	Death			5.7	Dermal LD_{50}, abraded skin
Ballantyne 1994								**HCN**
Rabbit (albino) 10 F	Once	5.4–7.6 mg CN^-/kg	LE, CS	Death			6.6	Dermal LD_{50}, intact skin
Ballantyne 1994								**NaCN**
Rabbit (albino) 9–10 F	Once	5.3–8.4 mg CN^-/kg	LE, CS	Death			5.9	Dermal LD_{50}, abraded skin (NaCN solution)
Ballantyne 1994								**NaCN**
Rabbit (albino) 6 F	Once	3.7–10.6 mg CN^-/kg	LE, CS	Death			6.3	Dermal LD_{50}, moist skin (NaCN powder)

Table 2-3. Levels of Significant Exposure to Cyanide – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ballantyne 1994								**NaCN**
Rabbit (albino) 6–12 F	Once	2.6–5.3 mg CN^-/kg	LE, CS	Death			3.9	Dermal LD_{50}, abraded skin (NaCN powder)

Cardio = cardiovascular; CN = cyanide; CS = clinical signs; F = female(s); HCN = hydrogen cyanide; KCN = potassium cyanide; LD_{50} = dose associated with 50% lethality; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NaCN = sodium cyanide; Neuro = neurological; NOAEL = no-observed-adverse-effect-level; PPE = personal protective equipment; Resp = respiratory

2.2 DEATH

It is well-established that death can occur following intentional or accidental cyanide poisoning, as documented in several reviews and regulatory documents (Asiah et al. 2014; Bhattacharya and Flora 2009; EPA 2006a, 2010; Geller et al. 2006; NIH/NINDS 2016a, 2016b; WHO 2004). Information contained in these sources is briefly reviewed below. Cyanide and cyanide compounds have been weaponized throughout history due to their lethality, with documented use during the Roman Empire, Franco-Prussian War, and both World Wars. Inhalation of cyanide fumes is often a contributing factor to fatalities related to fire smoke inhalation. Cyanide causes death by widespread inhibition of cellular oxygen utilization (see Section 2.21 for details). While cardiac abnormalities are common, death is almost always due to respiratory arrest due to CNS depression. Clinical signs that precede death include rapid breathing followed by slowed irregular breathing, light-headedness and giddiness, nausea and vomiting, confusion, restlessness and anxiety, hypotension, bradycardia, cyanosis, syncope, cardiac arrhythmia, stupor, spasms, convulsions, or coma. Metabolic acidosis precedes death in poisonings that are not immediately lethal. A comprehensive review of available case-reports or case-series reports that reviewed cyanide-related deaths is not included in this profile. Rather, this section is focused on available studies in humans and animals that estimated lethal exposure levels via the inhalation, oral, and/or dermal routes.

Based on analysis of available human and animal data, DOA (1976) estimated an average hydrogen cyanide concentration that would be fatal for humans within 30 minutes would be 622 ppm, with estimated total absorbed doses in fatal cases as low as 0.7 mg CN^-/kg (Rieders 1971). In one case, a worker exposed to 200 ppm hydrogen cyanide in a silverplating tank became unconscious and eventually died even though he had received antidotal therapy in a hospital (Singh et al. 1989). Three deep-sea trawler men died when exposed to toxic fumes (containing lethal concentrations of hydrogen cyanide, carbon dioxide, and hydrogen sulfide) from spoiled fish (Cherian and Richmond 2000); all three men collapsed within 1 minute of exposure. Cyanide exposure was confirmed in one of the men based on a cyanide concentration of 0.05 mg/L in a postmortem blood sample. In other cases, exposure to 270 ppm hydrogen cyanide led to immediate death, 181 ppm hydrogen cyanide exposure was fatal after 10 minutes, and 135 ppm hydrogen cyanide was fatal after 30 minutes in humans (Dudley et al. 1942). WHO (2004) determined that hydrogen cyanide concentrations of ≥110 ppm may lead to death within 30–60 minutes, and that 270 ppm hydrogen cyanide could be immediately fatal. Due to the detoxification rate of hydrogen cyanide (17 µg/kg/minute), the concentration that is lethal for 50% of the population

(LC_{50}) is higher for a longer exposure duration (e.g., 60 minutes) than for a shorter duration (e.g., 2 minutes) (NIH/NINDS 2016a, 2016b).

Levels of acute-duration exposure resulting in animal deaths were reported in multiple studies and LC_{50} values were determined for several species. Inhalation LC_{50} values of hydrogen cyanide in rats ranged from 143 ppm for 60 minutes to 3,417 ppm for 10 seconds (Ballantyne 1983a). Five-minute LC_{50} values of 503 ppm for rats and 323 ppm for mice were reported by Higgins et al. (1972). At lethal concentrations, rodents exhibited hyperactivity and asphyxia convulsions with death occurring within 20 minutes of exposure; gross pathology findings included pulmonary hemorrhage and congestion of the liver and kidney. The 30-minute LC_{50} value in mice for hydrogen cyanide was 166 ppm (Matijak-Schaper and Alarie 1982). Hume et al. (1995) reported 90% lethality in mice after exposure to 400 ppm hydrogen cyanide for 3 minutes, and Ma et al. (2021) reported 33% lethality in mice after exposure to 327 ppm hydrogen cyanide for 40 minutes. LC_{50} values for hydrogen cyanide in rabbits ranged from 188 ppm for 30 minutes to 2,200 ppm for 45 seconds (Ballantyne 1983a). Mortality was also reported in experiments with dogs exposed for acute (Haymaker et al. 1952) and intermediate durations (Valade 1952). Both studies used a small number of dogs for the different exposure regimens, so statistical significance could not be evaluated.

An average fatal oral dose of 1.52 mg CN^-/kg for humans has been calculated from case report studies of intentional or accidental poisonings (EPA 1987). Assuming a 70-kg individual, estimated lethal doses for specific cyanide compounds are 0.67–3.37 mg CN^-/kg as hydrogen cyanide and 0.86–1.43 mg CN^-/kg as potassium cyanide (Bhattacharya and Flora 2009). Suicide cases have involved ingestion of 100–600 g of potassium or sodium cyanide, absorbing up to about 3.5 mg CN^-/kg prior to death (Rieders 1971). The lowest fatal oral dose reported in humans was estimated as 0.56 mg CN^-/kg (form not specified), based on data obtained from the case history in a report by Gettler and Baine (1938). However, analytical measurements at the time of this study lacked the precision of current technology.

Several studies have calculated oral doses associated with 50% lethality (LD_{50} values) for animals following a single oral exposure to cyanide compounds. The LD_{50} values for sodium cyanide in rats ranged widely from 2.7 to 34 mg CN^-/kg; findings may be sex- and/or species-related but data are too limited to make a clear determination (Ballantyne 1988; Rice et al. 2018; Smyth et al. 1969). The highest LD_{50} value of 34 mg CN^-/kg was reported in male Sprague-Dawley rats (Rice et al. 2018), while the lowest value of 2.7 mg CN^-/kg was reported in female Porton rats (Ballantyne 1988). Ballantyne (1988) showed that values were similar in fasted (2.7 mg CN^-/kg) and unfasted (3 mg CN^-/kg) female Porton

rats; however, death occurred sooner in unfasted animals (17 minutes compared to 22 minutes). An intermediate value of 34 mg CN^-/kg was reported in rats of unspecified strain and sex (Rice et al. 2018). For calcium cyanide, the reported LD_{50} value in rats was 22 mg CN^-/kg (Smyth et al. 1969). Mortality was 95% in rats and mice that received a single gavage dose of 4 and 6 mg CN^-/kg, respectively, in the form of potassium cyanide in a volume of water equivalent to 5% of body weight (Ferguson 1962); mortality was lower (50% in rats and 35% in mice) when the same doses were delivered in a volume of water equivalent to 1.25% of body weight. In mice, LD_{50} values for potassium cyanide were similar in adults and juveniles, with values of 4.4 and 4.75 CN^-/kg in adult females and males, respectively, and values of 4.0 and 4.36 mg CN^-/kg in juvenile females and males, respectively (Sabourin et al. 2016). Acute LD_{50} values in rabbits were similar (2.34–2.7 mg CN^-/kg) regardless of whether the source was hydrocyanic acid, sodium cyanide, or potassium cyanide (Ballantyne 1983a, 1988).

Intermediate-duration drinking water and dietary studies did not report increased mortality in rats or mice, even at doses higher than those associated with mortality in acute-duration gavage studies. No exposure-related deaths were reported in rat or mice exposed to doses up to 12.5 or 28.8 mg CN^-/kg/day, respectively, in the drinking water for 13 weeks (NTP 1993; Tyner and Greeley 2023). Similarly, mortality was not increased in rats following dietary exposure to doses up to 47 mg CN^-/kg/day as potassium thiocyanate or 53 mg CN^-/kg/day as potassium cyanide for 11.5 months (Philbrick et al. 1979). Increased mortality was observed in rats exposed to 14.5 mg CN^-/kg/day as copper cyanide for 90 days (Gerhart 1986) and 2.6 mg CN^-/kg/day as potassium silver cyanide for 90 days (Gerhart 1987); these data are omitted from the LSE table because of the possible confounding effect of the metals. Hemolytic anemia, which was probably related to copper toxicity, was most likely responsible for observed deaths in rats exposed to copper cyanide (Gerhart 1986).

Since absorption of hydrogen cyanide through the skin is much slower than the lungs, the estimated LD_{50} value of 100 mg CN^-/kg for dermal exposure to hydrogen cyanide in humans is higher than estimated LC_{50} values via inhalation (Rieders 1971). In individuals wearing proper respiratory protection but lacking dermal protective gear, extremely high air concentrations of hydrogen cyanide (6,300–10,000 ppm) can be fatal through the dermal route (EPA 2006a).

Based on a series of LD_{50} studies in rabbits (Ballantyne 1994), dermal absorption (and the resulting toxicity) is increased through moist or abraded skin. Reported LD_{50} values for dermal exposure to cyanides in rabbits on dry skin include 6.6 mg CN^-/kg as hydrogen cyanide solution, 7.7 mg CN^-/kg as sodium cyanide solution, and 8.9 mg CN^-/kg as potassium cyanide solution; no lethality was observed

when sodium cyanide powder was applied to dry rabbit skin at doses up to 106 mg CN^-/kg (Ballantyne 1994). For moist skin, the dermal LD_{50} value for sodium cyanide powder was 6.3 mg CN^-/kg in rabbits. In abraded skin, dermal LD_{50} values in rabbits included 2.3 mg CN^-/kg for hydrogen cyanide solution, 5.9 mg CN^-/kg for sodium cyanide solution, 3.9 mg CN^-/kg for sodium cyanide powder, and 5.7 mg CN^-/kg for potassium cyanide solution (Ballantyne 1994). Similar differences in toxicity of various chemical forms of cyanide were observed after cyanide was applied to the inferior conjunctival sac of one eye (Ballantyne 1983a, 1983b, 1988). Transocular LD_{50} values were 1.0 mg CN^-/kg as hydrogen cyanide, 2.68 mg CN^-/kg as sodium cyanide, and 3.2 mg CN^-/kg as potassium cyanide. The deaths occurred within 3–12 minutes. Overt signs of toxicity in rabbits prior to death for both dermal or intraocular exposure included dyspnea, gasping, weakness, spasms, unsteadiness, convulsions, and coma. Deaths occurred also in guinea pigs when their skin was exposed to hydrogen cyanide; however, doses were not quantified (Fairley et al. 1934; Walton and Witherspoon 1926). It should be noted that none of the dermal studies reported the surface area to which the cyanide was applied. Similar to overt toxicity observed in rabbits, convulsions and coma preceded death in guinea pigs.

2.3 BODY WEIGHT

Body weight data in humans are limited to a single occupational exposure study. In an occupational setting, weight loss (mean of 5.6 kg) during employment was self-reported in 50% of workers formerly exposed to 15 ppm hydrogen cyanide for a mean duration of 11 months in a silver-reclaiming facility (Blanc et al. 1985). When workers were qualitatively stratified into low-, moderate-, and high-exposure levels based on job title, a dose-relationship between exposure and reported body weight loss was observed. This finding was associated with a reported loss of appetite in 58% of workers. Limitations of this study include lack of quantitative exposure levels for the low-, moderate-, and high-exposure groups, reliance on self-reporting of symptoms that occurred during employment that ceased 7–30 months prior to the assessment, and co-exposure to other chemicals during the silver-reclaiming process. Appetite loss was also reported in 25% of workers exposed to an unknown level of hydrogen cyanide vapor during heat treatment (case hardening) and electroplating for an unreported duration (Kumar et al. 1992). Body weight loss was not examined in this cohort.

No studies were located regarding body weight effects in animals after inhalation exposure to hydrogen cyanide. Decreased body weight (13%) was reported in rats intermittently exposed to 25 ppm cyanogen via inhalation for 6 months (Lewis et al. 1984). As discussed in Section 2.1, this study is not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Reports of body weight effects in animals exposed to cyanide compounds via drinking water are inconsistent. One study reported a 70% reduction body weight gain in male rats that ingested 3.6 mg CN^-/kg/day as potassium cyanide in drinking water for 15 days, noting that the effect was significant as early as the first week of treatment (Sousa et al. 2002). However, several other drinking water studies have not observed adverse effects on body weight in rats after exposure to doses up to 6.4 mg CN^-/kg/day as potassium thiocyanide or 12 mg CN^-/kg/day as potassium cyanide for 14 days (de Sousa et al. 2007) or up to 12.5 mg CN^-/kg/day as sodium cyanide in drinking water for 13 weeks (NTP 1993; Tyner and Greeley 2023). Additionally, no body weight effects were observed in mice exposed to doses up to 28.8 mg CN^-/kg/day as sodium cyanide in drinking water for 13 weeks (NTP 1993). It is difficult to interpret the adversity of the findings reported by Sousa et al. (2002) in the absence of absolute body weight and water intake data and lack of support from other available drinking water studies.

In intermediate-duration dietary studies, decreased body weight gains of 22–33% were reported in rabbits exposed to 15 mg CN^-/kg/day as sodium cyanide (Okolie and Iroanya 2003) and in rats and rabbits exposed to 53 or 20 mg CN^-/kg/day as potassium cyanide, respectively (Okolie and Osagie 1999, 2000; Philbrick et al. 1979). However, no adverse effects on body weight were observed in rats exposed to dietary potassium thiocyanate at doses up to 47 mg CN^-/kg/day for 11.5 months (Philbrick et al. 1979).

Findings from studies that employed bolus dosing (i.e., gavage, buccal bolus) also reported inconsistent findings for body weight effects following exposure to cyanide. When effects were observed, they were generally associated with doses below those associated with effects in dietary studies. This observation is likely because bolus administration may overwhelm detoxification processes.

Acute-duration bolus administration studies in animals do not report adverse effects on body weight following exposure to cyanide compounds, with no body weight effects in mice exposed once to doses up to 4.6 mg CN^-/kg as potassium cyanide (Hawk et al. 2016; Sabourin et al. 2016). Decreased body weight gains of 25–39% were reported in some intermediate-duration oral bolus administration studies, including rats exposed to ≥0.5 mg CN^-/kg/day as sodium cyanide (Oyewopo et al. 2021a) and in rabbits exposed to 1.2 mg CN^-/kg/day as potassium cyanide (Avais et al. 2014). In contrast, no adverse effects on body weight were observed in rats at oral bolus doses up to 0.56 mg CN^-/kg/day as potassium cyanide (Mathangi et al. 2011; Soto-Blanco et al. 2002) or 1.7 mg CN^-/kg/day as sodium cyanide (Shivanoor and David 2015). No effect on body weight gain was observed in rabbits exposed to 0.15 mg CN^-/kg/day via gavage as potassium cyanide for up to 3 months (Ozolua et al. 2007).

In a 90-day gavage study, reduced body weight gain was reported in male rats exposed to ≥4.35 mg CN^-/kg/day as copper cyanide, but not in those exposed to 1.45 mg CN^-/kg/day (Gerhart 1986). Additionally, decreased weight gain was found in male rats exposed to ≥2.6 mg CN^-/kg/day via gavage as potassium silver cyanide for 90 days (Gerhart 1987). Since the presence of the copper or silver may have contributed to the observed decreases in body weight, these data are omitted from the LSE table.

As discussed in Section 2.1, animal studies on cassava are discussed in this profile, but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Pregnant hamsters fed 1.0 mg CN^-/kg/day in cassava for 10 days during gestation had decreased body weight gain (Frakes et al. 1986). Body weights were decreased by 24% in rats exposed to 5.50 mg CN^-/kg/day as cassava in the diet for 28 days, compared to control, and exposed animals lost body weight during the exposure period (Udeme et al. 2015). However, Udeme et al. (2015) did not report food intake data.

No studies were located regarding body weight effects in animals after dermal exposure to cyanide.

2.4 RESPIRATORY

Respiratory effects, primarily respiratory irritation and breathing difficulties, have been reported following exposure to cyanide compounds via all routes examined in both humans and animals. With the exception of local irritation, effects are attributed to general toxic actions of cyanide (impaired cellular oxygen utilization; see Section 2.21) and/or cyanide-related CNS depression rather than direct toxic action on the respiratory tract.

In case reports of humans acutely exposed to high levels of hydrogen cyanide requiring hospitalization, the rate of respiration is initially increased followed by subsequent dyspnea (Chen and Rose 1952; Peden et al. 1986; Potter 1950). The levels of exposure in these accidental poisonings were not provided.

Subjective complaints of respiratory effects were reported in a cohort of 56 workers exposed to hydrogen cyanide vapor during heat treatment (case hardening) and electroplating, including throat irritation (16.1%), breathlessness (8.9%), and cough (12.5%); physical exam revealed throat congestion in 25% of workers (Kumar et al. 1992). Breathing difficulties were self-reported in some workers exposed to unspecified levels of hydrogen cyanide in the cassava processing industry (Janagam et al. 2008).

Reporting in these studies was inadequate to determine if reported findings were increased over unexposed individuals and/or attributable to exposure.

Results of other occupational studies evaluating potential associations between cyanide exposure and adverse respiratory effects are reported in Table 2-4. Cough and throat congestion were more prevalent in workers employed for >10 years, compared to those employed <10 years; other symptoms were not associated with duration of employment. Exposure levels were not reported in this study. In an occupational study of three electroplating factories, an increased incidence of subjective respiratory complaints, including dyspnea and throat irritation, was observed in 36 male workers exposed to sodium cyanide and copper cyanide for 5–15 years, compared to 20 unexposed referents (El Ghawabi et al. 1975). Measured mean concentrations of "cyanides" (not further characterized) in the three factories were 6.416–10.375 ppm. Dyspnea, cough, wheezing, sore throat, hemoptysis, epistaxis, nasal congestion, and altered sense of smell were also self-reported in 19–47% workers formerly exposed to 15 ppm hydrogen cyanide for a mean duration of 11 months in a silver-reclaiming facility (Blanc et al. 1985). When the 36 workers were qualitatively stratified into low-, moderate-, and high-exposure levels based on job title, a positive association between exposure and self-reported incidence of nasal congestion was observed; no association was observed for dyspnea (other respiratory complaints were not evaluated for exposure-response). Limitations of this study include lack of quantitative exposure levels for the low-, moderate-, and high-exposure groups and reliance on self-reporting of symptoms that occurred during employment that ceased 7–30 months prior to the assessment. In general, findings in these occupational studies were confounded by other known chemical co-exposures, including as metals, cleaners, and cutting oils.

Table 2-4. Results of Select Epidemiological Studies Evaluating Occupational Exposure to Cyanide and Respiratory Effects

Reference, study type, and population	Measure of exposure	Outcome evaluated	Result
Blanc et al. 1985 Cohort; 36 former workers from a silver-reclaiming facility; mean duration of employment was 11 months and mean duration elapsed since employment was 10.5 months (United States, Illinois)	Time-weighted average concentration of hydrogen cyanide (measured after plant ceased operations): 15 ppm REI estimated based on job title; no quantitative exposure levels: Low (n=7) Moderate (n=13) High (n=16)	Dyspnea	↔ (REI)
		Nasal congestion	↑ (REI)

Table 2-4. Results of Select Epidemiological Studies Evaluating Occupational Exposure to Cyanide and Respiratory Effects

Reference, study type, and population	Measure of exposure	Outcome evaluated	Result
Chatgtopadhyay et al. 2000 Prospective cohort; 24 workers exposed to cyanide fumes from a metal tempering plant (at initial investigation; mean exposure of 21.00 years), 17 workers at 2-year follow-up (mean exposure of 22.96 years), and 14 unexposed referents (India)	Not reported	Pulmonary function (when categorized by smoking status)	
		FEV_1	↔ (initial) ↔ (follow-up) ↔ (duration of exposure)
		$FEV_{1\%}$	↓ (initial, smokers) ↔ (follow-up) ↔ (duration of exposure)
		FEF	↓ (initial, smokers) ↔ (follow-up) ↔ (duration of exposure)
		$FEF_{25–75\%}$	↔ (initial) ↔ (follow-up) ↔ (duration of exposure)
		FVC	↔ (initial) ↔ (follow-up) ↔ (duration of exposure)
		VC	↔ (initial) ↔ (follow-up) ↔ (duration of exposure)
		PEFR	↔ (initial) ↓ (follow-up, smokers) ↔ (duration of exposure)
El Ghawabi et al. 1975 Cross-sectional; 36 male workers from three electroplating factories (9 from Factory A, 12 from Factory B, 15 from Factory C; employed 5–15 years) and 20 unexposed male referents (Egypt)	Mean (range) "cyanides" concentrations, ppm: Factory A: 10.375 (8.2–12.4) Factory B: 6.416 (4.2–8.8) Factory C: 8.083 (5.9–9.6) "Cyanides" measured were not further described; cyanide exposure evolved from plating bath containing sodium cyanide and copper cyanide.	Self-reported respiratory complaints (dyspnea, throat irritation)	↑ (workers versus referents)

↑ = association; ↓ = inverse association; ↔ = no association; FEF = forced expiratory flow; $FEF_{25–75\%}$ = forced mid expiratory flow; FEV_1 = forced expiratory volume in 1 second; $FEV_{1\%}$ = forced expiratory volume in 1 second expressed as a percentage of forced vital capacity; FVC = forced vital capacity; PEFR = peak expiratory flow rate; REI = relative exposure index; VC = vital capacity

Chatgtopadhyay et al. (2000) reported decreases in several measures of pulmonary function in 24 workers exposed to an unreported level of cyanide fumes for an average of 21 years in a metal tempering plant,

compared to 14 unexposed referents. Pulmonary function worsened over the next 2 years in 17 workers with follow-up data. However, once workers and referents were divided by smoking status, the only differences that remained included the following parameters in smoking workers, compared to smoking referents: (1) decreased forced expiratory volume in 1 second as the percentage of forced vital capacity ($FEV_{1\%}$) at the initial examination (but not follow-up); (2) decreased forced expiratory flow (FEF) at the initial examination; (but not follow-up); and (3) decreased peak expiratory flow rate (PEFR) at the follow-up examination. No differences were noted between nonsmoking workers and nonsmoking referents.

Data for respiratory effects in animals following inhalation exposure to hydrogen cyanide are limited; most studies had major limitations. Following exposure to a series of hydrogen cyanide concentrations for 30 minutes, the concentration associated with a 50% reduction in the respiratory rate of mice was calculated at 63 ppm hydrogen cyanide (Matijak-Schaper and Alarie 1982). Decreased respiratory rate was attributed to depression of the respiratory center. Exposure to 327 ppm hydrogen cyanide for 40 minutes resulted in gasping and labored breathing in mice that survived that persisted for up to 60 minutes post-exposure (Ma et al. 2021). Other identified studies in dogs and monkeys reported severe respiratory effects but are not included in the LSE table due to inadequate study design (low animal number and/or lack of concurrent control) and/or poor reporting of study design and results. Acute-duration studies reported asphyxia and pulmonary edema in dogs at concentrations of 149–633 ppm hydrogen cyanide for 2–10 minutes (Haymaker et al. 1952) and severe dyspnea in monkeys exposed to ≥100 ppm hydrogen cyanide for 30 minutes (Purser et al. 1984). In an intermediate-duration study in dogs, dyspnea was reported following exposure to 45 ppm hydrogen cyanide for 30 minutes a day at 2–8-day intervals for 28–96 days (Valade 1952).

Nasal irritation was reported in volunteers exposed to 16 ppm cyanogen for 6–8 minutes (McNerney and Schrenk 1960). No effects were reported at 8 ppm cyanogen. In laboratory animals, asphyxia was observed in rats exposed to 250 ppm cyanogen for 7.5–120 minutes (McNerney and Schrenk 1960). In intermediate-duration studies, no respiratory effects were reported in rats exposed to 25 ppm cyanogen for 6 months, and a decrease in total lung moisture content was the only finding in monkeys exposed to 11 ppm cyanogen, also for 6 months (Lewis et al. 1984). As discussed in Section 2.1, these studies are not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Breathing irregularities have also been reported in humans after oral cyanide poisoning. Stertorous, deep, and rapid breathing was reported in a man who ingested approximately 15 mg CN^-/kg as potassium cyanide in a suicide attempt (Liebowitz and Schwartz 1948). Shortness of breath and dyspnea were observed in two reports of suicide attempts with potassium cyanide; one man ingested 7.6 mg CN^-/kg (Goodhart 1994) and the other man ingested 0.57 mg CN^-/kg (Saincher et al. 1994). A man admitted to a hospital after ingesting an unknown amount of sodium cyanide ceased breathing (Grandas et al. 1989). A woman who ingested an unknown amount of cyanide developed acute respiratory distress syndrome and arteriolization (elevated oxyhemoglobin saturation) of the ventral venous blood (Martin-Bermudez et al. 1997). Dyspnea developed in a woman 20 minutes after eating 30 apricot pits (~15 g), resulting in an estimated cyanide exposure between 0.026 and 0.234 mg CN^-/kg (Suchard et al. 1998). Tachypnea was also reported in children who were poisoned by cyanide after ingesting apricot pits (Lasch and El Shawa 1981).

Respiratory effects in animals exposed via drinking water or dietary ingestion were limited, and studies with reliable study designs and data reporting did not report adverse effects. No effects on lung weights or histology were observed in rat dams given up to 12 mg CN^-/kg/day as potassium cyanide or 6.43 mg CN^-/kg/day as potassium thiocyanide in drinking water on gestation days (GDs) 6–20 (De Sousa et al. 2007). Similarly, no exposure-related effects on lung weight or histology were observed in rats or mice exposed to doses up to 12.5 or 28.8 mg CN^-/kg/day as sodium cyanide, respectively, in drinking water for 13 weeks (NTP 1993).

In contrast, Okolie and Iroanya (2003) qualitatively reported evidence of pulmonary edema (thickened alveolar walls and congestion) and evidence of tissue damage (increased lactate dehydrogenase [LDH] in the lung tissue) in rabbits that ingested 15 mg CN^-/kg/day as sodium cyanide in feed for 4 weeks. However, due to lack of quantitative histopathology data reporting, statistical significance of the findings could not be determined. Therefore, these findings are omitted from the LSE table. In a 2-year study in rats, no respiratory effects were reported at a target dietary dose of 10.4 mg CN^-/kg/day as hydrogen cyanide (Howard and Hanzal 1955). However, there is uncertainty in the dose because evaporation of the cyanide from the feed resulted in unstable cyanide levels throughout the experiment. Due to uncertainty in dose estimates for Howard and Hanzal (1955), this study is not included in the LSE table.

A limited number of gavage studies in animals reported respiratory effects at doses below those associated with effects in dietary studies. This observation is likely because bolus administration may overwhelm detoxification processes. In an acute-duration study, labored respiration was observed in

adult and juvenile mice given single gavage doses ≥3.2 mg CN^-/kg/day as potassium cyanide (Sabourin et al. 2016). Labored respiration was reported in rats treated by gavage with 4.35 mg CN^-/kg/day as copper cyanide or 0.8 mg CN^-/kg/day as a form of potassium silver cyanide for 90 days (Gerhart 1986, 1987). Due to the unknown contribution of copper and silver to observed respiratory effects in the Gerhart (1986, 1987) studies, these data are omitted from the LSE table.

In dermal exposure studies in humans, breathing irregularities, including Cheyne-Stokes respiration (atypical breathing pattern defined by cycles of slow, deep breathing or apnea followed by rapid, short breaths/hyperventilation) (Rudrappa et al. 2023), developed in two persons who fell into cisterns containing copper cyanide or potassium cyanide (Dodds and McKnight 1985; Trapp 1970) and one person whose hands were exposed to hydrogen cyanide (Potter 1950).

Rapid breathing was reported as the first sign of toxicity in rabbits that received 0.9 mg CN^-/kg as hydrogen cyanide, 1.69 and 2.1 mg CN^-/kg as sodium cyanide, and 2.5 mg CN^-/kg as potassium cyanide in their conjunctival sacs (Ballantyne 1983b, 1988). Similarly, labored or rapid breathing preceded coma and death in guinea pigs exposed dermally to unknown doses of hydrogen cyanide (Fairley et al. 1934; Walton and Witherspoon 1926).

Mechanisms of Respiratory Toxicity. Clinical signs of labored or difficult breathing are most likely due to general mechanisms of cyanide toxicity (histotoxic anoxia), in which cells are unable to utilize oxygen (see Section 2.21 for details), rather than a direct effect of the respiratory system. However, Bhattacharya et al. (1994) demonstrated an initial increased air flow, transthoracic pressure, and tidal volume accompanied by a significant decrease in pulmonary phospholipids following inhalation of hydrogen cyanide in rats. This study also showed that hydrogen cyanide exhibited a direct effect on pulmonary cells in rats.

Respiratory system effects may also be secondary to neurotoxic effects of cyanide, such as CNS depression (see Section 2.15). Chao et al. (1996) investigated the possibility that cyanide had an effect on motor neurons that was independent of respiratory impairment. In mouse triangularis sterni and diaphragm nerve-muscle preparations under glucose-free conditions, 10 µM sodium cyanide increased spontaneous transmitter release. This was correlated with a depression of adenosine triphosphate (ATP)-sensitive potassium currents, an effect that was antagonized by diazoxide, an opener of ATP-sensitive K^+ channels. The study authors suggested that cyanide causes depolarization of motor nerve terminals via its effect on the ATP-sensitive K^+ channels. Cassel et al. (1994) examined the *in vitro* effects of sodium

cyanide on two forms of monoamine oxidase (MAO), an enzyme important in regulation of biogenic amines in the brain and peripheral tissue. In striatal tissue, cyanide produced a dose-dependent increase in the activity of MAO-A but not MAO-B. Greer and Carter (1995) investigated the effects of hydrogen cyanide on the neural mechanisms controlling breathing. Cyanide, at concentrations considered lethal *in vivo*, caused a modest depression of the frequency and amplitude of inspiratory rhythmic discharge. The neuronal network underlying respiration continued to function for hours in the presence of very high concentrations of cyanide. The study authors hypothesized that the rapid suppression of breathing caused by cyanide *in vivo* is due to changes in neuronal excitability in respiratory centers in the CNS.

2.5 CARDIOVASCULAR

There is limited evidence of cardiovascular effects, primarily bradycardia and irregular heartbeat, following exposure to cyanide compounds in both humans and animals. Some of these findings are attributed to general toxic actions of cyanide (impaired cellular oxygen utilization; see Section 2.21) and/or cyanide-related CNS depression rather than direct toxic action on the cardiovascular system.

Wexler et al. (1947) reported that four men who were executed via inhalation of hydrogen cyanide gas (concentration not reported) had a distinct slowing of the heart rate within 1–3 minutes of exposure, with further changes in the heart rate, sinus irregularities, and audio-visual dissociation. Palpitations and hypotension were the most frequently reported cardiovascular effects in patients after accidental inhalation poisoning with cyanide; however, exact exposure levels were not known (Peden et al. 1986).

Subjective complaints of chest pain have been reported in some workers occupationally exposed to cyanide; it is unclear if this nonspecific complaint is directly related to potential cardiovascular effects. Subjective complaints of chest pain were reported in a 13/56 workers exposed to an unknown concentration of hydrogen cyanide vapor during heat treatment (case hardening) and electroplating (Kumar et al. 1992). In another occupational study, 7/36 workers exposed to an unspecified form of cyanide at mean concentrations of 6.416–10.375 ppm for 5–15 years from Egyptian electroplating factories complained of precordial pain, compared to 1/20 unexposed referents (El Ghawabi et al. 1975). The source of cyanide exposure was an electroplating bath containing sodium cyanide and copper cyanide. In 36 workers formerly exposed to 15 ppm hydrogen cyanide for a mean duration of 11 months in a silver-reclaiming facility, 31% of workers recalled chest pain and 14% of workers recalled palpitations during employment (Blanc et al. 1985). When workers were qualitatively stratified into low-, moderate-, and high-exposure levels based on job title, a dose-relationship between exposure and self-

reported incidence of chest pain was observed (incidence of palpitations was not evaluated for exposure-response). Limitations of this study include lack of quantitative exposure levels for the low-, moderate-, and high-exposure groups and reliance on self-reporting of symptoms that occurred during employment that ceased 7–30 months prior to the assessment. Additionally, findings in both studies are confounded by other known chemical co-exposures, including metals, cleaners, and cutting oils.

A limited number of studies evaluated cardiovascular endpoints in animals following inhalation exposure to cyanide compounds. Bradycardia, arrhythmias, and T-wave abnormalities were observed in monkeys exposed to 100 ppm hydrogen cyanide for 30 minutes (Purser et al. 1984). While these data suggest cyanide-related changes in cardiac function, use of a single animal per exposure level and lack of a concurrent control preclude inclusion in the LSE table. Increased cardiac-specific creatine phosphokinase activity was measured in blood samples from rats 2 hours after 12.5 minutes of exposure to 200 ppm hydrogen cyanide for 20 days at 4-day intervals between exposures (O'Flaherty and Thomas 1982). However, no treatment-related changes were found in the hearts at histopathology. In addition, no cardiovascular effects were reported at necropsy in rats and monkeys intermittently exposed to 25 ppm cyanogen for 6 months (Lewis et al. 1984). As discussed in Section 2.1, cyanogen studies are not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Several case studies also reported cardiovascular effects in humans after oral exposure to cyanide. Weak, shallow pulse and inaudible heart sounds were observed in a comatose man on hospital admission after ingestion of ≈15 mg CN^-/kg as potassium cyanide (Liebowitz and Schwartz 1948). Following gastric lavage and glucose infusion, the pulse rate and blood pressure became elevated. An enlarged heart was noted. No cardiovascular effects were reported during the recovery. In another study, children poisoned by apricot pits had hypotension upon hospital admission (Lasch and El Shawa 1981).

Cardiovascular function was not evaluated in animal studies following drinking water or dietary exposure. No treatment-related changes in heart weight or histology were seen in rats or mice exposed to doses up to 12.5 or 28.8 mg CN^-/kg/day, respectively, as sodium cyanide in the drinking water for 13 weeks (NTP 1993). No treatment-related effects on heart histopathology and no change in cardiac tissue levels of aspartate aminotransferase (AST) or alkaline phosphatase (ALP) were observed in male rabbits that ingested 20 mg CN^-/kg/day as potassium cyanide via the diet for 40 weeks (Okolie and Osagie 2000). While no changes in heart weight or histology were observed in rats exposed to an estimated dose of 10.4 mg CN^-/kg/day as hydrogen cyanide in their feed for 2 years, the reliability of the dose is low

because evaporation of the cyanide from the feed resulted in unstable cyanide levels throughout the experiment and uncertainties as to the dose-response for cyanide (Howard and Hanzal 1955). Due to low confidence in dose estimates, the 2-year dietary study is omitted from the LSE table.

Cardiovascular function was assessed in animal studies that employed bolus dosing (i.e., gavage, buccal bolus). It is noted that histopathological changes in the cardiovascular system were observed at doses below those eliciting no effects following drinking water or dietary exposure. This observation is likely because bolus administration may overwhelm detoxification processes. These studies are discussed below.

An acute, single gavage dose of 3.2 mg CN^-/kg/day as potassium cyanide to adult mice resulted in decreased systolic, diastolic, and mean arterial blood pressure and decreased heart rate that were observed beginning immediately upon dosing through 1 hour post dosing (Hawk et al. 2016). Following dose administration, four mice (two mice per sex) experienced cardiovascular events on the electrocardiogram (ECG); however, it was unclear whether these were treatment-related as the incidence was low and the events were varied. There were no effects noted on heart weight or histopathology in either adult or juvenile mice exposed once to 3.2 mg CN^-/kg/day as potassium cyanide; cardiovascular function was not assessed in juvenile mice (Hawk et al. 2016). In another study, a single gavage dose of up to 4.6 mg CN^-/kg/day as potassium cyanide did not result in any weight or histological changes to the heart nor any significant effects on serum levels of biomarkers of cardiac damage (Sabourin et al. 2016). In a 10-day gavage study, rats given 12 mg CN–/kg/day had increased vascular resistance in the brain characterized by decreased lumen size and arterial wall cellular degeneration in the middle cerebral artery. This resulted in responsive dilation of the common carotid artery (increased diameter) (Ogundele et al. 2014a). Following a 10-day recovery period, attenuation of cyanide-related vascular effects was observed. There were no treatment-related effects on contractile strength or electrophysiology of the aortic ring in rabbits following gavage of 0.15 mg CN^-/kg/day as potassium cyanide for 25 days (Ozolua et al. 2007). No significant histopathological changes were observed in rats exposed to 2.6 or 7.8 mg CN^-/kg/day as potassium silver cyanide for 90 days (Gerhart 1987).

As discussed in Section 2.1, animal studies on cassava are discussed in this profile, but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Dogs fed a diet of cassava ingested an estimated 1.04 mg CN^-/kg/day for 14 weeks and exhibited hemorrhage, pyknotic nuclei, and swelling of muscle fibers in the myocardium (Kamalu 1993). Dogs similarly fed rice to which 1.04 mg CN^-/kg food was added (sodium cyanide was added to release hydrogen cyanide during the

cooking process) did not show any apparent cardiovascular effects (Kamalu 1993), suggesting that observed cardiovascular effects in cassava-fed dogs were attributable to other compounds found in cassava root and/or interactions between cyanide and other compounds. However, findings in this study were confounded by concurrent diseases in study animals requiring pharmaceutical intervention (Kamalu 1991, 1993); therefore, the sodium cyanide study is omitted from the LSE table.

In a dermal exposure study, peripheral vasoconstriction and gross plasma extravasation were reported in a man who accidentally fell into a cistern with hot copper cyanide (Dodds and McKnight 1985). Palpitations were recorded in three men who wore respiratory masks while working in an atmosphere containing 20,000 ppm hydrogen cyanide for 8–10 minutes (Drinker 1932). The masks were reported to give excellent respiratory protection. Therefore, the effects seen in these men may have been due to dermal exposure.

No studies were located regarding cardiovascular effects in animals after dermal exposure to cyanide.

Mechanisms of cardiovascular toxicity. While some of the reported cardiovascular effects associated with cyanide exposure may be attributable to general cyanide histotoxic anoxia (see Section 2.21) or secondary to CNS depression (see Section 2.15), results of *in vitro* studies suggest an interaction between calcium ions and cyanide in cardiovascular effects (Allen and Smith 1985; Robinson et al. 1985a). It has been demonstrated that exposure to cyanide in metabolically depleted ferret papillary muscle eventually resulted in elevated intracellular calcium levels, but only after a substantial contracture develops (Allen and Smith 1985). The study authors proposed that intracellular calcium may precipitate cell damage and arrhythmias. Cheung et al. (2019) demonstrated that, *in vitro*, cyanide induces calcium influx by activating protein kinase C epsilon, which phosphorylates the L-type calcium channel on myocytes (Cheung et al. 2019). Additionally, a number of gene expression changes in the transcriptome from pathways associated with cardiac injury, such as angiogenesis, cardiac contractility, and fibrogenesis, were observed in mice following exposure to 327 ppm hydrogen cyanide for 40 minutes, a concentration that was lethal to 8/24 mice (Ma et al. 2021).

Franchini and Krieger (1993) produced selective denervation of the aortic and carotid bifurcation areas, and confirmed the carotid body chemoreceptor origin of cardiovascular, respiratory, and certain behavioral responses to cyanide in rats. Bradycardia and hyperventilation induced by cyanide are typical responses evoked by carotid body chemoreceptor stimulation (Franchini and Krieger 1993).

2.6 GASTROINTESTINAL

Gastrointestinal effects reported in occupational studies of cyanide exposure are probably provoked by CNS effects and/or by irritation of the gastric mucosa in cases in which the gas is swallowed during breathing. In an occupational hygiene study in three electroplating factories, an increased incidence of self-reported vomiting was observed in 36 male workers exposed to sodium cyanide and copper cyanide for 5–15 years, compared to 20 unexposed referents (El Ghawabi et al. 1975). Measured mean concentrations of "cyanides" (not further characterized) were 6.416–10.375 ppm. Nausea or vomiting was also reported in 69% of workers formerly exposed to 15 ppm hydrogen cyanide for a mean duration of 11 months in a silver reclaiming facility (Blanc et al. 1985). When the 36 workers were qualitatively stratified into low-, moderate-, and high-exposure levels based on job title, a dose-relationship between exposure and reported incidence of nausea and vomiting was observed. Limitations of this study include lack of quantitative exposure levels for the low-, moderate-, and high-exposure groups and reliance on self-reporting of symptoms that occurred during employment that ceased 7–30 months prior to the assessment. Additionally, findings in both studies are confounded by other known chemical co-exposures, including metals, cleaners, and cutting oils.

Information regarding gastrointestinal effects in animals exposed via inhalation is limited to a report of vomiting in dogs exposed to 45 ppm hydrogen cyanide for 28–96 days (Valade 1952). This study is omitted from the LSE table due to poor reporting of study design and results and lack of a concurrent control group.

Gastrointestinal effects observed in acute oral cyanide poisoning cases in humans are attributed to alkaline properties of cyanide compounds, resulting in corrosive effects in the gastrointestinal tract. Vomiting was reported in children who ingested a large number of apricot pits (Lasch and El Shawa 1981) and in a man who ingested 7.6 mg CN^-/kg in a suicide attempt (Goodhart 1994). Gastrointestinal spasms were reported in a man who accidentally ingested (and inhaled) an unknown amount of potassium cyanide (Thomas and Brooks 1970). Gastric surgery for extensive necrosis had to be performed in a man after he ingested an unknown amount of sodium cyanide (Grandas et al. 1989).

Data pertaining to potential gastrointestinal effects in animals following oral exposure to cyanide are limited, but do not suggest that the gastrointestinal tract is a primary target of cyanide toxicity. No histopathological changes were observed in the gastrointestinal tract in rats or mice exposed to doses up to 12.5 or 28.8 mg CN^-/kg/day as sodium cyanide, respectively, in drinking water for 13 weeks (NTP

1993). Diarrhea was observed in rats treated orally with 14.5 mg CN^-/kg/day copper cyanide for 90 days (Gerhart 1986). However, since diarrhea has been observed in both humans and animals following oral exposure (ATSDR 2024), the diarrhea was probably due to the toxicity of copper. Therefore, gastrointestinal data from Gerhart (1986) are omitted from the LSE table. No gastrointestinal effects were found in rats exposed to 7.8 mg CN^-/kg/day as potassium silver cyanide for 90 days (Gerhart 1987). Chronic intestinal inflammation occurred in dogs exposed to ≥0.27 mg CN^-/kg/day as sodium cyanide via capsules for 14.5 months (Hertting et al. 1960). However, this study is omitted from the LSE table due to lack of concurrent control and use of only one animal per dose group.

Reduced height of the stratum corneum of the oral mucosa was observed in hamsters administered 14.7 mg CN^-/kg/day as potassium cyanide directly to the cheek pouch mucosa for 90 days (Salum et al. 2006). No other histological changes (e.g., epithelial changes, inflammation) were evident. The biological relevance of this portal-of-entry effect is unclear.

No studies were located regarding gastrointestinal effects in humans after dermal exposure to cyanide. Acute-duration dermal exposure of guinea pigs to an unknown concentration of hydrogen cyanide resulted in submucous hemorrhages in the stomach as observed at necropsy (Fairley et al. 1934).

2.7 HEMATOLOGICAL

There is limited evidence of altered hematological endpoints following occupational exposure to cyanide in electroplating plants; however, findings are confounded by co-exposure to copper, a known hematotoxic agent. In an occupational hygiene study in three electroplating factories, increases in hemoglobin levels and percent lymphocytes were observed in 36 male workers exposed to sodium cyanide and copper cyanide for 5–15 years, compared to 20 unexposed referents (El Ghawabi et al. 1975). Additionally, punctate basophilia of erythrocytes, which indicated toxic poisoning, was present in 28 of 36 subjects. Measured mean concentrations of "cyanides" (not further characterized) were 6.416–10.375 ppm. However, exposure to copper, a known hematotoxic agent, also occurred during the electroplating operations. In another study (Kumar et al. 1992), an increase in neutrophil values, an increase in erythrocyte sedimentation rate, and a decrease in hemoglobin levels (compared to normal clinical ranges) were noted in 34 male workers exposed to unspecified concentrations of hydrogen cyanide for an unspecified duration during case hardening and electroplating.

No studies were located regarding hematological effects in animals after inhalation exposure to hydrogen cyanide. No hematological effects were found in rats or monkeys intermittently exposed to 25 ppm cyanogen for 6 months (Lewis et al. 1984). As discussed in Section 2.1, this study is not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Information regarding hematological effects in humans after oral exposure to cyanide is limited. In a case report, no adverse hematologic effects were reported in a man who ingested 15 mg CN^-/kg as potassium cyanide (Liebowitz and Schwartz 1948).

A limited number of studies evaluated hematological effects in animals following oral exposure. As discussed below, no adverse effects were observed in drinking water studies. Effects observed at lower doses in gavage studies may be attributable to overwhelming detoxification processes and/or other cations in the administered compound (e.g., copper).

No treatment-related hematological effects were observed in rats or mice exposed to doses of up to 12.5 or 28.8 mg CN^-/kg/day, respectively, as sodium cyanide in drinking water for 13 weeks (NTP 1993; Tyner and Greeley 2023). Decreased erythrocyte counts, hemoglobin levels, packed cell volume, and mean corpuscular volume were observed in rabbits exposed to 1.2 mg CN^-/kg/day as potassium cyanide via gavage for 40 days (Avais et al. 2014).

Hemolytic anemia, characterized by decreased erythrocytes, hemoglobin concentrations, and hematocrit, was observed in rats given a gavage dose of 14.5 mg CN^-/kg/day as copper cyanide for 90 days (Gerhart 1986). The diagnosis of anemia was supported by microscopic findings of pigmentation of the spleen and liver, presence of hemoglobin in the cytoplasm of the renal convoluted tubule epithelium, and hyperplasia of hematopoietic tissue (spleen and bone marrow). Decreased hemoglobin was observed also at 4.35 mg CN^-/kg/day after 90 days. Since hemolytic anemia is characteristic of copper toxicity; it is unclear whether the hematological effects can be partially attributed to copper toxicity rather than to cyanide toxicity; thus, the data are omitted from the LSE table. Increased mean corpuscular volume, mean corpuscular hemoglobin concentration, and spleen weights were indicative of hematological effects in rats exposed to 7.8 mg CN^-/kg/day as potassium silver cyanide for 90 days by gavage (Gerhart 1987). No effects were found at 2.6 mg CN^-/kg/day. Due to unknown contribution of silver to the hematological effects, these data are omitted from the LSE table.

No studies were located regarding hematological effects in humans or animals after dermal exposure to cyanide.

2.8 MUSCULOSKELETAL

Data regarding potential musculoskeletal effects in humans or animals after exposure to cyanide or cyanide compounds are extremely limited.

Muscular rigidity was observed in humans after acute oral cyanide poisoning (Grandas et al. 1989) and rhabdomyolysis, a clinical syndrome characterized by skeletal muscle injury, was observed in a man who ingested 0.57 mg CN^-/kg as potassium cyanide in a suicide attempt (Saincher et al. 1994). Clinical laboratory findings of increased serum creatinine and serum creatine kinase in this case are evidence of the observed breakdown of muscle fibers.

No musculoskeletal effects were observed at necropsy in rats or monkeys intermittently exposed to 25 ppm cyanogen for 6 months (Lewis et al. 1984). As discussed in Section 2.1, this study is not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

2.9 HEPATIC

Data pertaining to potential hepatotoxicity in humans are limited to two occupational studies. An increase in serum ALP was noted in 10 of the 15 electroplating and case hardening workers exposed to an unspecified concentration of hydrogen cyanide for an unspecified duration, compared to normal clinical ranges (Kumar et al. 1992). Serum bilirubin was found to be within the normal range in all 15 workers. Serum AST was marginally elevated in 20 workers exposed to unspecified levels of hydrogen cyanide in the cassava processing industry, compared to 20 age-matched referents; however, AST levels in workers were within the normal clinical range (Janagam et al. 2008). Serum alanine aminotransferase (ALT) and total protein levels were comparable between workers and unexposed referents. Cassava workers showed an altered lipid profile, compared with unexposed referents, with elevated total serum cholesterol, triglycerides, high-density lipoproteins (HDL), and very low-density lipoproteins (VLDL); levels of low-density lipoproteins (LDL) were comparable between groups (Janagam et al. 2008). Of these findings, the study authors proposed that the elevated triglycerides show the most clinical relevance and are likely due to altered energy metabolism (decreased glycolysis) associated with cyanide exposure.

No studies were located regarding hepatic effects in animals after inhalation exposure to hydrogen cyanide. No changes in clinical chemistry or liver histology were observed in rats or monkeys exposed to 25 ppm cyanogen for 6 months (Lewis et al. 1984). As discussed in Section 2.1, these studies are not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

There is inconsistent evidence of hepatotoxicity in animals following exposure to cyanide via drinking water, with findings in short-duration studies not confirmed in longer-duration studies. Acute-duration oral exposure to potassium cyanide at doses ≥1.2 mg CN^-/kg/day in drinking water on GDs 6–20 resulted in mild hepatic vacuolation and mild-to-moderate hepatic congestion in 100% of examined dams (de Sousa et al. 2007). These effects persisted in dams similarly treated on GDs 6–20 but sacrificed on postnatal day (PND) 22 after a 3-week recovery period. Although incidences were not reported in this study, the study authors only reported severity scores when all animals analyzed showed the same alteration. Therefore, it is unclear if hepatic lesions were observed in some animals (but not all) at the lowest dose tested (0.4 mg CN^-/kg/day); thus, a NOAEL for hepatic effects could not be established for this study. All dams similarly exposed to 6.4 mg CN^-/kg/day as potassium thiocyanate also exhibited mild hepatocyte vacuolation and bile duct proliferation at GD 20 and PND 22; again, it is unclear if hepatic lesions were observed in some (but not all) of the dams exposed to lower potassium thiocyanate doses (0.2 or 0.6 mg CN^-/kg/day). In a 15-day study, severe cytoplasmic vacuolization of hepatocytes was observed in male rats that ingested 3.6 mg CN^-/kg/day as potassium cyanide in drinking water (Sousa et al. 2002); hepatic effects were reportedly minimal at 0.36–1.2 mg CN^-/kg/day and absent at 0.12 mg CN^-/kg/day. However, since incidence data for histopathological lesions were not provided, significance of the findings cannot be determined; therefore, these data are omitted from the LSE table. In contrast to the 14- to 15-day studies, no adverse effects on liver weight or histology were observed in rats or mice exposed to up to 12.5 or 28.8 mg CN^-/kg/day, respectively, as sodium cyanide in the drinking water for 13 weeks (NTP 1993; Tyner and Greeley 2023). Drinking water studies did not find any exposure-related changes in hepatic clinical chemistry values (Sousa et al. 2002; NTP 1993; Tyner and Greeley 2023).

Liver damage was reported in rabbits exposed to dietary sodium or potassium cyanide. Based on the limited database, it is unknown if these findings represent a species (rabbit versus rodent) or route (dietary versus drinking water) susceptibility. Liver damage was observed in rabbits that ingested 15 mg CN^-/kg/day from sodium cyanide in feed for 4 weeks, as indicated by increased serum enzyme activities (ALP, ALT, LDH), histopathological lesions (necrosis, fatty degeneration, and congestion), and decreased hepatic enzyme ALP activities (Okolie and Iroanya 2003). Increased serum levels of ALT,

ALP, LDH, and sorbitol dehydrogenase (SDH) and focal hepatocellular congestion and necrosis were also observed in male rabbits that ingested 20 mg CN^-/kg/day as potassium cyanide via the diet for 10 months (Okolie and Osagie 1999). For both studies, quantitative data were only provided for serum enzyme data. Since incidence data were not reported for liver lesions, the significance of the findings could not be determined. Therefore, histological findings from the rabbit studies are omitted from the LSE table.

In rodents, dietary cyanide exposure is limited to a single chronic-duration study in which no hepatic effects were observed in rats following exposure to an estimated dose of 10.4 mg CN^-/kg/day as hydrogen cyanide in their feed for 2 years (Howard and Hanzal 1955). In this study, the reliability of the dose is low because evaporation of the cyanide from the feed resulted in unstable cyanide levels throughout the experiment and uncertainties as to the dose-response for cyanide. Due to low confidence in dose estimates, this study in rats is omitted from the LSE table.

A gavage study in rabbits supports that this species may be susceptible to hepatotoxicity following exposure to cyanide; however, susceptibility may be attributable to overwhelming the detoxification processes. Increased serum levels of ALT, AST, ALP, bilirubin, and LDH, along with decreased serum levels of total serum albumin and protein, were observed in rabbits given 1.2 mg CN^-/kg/day as potassium cyanide via gavage for 40 days (Avais et al. 2018). Liver histology was not assessed in this study. No effects were observed on serum levels of ALT or AST in rabbits gavaged with very low doses of 0.15 mg CN^-/kg/day as potassium cyanide for 25 days (Ozulu et al. 2007).

In bolus studies in rodents, no changes in liver weight or histology were observed in mice at single oral doses up to 4.6 mg CN^-/kg/day as potassium cyanide (Hawk et al. 2016; Sabourin et al. 2016). In rats exposed 0.56 mg CN^-/kg/day as potassium cyanide for 90 days via gavage, increases in hepatic microgranuloma, spotty necrosis, and portal inflammation were qualitatively described (Mathangi et al. 2011). However, due to lack of quantitative histopathology data reporting, statistical significance of the findings could not be determined. Therefore, these findings are omitted from the LSE table. There were no reported changes in liver weights in this study. Rats treated for 90 days by gavage with 14.5 mg CN^-/kg/day as copper cyanide had increased serum levels of ALT, AST, bilirubin, and ALP, and decreased globulin levels in the blood (Gerhart 1986). Liver necrosis was observed in low incidences (not quantified) in both sexes at 14.5 mg CN^-/kg/day and in females at 4.35 mg CN^-/kg/day. The hepatic effects of copper cyanide could possibly be due to the toxicity of copper rather than of cyanide and are

therefore omitted from the LSE table. No hepatic effects were reported in rats exposed by gavage to 7.8 mg CN^-/kg/day as potassium silver cyanide for 90 days (Gerhart 1987).

As discussed in Section 2.1, animal studies on cassava are discussed in this profile, but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Serum levels of ALT, AST, and ALP were elevated in rats exposed to ≥5.50 mg CN^-/kg/day as cassava in the diet for 28 days (Okafor et al. 2006; Udeme et al. 2015). No adverse changes in serum hepatic enzymes were noted in rats similarly exposed to 6.27 mg CN^-/kg/day for 7 days (Okafor et al. 2006). Periportal vacuolation and congestion were observed in the livers of dogs fed 1.04 mg CN^-/kg/day in rice as cassava for 14 weeks (Kamalu 1993). Dogs similarly fed rice to which 1.04 mg CN^-/kg food was added (sodium cyanide was added to release hydrogen cyanide during the cooking process) did not show any apparent hepatic effects (Kamalu 1993), suggesting that observed hepatic effects in cassava-fed dogs were attributable to other compounds found in cassava root and/or interactions between cyanide and other compounds. However, findings in this study were confounded by concurrent diseases in study animals requiring pharmaceutical intervention (Kamalu 1991, 1993); therefore, the sodium cyanide study is omitted from the LSE table.

No studies were located regarding hepatic effects in humans or animals after dermal exposure to cyanide.

2.10 RENAL

Data pertaining to potential renal toxicity in humans following inhalation exposure are limited. Serum creatinine was elevated in 20 workers exposed to unspecified levels of hydrogen cyanide in the cassava processing industry, compared to 20 age-matched referents; however, levels in workers were within the normal clinical range (Janagam et al. 2008). In a case report, anuria followed by polyuria was observed in a man who was occupationally exposed to 200 ppm hydrogen cyanide for an unspecified length of time (Singh et al. 1989).

No studies were located regarding renal effects in animals after inhalation exposure to hydrogen cyanide. No histopathological changes were observed in kidneys of rats or monkeys intermittently exposed to 25 ppm cyanogen for 6 months (Lewis et al. 1984). As discussed in Section 2.1, these studies are not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Data pertaining to potential renal toxicity in humans following oral exposure are limited to a single case report of albuminuria in a man during the first 2 days after ingestion of 15 mg CN^-/kg as potassium cyanide in a suicide attempt (Liebowitz and Schwartz 1948).

Studies reporting renal effects in animals exposed to cyanide via drinking water and the diet are mixed. Multiple studies reported histological lesions in the kidney, but few reported incidence data, precluding independent assessment of the significance of the findings. Therefore, histopathology data from drinking water and dietary studies discussed below that lack quantitative data are omitted from the LSE table.

No renal lesions were observed in rat dams exposed to doses up to 12 mg CN^-/kg/day as potassium cyanide or up to 6.4 mg CN^-/kg/day as potassium thiocyanide in drinking water on GDs 6–20 (de Sousa et al. 2007). In a 15-day study, renal congestion and cytoplasmic vacuolization of the proximal tubular epithelium (moderate-to-severe) were qualitatively reported in male rats exposed to 1.2–3.6 mg CN^-/kg/day as potassium cyanide in drinking water (Sousa et al. 2002); lesions were reportedly minimal in severity at 0.3 mg CN^-/kg/day and absent at 0.12 mg CN^-/kg/day. In contrast, there were no treatment-related changes in kidney weights or histology in rats or mice exposed to up to 12.5 or 28.8 mg CN^-/kg/day as sodium cyanide in the drinking water for 13 weeks (NTP 1993; Tyner and Greeley 2023). Drinking water studies did not find any exposure-related changes in renal clinical chemistry values in rodents (NTP 1993; Sousa et al. 2002; Tyner and Greeley 2023).

Renal damage was reported in rabbits exposed to dietary sodium or potassium cyanide. Based on the limited database, it is unknown if these findings represent a species (rabbit versus rodent) or route (dietary versus drinking water) susceptibility. In rabbits, evidence of tissue damage (increased LDH in kidney tissue) was observed, and renal tubular and glomerular necrosis were qualitatively reported following dietary exposure to 15 mg CN–/kg/day as sodium cyanide for 4 weeks (Okolie and Iroanya 2003) or 20 mg CN^-/kg/day as potassium cyanide for 40 weeks (Okolie and Osagie 1999). In the 40-week study, increased serum levels of creatinine and urea were also observed.

In rodents, dietary cyanide exposure is limited to a single chronic-duration study in which no renal effects were observed in rats following exposure to an estimated dose of 10.4 mg CN^-/kg/day as hydrogen cyanide in their feed for 2 years (Howard and Hanzal 1955). In this study, the reliability of the dose is low because evaporation of the cyanide from the feed resulted in unstable cyanide levels throughout the experiment and uncertainties as to the dose-response for cyanide. Due to low confidence in dose estimates, this study is omitted from the LSE table. Cloudy swelling of epithelial cells of renal tubules

was reported in three dogs exposed to cyanide via capsule for 14.5 months; each dog was exposed to a different dose of sodium cyanide (ranging from 0.27 to 1.68 mg CN^-/kg/day) (Hertting et al. 1960). However, this study is omitted from the LSE table due to lack of concurrent control and use of only one animal per dose group.

Consistent with drinking water and dietary studies, renal findings following exposure to cyanide compounds via bolus dosing (i.e., gavage, buccal bolus) are inconsistent between studies and species. Any observed increases in susceptibility via this route may be attributable to overwhelming detoxification processes and/or other cations in the administered compound (e.g., copper). These studies are discussed below.

A single gavage dose of up to 4.6 mg CN^-/kg/day as potassium cyanide resulted in minimal-to-mild acute tubular necrosis in adult male mice sacrificed 24 hours post-exposure (1/4) or 7 days post-exposure (2/4); lesions were not observed 28 days post-exposure (Sabourin et al. 2016). However, in the same study, Sabourin et al. (2016) found no treatment-related renal effects changes in adult female mice. In similarly exposed juvenile mice, single gavage doses up to 4.16 mg CN^-/kg/day as potassium cyanide did not induce changes in kidney weight or histology (Sabourin et al. 2016). Hawk et al. (2016) found no treatment related renal effects in adult or juvenile mice exposed to a single oral dose of 3.2 mg CN^-/kg/day as potassium cyanide. Rabbits administered 1.2 mg CN^-/kg/day as potassium cyanide via gavage for 40 days exhibited increased serum levels of creatinine, urea, and uric acid, and decreased serum levels of albumin and total protein; renal histology was not assessed in this study (Avais et al. 2018). No effects on kidney weights or histology were observed in rats given gavage doses of 0.56 mg CN^-/kg/day as potassium cyanide for 90 days (Mathangi et al. 2011). Decreased kidney weights were observed in rats treated with 14.5 mg CN^-/kg/day as copper cyanide for 90 days (Gerhart 1986); no changes were reported at 4.35 mg/kg/day. However, as copper toxicity may have contributed to observed kidney effects, these data are omitted from the LSE table. Increased blood urea nitrogen was found in rats exposed to 7.8 mg CN^-/kg/day as potassium silver cyanide, but not at 2.6 mg CN^-/kg/day (Gerhart 1987). The contribution of silver to this effect is not known; therefore, these data are omitted from the LSE table.

Data pertaining to potential renal toxicity in humans following dermal exposure are limited to a single case report of transitory oliguria (scanty urination) in a patient who accidentally fell into a cistern containing 1,000 gallons of hot copper cyanide and remained there for 3 minutes before being rescued (Dodds and McKnight 1985). No studies were located regarding renal effects in animals after dermal exposure to cyanide.

2.11 DERMAL

Data pertaining to potential dermal effects of cyanide exposure in humans are limited to occupational exposure studies. Reported dermal effects in these studies may be due to direct dermal exposure to vapors, rather than due to systemic effects of cyanide. Brick-red chemical burns on the skin were observed in a man who was occupationally exposed to 200 ppm hydrogen cyanide for an unspecified length of time (Singh et al. 1989). Skin rash was reported in 42% of workers exposed to 15 ppm hydrogen cyanide for an average of 11 months in a silver-reclaiming facility, with 25% of workers reporting persistent rash at a mean duration of 10.5 months post-employment (Blanc et al. 1985). When the 36 workers were qualitatively stratified into low-, moderate-, and high-exposure levels based on job title, a dose-relationship was observed between exposure and self-reported incidence of persistent skin rash. In contrast, dermatitis was not reported in 36 male workers exposed to mean concentrations of an unspecified form of cyanide of 6.416–10.375 ppm for 5–15 years (El Ghawabi et al. 1975). The source of cyanide exposure was electroplating bath fluid containing sodium cyanide and copper cyanide.

No studies were located regarding dermal effects in animals after inhalation exposure to hydrogen cyanide. No dermal lesions were found in rabbits exposed to 5,000 ppm cyanogen for 8 hours (McNerney and Schrenk 1960). As discussed in Section 2.1, these studies are not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Data pertaining to dermal effects in animals following oral exposure are limited. No histopathological changes of the skin were observed in rats or mice following exposure to doses up to 12.5 or 28.8 mg CN^-/kg/day as sodium cyanide, respectively, in drinking water for 13 weeks (NTP 1993). During intermediate-duration exposure, discolored inguinal fur was found in rats exposed for 90 days to 14.5 mg CN^-/kg/day by gavage as copper cyanide (Gerhart 1986) and to 2.6 mg CN^-/kg/day as potassium silver cyanide (Gerhart 1987). However, as these findings were likely due to the metal components of the administered compounds, these data are omitted from the LSE table.

No standard dermal irritation studies in animals with exposure level data were identified for cyanide compounds. Vascular congestion was reported in the skin of guinea pigs after exposure to unknown doses of hydrogen cyanide for 65 minutes (Fairley et al. 1934).

2.12 OCULAR

Evidence from humans and animals indicate that direct ocular exposure to cyanide (or cyanide compounds, including vaporized forms) is irritating to the eyes. Evidence for ocular effects from systemic exposure to cyanide (e.g., via oral exposure) are extremely limited.

In occupational studies, eye irritation was reported by 58% of workers exposed to 15 ppm hydrogen cyanide (Blanc et al. 1985) and 25% of workers exposed to mean concentrations of an unspecified form of cyanide of 6.416–10.375 ppm (El Ghawabi et al. 1975). The source of cyanide exposure in the study by El Ghawabi et al. (1975) was electroplating bath fluid containing sodium cyanide and copper cyanide. Subjective complaints of eye irritation were also reported in a cohort of 56 workers exposed to hydrogen cyanide vapor during heat treatment (case hardening) and electroplating, including lacrimation (17.8%) and congestion of the eyes (14.3%); physical exam revealed conjunctivitis (8.9%) and exophthalmos (3.6%) in some workers (Kumar et al. 1992). Exposure levels were not reported in this study. Eye irritation was also reported in 15/20 (75%) of workers exposed to an unspecified concentration of hydrogen cyanide in the cassava processing industry (Janagam et al. 2008). The ocular effects reported in occupational studies may not be due solely to cyanide exposure, as workers may be exposed to a variety of chemicals that are irritating to the eyes.

Cyanogen caused eye irritation in volunteers during acute-duration exposure to 16 ppm (8 ppm cyanide) (McNerney and Schrenk 1960). No effect was observed in those exposed to 8 ppm cyanogen (4 ppm cyanide). Information regarding ocular effects in animals after inhalation exposure is limited to a report of eye irritation in rats acutely exposed (7.5–120 minutes) to 250 ppm cyanogen (125 ppm cyanide) (McNerney and Schrenk 1960). As discussed in Section 2.1, this study is not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

Macular degeneration and optic atrophy were reported in 20 West Africans who ingested cassava over an unspecified period (van Heijst et al. 1994). The mean levels of thiocyanate and cyanide in these patients were elevated but were not statistically different from controls (hospital staff). Individuals with other neurological lesions in addition to ocular effects had significantly elevated serum levels of thiocyanate and cyanide. The study authors indicated that nutritional deficiencies in the study population contributed to neuropathy. See Section 2.15 for more details on neurological effects associated with cassava ingestion.

Data on ocular effects following oral exposure in animals are limited. No exposure-related ophthalmological effects or histopathological lesions in the eye or optic nerve were reported in male rats exposed to doses up to 11.50 mg CN^-/kg/day as sodium cyanide in the drinking water for 13 weeks (Tyner and Greeley 2023). In gavage studies, ocular opacity was noted in rats exposed to 2.6 mg CN^-/kg/day as potassium silver cyanide for 90 days (Gerhart 1987). However, since it is likely that opacity resulted from deposition of silver, these data are omitted from the LSE table. No pathological findings were observed during ophthalmological examination of rats exposed to 14.5 mg CN^-/kg/day as copper cyanide for 90 days (Gerhart 1986).

Cyanide toxicity was tested in rabbits by applying 1.69–5.28 mg CN^-/kg/day as sodium cyanide to the inferior conjunctival sac of one eye (Ballantyne 1983b, 1988). Irritation, lacrimation, and conjunctival hyperemia were present immediately after the treatment. Keratitis developed in some rabbits after a cyanide application of 0.9 mg CN^-/kg as hydrogen cyanide, 2.1 mg CN^-/kg as sodium cyanide, or 2.5 mg CN^-/kg as potassium cyanide.

2.13 ENDOCRINE

The endocrine system, specifically the thyroid gland, has been identified as a potential target of toxicity in workers with occupational exposure to cyanide and populations with elevated cyanide exposure associated with dietary cassava intake. In animal studies, the thyroid is one of the most sensitive targets of oral toxicity; thyroid toxicity has not been evaluated following inhalation or dermal exposure in animals. Mechanistic data indicate that observed thyroid toxicity is attributable to the metabolite, thiocyanate, which competes with iodine for binding to the sodium-iodine symporter. Based on systematic review, the thyroid is a presumed target of toxicity following oral exposure based on a low level of evidence in humans, a moderate level of evidence in animals, and supporting mechanistic data (see Appendix C). Systematic review was not conducted for inhalation exposure due to inadequate dose-response data for that exposure route.

Epidemiological data pertaining to thyroid effects in occupationally exposed workers are presented in Table 2-5. Mean thyroid stimulating hormone (TSH) levels were 29% higher in a group of 36 male workers formerly exposed to 15 ppm hydrogen cyanide for a mean duration of 11 months in a silver-reclaiming facility, compared to 100 laboratory reference values (Blanc et al. 1985). Serum triiodothyronine (T3) levels were comparable to reference values in the entire cohort. However, a different pattern was observed in a subgroup of the 16 highest exposed workers (determined by job title).

This subgroup had mildly elevated serum T3 levels (8%) relative to laboratory reference values; however, serum TSH levels were comparable to laboratory reference values. No exposure-related changes were observed for serum thyroxine (T4). The study investigators indicated that the absence of T4 abnormalities could be accounted for by the time lapse between exposure and examination (median 10.5 months) (Blanc et al. 1985). No evidence of thyroid enlargement was observed in the 36 formerly exposed workers. Limitations of this study include lack of quantitative exposure levels for the low-, moderate-, and high-exposure groups, reliance on self-reporting of symptoms that occurred during employment that ceased 7–30 months prior to the assessment, and co-exposure to other chemicals during the silver-reclaiming process.

Table 2-5. Results of Epidemiological Studies Evaluating Occupational Exposure to Cyanide and Thyroid Effects

Reference, study type, and population	Measure of exposure	Outcome evaluated	Result
Banerjee et al. 1997 Cross-sectional study; 35 male workers exposed to cyanide in electroplating factory for >5 years and 35 unexposed referents (India)	Mean±standard deviation serum thiocyanate concentrations: Exposed: 316±15 µmol/L Referents: 90.8±9.02 µmol/L	Serum T3	↓ (exposed versus referent)
		Serum TSH	↑ (exposed versus referent)
		Serum T4	↓ (exposed versus referent)
Blanc et al. 1985 Retrospective cohort; 36 former workers from a silver-reclaiming facility (mean duration of employment was 11 months; mean duration elapsed since employment was 10.5 months) and 100 laboratory control referents (United States, Illinois)	Time-weighted average concentration of hydrogen cyanide (measured after plant ceased operations): 15 ppm Relative exposure index (REI) estimated based on job title; no quantitative exposure levels: Low (n=7) Moderate (n=13) High (n=16)	Serum T3	↔ (exposed versus referent) ↑ (high versus referent)
		Serum TSH	↑ (exposed versus referent) ↔ (high versus referent)
		Serum T4	↔ (exposed versus referent)
		Enlarged thyroid	↔

Table 2-5. Results of Epidemiological Studies Evaluating Occupational Exposure to Cyanide and Thyroid Effects

Reference, study type, and population	Measure of exposure	Outcome evaluated	Result
El Ghawabi et al. 1975 Cross-sectional; 36 male workers from three electroplating factories (9 from Factory A, 12 from Factory B, 15 from Factory C; employed 5–15 years) and 20 unexposed male referents (Egypt)	Mean (range) "cyanides" concentrations, ppm: Factory A: 10.375 (8.2–12.4) Factory B: 6.416 (4.2–8.8) Factory C: 8.083 (5.9–9.6) "Cyanides" measured were not further described; cyanide exposure evolved from plating bath containing sodium cyanide and copper cyanide.	Thyroid enlargement	↑ (workers versus referents)
		Thyroid iodine uptake	↑ (workers versus referents)

↑ = association; ↓ = inverse association; ↔ = no association; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

Thyroid effects were also noted in cross-sectional studies of electroplating workers; however, exposure to other chemicals such as cleaners and cutting oils also occurs during electroplating operations. Thyroid enlargement was present in 20 of 36 male workers exposed to mean concentrations of an unspecified form of cyanide of 6.416–10.375 ppm (El Ghawabi et al. 1975). The source of cyanide exposure was electroplating bath fluid containing sodium cyanide and copper cyanide. Additionally, thyroid ^{131}I uptake was significantly higher in the 36 male workers, compared to 20 unexposed male referents. The study authors proposed that this finding may be due to thiocyanate's ability to block iodine uptake and also compete with I^- as a substrate for the thyroid peroxidase, resulting in less "organification" of I_2 (decreasing the iodination of tyrosine to form iodotyrosine) by the thyroid gland. Since the workers were away from work on the 2 days preceding the test, the results may be explained on the basis of acute cyanide withdrawal, as with other anti-thyroid agents, where sudden cessation of the drug leads to rapid accumulation of iodine in the iodine-depleted gland (El Ghawabi et al. 1975). In another study, serum T3 and T4 levels were decreased and serum TSH levels were elevated in 35 nonsmoking male workers exposed to cyanide in an electroplating factory for >5 years, compared to 35 unexposed referents (Banerjee et al. 1997). External exposure concentrations were not reported, but mean serum thiocyanate concentrations were 316 and 90.8 µmol/L in workers and referents, respectively.

No inhalation studies in animals evaluating potential effects on the thyroid gland or other endocrine organs were identified.

Chronic-duration oral exposure to cyanide in humans who eat cassava as a main carbohydrate source of their diet has been associated with thyroid toxicity. The incidence of endemic goiter correlated with cassava intake in the Congo, where thyroid uptake of radioiodine was decreased in the goitrous area, compared with the controls (Delange and Ermans 1971). In another study, altered thyroid hormone parameters were measured in a village in Mozambique where an epidemic of spastic paraparesis was found, which was related to ingestion of cassava (Cliff et al. 1986). Increases in thyroid stimulating hormone levels and the ratio of T3 to T4 were detected in serum; consistent with these measurements, the study authors calculated a decrease in the index of free thyroxine (FT4I) and an increase in free triiodothyronine (FT3I). However, the incidence of endemic goiter was not elevated in this village. Examined individuals also had very high levels of thiocyanate in serum and urine (Cliff et al. 1986). Congenital hypothyroidism has been observed in some children who were exposed to increased thiocyanate levels because of the maternal cassava diet during pregnancy, as reviewed by Ermans et al. (1980). While observed effects may be associated with cyanide exposure, findings are confounded by exposures to several other known compounds that occur with cassava consumption. Additionally, individuals who rely heavily on cassava as a main source of carbohydrates in their diet may suffer from nutritional deficiencies (Makene and Wilson 1972; Osuntokun 1972; Osuntokun et al. 1969), which may further confound findings.

Thyroid effects were also found in some animals following oral exposure to cyanide via drinking water. Dose-related increases in the number of resorption vacuoles in the thyroid gland were observed in all male rats that ingested 0.12–3.6 mg CN^-/kg/day as potassium cyanide in drinking water for 15 days (Sousa et al. 2002). This finding is generally associated with increased activity of the thyroid; however, since plasma levels of T3 and T4 were unaffected by treatment, the findings are of unclear biological significance. Similarly, de Sousa et al. (2007) reported dose-related increases in the number of resorption vacuoles in female rat dams exposed to potassium thiocyanate or potassium cyanide in drinking water at doses ≥0.21 or 0.4 mg CN^-/kg/day, respectively, on GDs 6–20. However, the biological significance of the findings could not be determined in the absence of additional thyroid lesions or evaluation of serum thyroid hormone levels in rat dams. Therefore, NOAEL/LOAEL determinations could not be made for thyroid effects reported by Sousa et al. (2002) or de Sousa et al. (2007). In contrast to these studies, no histopathological lesions to the thyroid were observed in rats or mice exposed to dose up to 12.5 or 28.8 mg CN^-/kg/day, respectively, as sodium cyanide in drinking water for 13 weeks (NTP 1993; Tyner and Greeley 2023). However, elevated absolute and relative thyroid weights and decreased serum T4 levels were reported in rats at 11.50 mg CN^-/kg/day, but not at doses ≤3.96 mg CN^-/kg/day (Tyner and

Greeley 2023). These effects were no longer observed after a 70-day recovery period. The 13-week study by NTP (1993) did not assess thyroid weight or serum thyroid hormone levels.

Dietary studies in rats support altered thyroid function following intermediate-duration exposure to cyanide. Rats fed a diet containing 53 mg CN^-/kg/day as potassium cyanide for 4 months had a significant decrease in plasma T4 levels and thyroid T4 secretion rates; at 11 months, treated rats showed no significant decreases in plasma T4 concentrations, but T4 secretion rates remained depressed (Philbrick et al. 1979). Relative thyroid weights were also elevated at necropsy at 11.5 months; however, this finding is confounded by a concurrent decrease in body weight gain and lack of absolute thyroid weight data reporting. In rats similarly exposed to diets containing 47 mg CN^-/kg/day as potassium thiocyanate, decreased serum T4 was observed at both 4 and 11 months, with decreased thyroid T4 secretion rates at 4 months only (Philbrick et al. 1979).

Data from animal studies that employed bolus dosing (i.e., gavage, buccal bolus) are limited. Findings reported at doses below those associated with effects in drinking water and dietary studies may be attributable to overwhelming detoxification processes by bolus dosing.

No exposure-related changes in thyroid weight or histology were observed in mice exposed once to potassium cyanide at bolus doses up to 4.6 mg CN^-/kg as potassium cyanide; serum thyroid hormone levels were not assessed in these studies (Hawk et al. 2016; Sabourin et al. 2016). Additionally, no exposure-related changes in plasma levels of T3 and T4 or thyroid histology were observed in rats exposed to gavage doses up to 0.24 mg CN^-/kg/day as potassium cyanide for 3 months (Soto-Blanco et al. 2002). However, increased serum T3 and T4 levels were reported in rabbits exposed to 1.2 mg CN^-/kg/day as potassium cyanide for 40 days via gavage (Avais et al. 2018). Thyroid histology was not evaluated in the rabbit study.

There is limited evidence of toxicity in other endocrine organs following oral exposure to cyanide. Acute-duration oral exposure to potassium cyanide at 12 mg CN^-/kg/day in drinking water on GDs 6–20 resulted in moderate pancreas islet cell vacuolation in 100% of examined dams (de Sousa et al. 2007). These effects were transient and were no longer apparent in dams similarly treated on GDs 6–20, but sacrificed on PND 22 after a 3-week recovery period. Although incidences were not reported in this study, the study authors only reported severity scores when all animals analyzed showed the same alteration. Therefore, it is unclear if pancreatic lesions were observed in some animals (but not all) at the lower doses (≤1.2 mg CN^-/kg/day); thus, a NOAEL for pancreatic effects could not be established for this

study. However, elevated serum glucose levels were observed at 12 mg CN^-/kg/day only; glucose levels at ≤1.2 mg CN^-/kg/day were comparable to control. Pancreatic lesions and alterations in serum glucose levels were not observed in dams similarly exposed to 6.4 mg CN^-/kg/day as potassium thiocyanate (de Sousa et al. 2007). In longer-duration studies, no exposure-related lesions were observed in the pancreas, adrenal gland, or pituitary glands of rats or mice exposed to doses up to 12.5 or 28.8 mg CN^-/kg/day as sodium cyanide for 13 weeks (NTP 1993). The histology of the pancreas was unaffected in male rabbits that ingested 20 mg CN^-/kg/day as potassium cyanide via the diet for 40 weeks (Okolie and Osagie 2000).

As discussed in Section 2.1, animal studies on cassava are discussed in this profile, but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Lesions in the adrenal gland (swelling of the adrenal cortex, hemorrhage, and fibrosis) and pancreas (hemorrhage, necrosis, fibrosis, and atrophy of the acinar tissue; fibrosis of islets of Langerhans) were observed in the dogs fed 1.04 mg CN^-/kg/day in rice as cassava for 14 weeks (Kamalu 1991, 1993). Similar effects were observed in dogs fed rice containing the same concentration of cyanide (sodium cyanide was added to release hydrogen cyanide during the cooking process), although hemorrhage was not observed in the pancreas and fibrosis was more prominent (Kamalu 1991, 1993). Dogs fed rice plus cyanide, but not dogs fed cassava diet, also showed decreased serum T3 levels and thyroid enlargement (Kamalu and Agharanya 1991). However, findings in this study were confounded by concurrent diseases in study animals requiring pharmaceutical intervention (Kamalu 1991, 1993); therefore, the sodium cyanide study is also omitted from the LSE table.

No studies were located regarding endocrine effects in humans or animals after dermal exposure to cyanide.

Mechanisms of Thyroid Toxicity. Thyroid effects following cyanide exposure can result from the interference of thiocyanate, a metabolite of cyanide, with iodine uptake and utilization in the thyroid gland (VanderLaan and Bissell 1946). In addition, thiocyanate may inhibit the iodination process, thus interfering with the binding of glandular iodine and reducing the formation of thyroxine (Ermans et al. 1972). This mechanism of action (competitive inhibition of the sodium-iodine symporter) has been proposed for other thyroid disrupting compounds, such as perchlorate, nitrate, chlorate, and fluoroborate (EPA 2010). Thiocyanate has a higher binding affinity for the sodium iodine symporter than the physiological ligand, iodine; however, it has a lower affinity than some other compounds (e.g., perchlorate) (De Groef et al. 2006; Tonacchera et al. 2004). This mode of action indicates that thyroid disruption will only be clinically relevant when thiocyanate levels are such that homeostatic processes

(e.g., stimulation of thyroid hormone production) are overwhelmed and clinical evidence of hypothyroidism is detected, such as thyroid gland enlargement, decreased thyroid hormones, and increased TSH (EPA 2010).

Fukayama et al. (1992) studied the antithyroid action of thiocyanate in a culture system of thyroid follicles. Thiocyanate concentrations equivalent to serum levels in smokers showed three independent antithyroid actions, including inhibition of iodide transport, inhibition of binding of iodide in the thyroid, and increased iodide efflux. The discrepancy in the potency of the antithyroid activity of thiocyanate *in vivo* and *in vitro* appears to be due to the presence of iodide and moieties such as the perchlorate ion, which is known to alter the effect of thiocyanate on the thyroid (Van Middlesworth 1986).

2.14 IMMUNOLOGICAL

Data pertaining to immunological effects in humans after exposure to cyanide are limited to a single cross-sectional study evaluating lymphocyte subpopulations in 17 male automotive painting workers exposed to inorganic cyanide compounds for 5–20 years, compared to 5 unexposed male referents (Haleem and Hussein 2024). Details on the referents were limited to the information on sex and age; mean ages were 33.11 years for exposed workers and 33.4 years for referents. The mean hydrogen cyanide level in workplace air was 2.8 ppm and mean plasma thiocyanate levels were 0.54 µM in referents, 1.78 µM in workers aged 22–33 years, and 1.99 µM in workers aged 33–44 years. Compared to unexposed referents, the percentage of CD3+ and CD4+ lymphocytes was decreased and the percentage of CD8+ lymphocytes and natural killer cells was increased. No difference was observed in the CD4+:CD8+ ratio. It is noted that automotive painting workers are exposed to numerous chemicals that were not accounted for in this analysis, and no analysis was conducted to determine if lymphocyte levels were associated with plasma thiocyanate levels.

No animal studies evaluating the function of the immune system in animals following exposure to cyanide via any route were identified.

A limited number of drinking water studies in animals evaluated potential effects of cyanide exposure on weight and/or histology of immune organs; no adverse effects were observed. No histological lesions were observed in the spleen of rat dams exposed to drinking water doses up to 12 mg CN^-/kg/day as potassium cyanide or 6.4 mg CN^-/kg/day as potassium thiocyanate on GDs 6–20 (de Sousa et al. 2007). No exposure-related changes in immune organ weight and/or histology (thymus, spleen, lymph nodes,

bone marrow) were noted in rats or mice exposed to doses up to 12.5 or 28.8 mg CN^-/kg/day as sodium cyanide, respectively, in drinking water for 13 weeks (NTP 1993).

2.15 NEUROLOGICAL

Data from both human and animal studies indicate that the CNS is a primary target for cyanide toxicity. Numerous plausible mechanisms for CNS toxicity have been proposed. Based on systematic review, the neurological system is a known target of cyanide toxicity in humans following oral exposure based on a high level of evidence from humans and animals. Systematic review was not conducted for inhalation exposure due to inadequate dose-response data for that exposure route.

Acute-duration inhalation exposure of humans to fatal levels of hydrogen cyanide causes a brief stage of CNS stimulation followed by depression, convulsions, coma with abolished deep reflexes and dilated pupils, paralysis, and in some cases, death (Bonsall 1984; Chen and Rose 1952; Peden et al. 1986; Potter 1950; Singh et al. 1989). Although clinical symptoms of cyanide poisoning are well recognized, specific dose-response data are generally not known. Acute-duration exposure to lower concentrations can cause lightheadedness, breathlessness, dizziness, numbness, and headaches (Lam and Lau 2000; Peden et al. 1986). Impaired short-term memory was reported as a delayed effect in a female 1 year after treatment for convulsions following acute-duration exposure to cyanide gas (Lam and Lau 2000). Slight loss of peripheral vision was the only persistent finding from a case report of a man who had been exposed to 452 ppm hydrogen cyanide for 13 minutes while cleaning a chemical tank (Bonsall 1984).

Severe neurological effects such as hemiparesis and hemianopia have been reported in case reports of chronic-duration occupational exposure to potassium cyanide and other chemicals (Sandberg 1967). Milder effects (headache, dizziness) were self-reported in some workers exposed to unspecified levels of hydrogen cyanide in the cassava processing industry (Janagam et al. 2008).

Epidemiological data pertaining to neurological effects in occupationally exposed workers are presented in Table 2-6. In an occupational hygiene study in three electroplating factories from Egypt, an increased incidence of subjective neurological complaints, including headache, weakness, changes in taste and smell, and dizziness, was observed in 36 male workers exposed to sodium cyanide and copper cyanide for 5–15 years, compared to 20 unexposed referents (El Ghawabi et al. 1975). Measured mean concentrations of “cyanides” (not further characterized) were 6.416–10.375 ppm. Two to three workers also reported salivation, disturbances of accommodation, and psychotic episodes, none of which were

reported in the referent group (El Ghawabi et al. 1975). A high percentage of 36 workers formerly exposed to 15 ppm hydrogen cyanide for a mean duration of 11 months in a silver-reclaiming facility also recalled adverse neurological symptoms during employment, including headache (72%), dizziness (72%), and easy fatigue (47%) (Blanc et al. 1985). Other effects reported at lower incidence (14–26%) included disturbed sleep, ringing in ears, paresthesia in extremities, and syncope. When the 36 workers were qualitatively stratified into low-, moderate-, and high-exposure levels based on job title, a dose-relationship between exposure and self-reported incidence a "neurological symptom complex" (headache, dizziness, dyspnea, nausea or vomiting, syncope) was found, as well as the following individual neurological symptoms: syncope, disturbed sleep, paresthesia in extremities, and syncope. When current symptoms were assessed in former workers, who on averaged ceased working 10.5 months prior to assessment, 33% still reported frequent headache (at least 10 times/month) and a dose-relationship between prior exposure and persistent headache was observed.

Table 2-6. Results of Epidemiological Studies Evaluating Occupational Exposure to Cyanide and Neurological Effects

Reference, study type, and population	Measure of exposure	Outcome evaluated	Result
Blanc et al. 1985 Retrospective cohort; 36 former workers from a silver-reclaiming facility; mean duration of employment was 11 months and mean duration elapsed since employment was 10.5 months (United States, Illinois)	Time-weighted average concentration of hydrogen cyanide (measured after plant ceased operations): 15 ppm REI estimated based on job title; no quantitative exposure levels: Low (n=7) Moderate (n=13) High (n=16)	Neurological symptom complex (headache, dizziness, dyspnea, nausea or vomiting, syncope)	↑ (REI)
		Headache	↔ (REI, during employment) ↑ (REI, residual, post-employment)
		Dizziness	↔ (REI)
		Syncope	↑ (REI)
		Disturbed sleep	↑ (REI)
		Paresthesia of extremities	↑ (REI)
		Easy fatigue	↑ (REI)
Chandra et al. 1988 Retrospective cohort; 111 workers from two electroplating and heat treatment plants (40 from Factory A, 71 from Factory B; exposed 5–19 years) and 30 unexposed referents (India)	Range of thiocyanate in urine, mg/100 mL: Factory A: 0.10–8.10 Factory B: 0.22–5.23 Control: 0.3–3.8	Presence of cyanide "disease"[a]	↑ (concentration × duration)

Table 2-6. Results of Epidemiological Studies Evaluating Occupational Exposure to Cyanide and Neurological Effects

Reference, study type, and population	Measure of exposure	Outcome evaluated	Result
El Ghawabi et al. 1975 Cross-sectional; 36 male workers from three electroplating factories (9 from Factory A, 12 from Factory B, 15 from Factory C; employed 5–15 years) and 20 unexposed male referents (Egypt)	Mean (range) "cyanides" concentrations, ppm: Factory A: 10.375 (8.2–12.4) Factory B: 6.416 (4.2–8.8) Factory C: 8.083 (5.9–9.6) "Cyanides" measured were not further described; cyanide exposure evolved from plating bath containing sodium cyanide and copper cyanide	Self-reported neurological complaints (headaches, weakness, changes in taste and smell, dizziness)	↑ (workers versus referents)
		Self-reported neurological complaints (salivation disturbances of accommodation, psychosis)	↔ (workers versus referents)
Knoblauch et al. 2020 Cross-sectional; 189 artisanal and small-scale gold miners (99 that utilized cyanide leaching, 90 that did not use cyanide) and 90 non-miners from the nearby community (Burkina Faso)	Mean blood lactate levels, mmol/L[b]: Cyanide miners: 4.7 Non-cyanide miners: 3.4 Non-miners: 2.8	Self-reported symptoms in past 2 weeks: Bizarre behavior	↑ (lactate levels)
		Changes in taste	↑ (lactate levels)
		Memory loss	↑ (lactate levels)
Kumar et al. 1992 Cross-sectional; 56 male workers exposed to hydrogen cyanide in copper electroplating and case hardening and 26 unexposed referents (India)	Hydrogen cyanide (unspecified concentration)	Impaired immediate memory (Benton visual retention test)	↑ (workers versus referents)
		Impaired short-term memory (Benton visual retention test)	↑ (workers versus referents)
		Impaired visual ability (Koh's block test)	↑ (workers versus referents)
		Impaired visual learning (Digit symbol test)	↑ (workers versus referents)
		Impaired psychomotor ability (Mirror drawing test)	↑ (workers versus referents)

[a]Cyanide disease was defined by three categorization schemes: biochemical categorization (urinary thiocyanate levels), clinical categorization (self-reporting of headache, giddiness, lacrimation, itching of the eyes, congestion of eyes, and coated tongue), and behavioral categorization (based on functional testing of delayed memory, visual ability, visual learning, and psychomotor ability). Results were reported only in terms of "healthy," "moderate," or "diseased," with no further information regarding specific neurological findings.
[b]Serum lactate levels were used as a biomarker of cyanide exposure. Normal levels are 0.5–1.5 mmol/L.

↑ = association; ↓ = inverse association; ↔ = no association; REI = relative exposure index

An association between both the concentration and duration of exposure to hydrogen cyanide and "cyanide disease" was reported in workers from two electroplating factories from India (Chandra et al.

1988). Cyanide disease was defined by three categorization schemes: biochemical categorization (urinary thiocyanate levels), clinical categorization (self-reporting of headache, giddiness, lacrimation, itching of the eyes, congestion of eyes, and coated tongue), and behavioral categorization (based on functional testing of delayed memory, visual ability, visual learning, and psychomotor ability). Results were reported only in terms of "healthy," "moderate," or "diseased," with no further information regarding neurological findings. In both factories, "cyanide-hours" were positively associated with diagnosis of "cyanide disease;" however, due to lack of detailed reporting on clinical signs or results of behavioral testing, specific details regarding observed neurological effects cannot be ascertained. Subjective complaints of neurological effects were also reported in another study of 56 Indian workers exposed to an unspecified concentration of hydrogen cyanide during heat treatment (case hardening) and electroplating, including headache (26.6%), tiredness (8.9), and giddiness or dizziness (8.9%) (Kumar et al. 1992). Giddiness and dizziness were more prevalent in workers employed for >10 years, compared to those employed <10 years; other symptoms were not associated with duration of employment. Neurobehavioral examinations showed impaired performance on measures of immediate and short-term memory (Benton visual retention test), visual ability (Koh's block test), visual learning (Digit symbol test), and psychomotor ability (Mirror drawing test) in the workers, compared with 26 unexposed referents (Kumar et al. 1992).

Self-reported neurological complaints (bizarre behavior, changes in taste, and memory loss) were also associated with cyanide exposure in a cross-sectional study in artisanal and small-scale gold miners from Burkina Faso in West Africa who utilized potassium cyanide during vat leaching (Knoblauch et al. 2020). These complaints were positively associated with blood lactate levels, which were used as a biomarker of cyanide exposure. The study subjects included 99 miners who used cyanide in the leaching process, 90 who did not use cyanide in the leaching process, and nearby community members who were not miners. While blood lactate levels were (as expected) highest in miners using cyanide, followed by miners not utilizing cyanide, then community members; lactate levels in the community were still above normal clinical ranges for blood lactate levels. The study authors indicated that this is a limitation of the study, as the community members are not true unexposed referents and likely had elevated levels due to direct or indirect exposure to cyanide due to the proximity to the mines. However, there are other possible explanations for elevated blood lactate levels in the groups without direct cyanide exposure (disease or injury, pharmaceutical agents, substance abuse), none of which were controlled for in this study.

The CNS is also a primary target for toxicity in animals following acute-duration inhalation exposure to hydrogen cyanide. While these studies are useful for hazard identification, due to various limitations (unreported exposure levels, lack of concurrent control, inadequate study reporting, and/or inadequate animal number), all but one of these acute-duration inhalation studies are omitted from the LSE table. The only study with adequate design and data reporting showed overt clinical signs of neurotoxicity in mice that survived exposure to 327 ppm hydrogen cyanide for 40 minutes that persisted for up to 60 minutes post exposure (Ma et al. 2021). Observed effects included lethargy, loss of righting reflex, convulsions, and tremors; lower concentrations were not evaluated in this study. In other studies, rats exposed to unspecified concentrations of hydrogen cyanide and kept unconscious for 20–60 minutes developed lesions of various degrees in the brain (Hirano et al. 1967; Levine 1969; Levine and Stypulkowski 1959). Necrosis was found mainly in the mid-sagittal sections of the brain. Demyelination was also reported and morphological signs indicative of remyelination were reported in rats several months after cyanide intoxication (Hirano et al. 1968), but it was apparent that this process was slow and incomplete. Acute-duration exposure of dogs for 2–10 minutes, each to a different concentration ranging from 149 to 633 ppm hydrogen cyanide resulted in motor incoordination, muscular rigidity, and coma (Haymaker et al. 1952). Extensive necrosis in the grey matter of the neural system was observed at necropsy. After acute-duration exposure (up to 30 minutes) to 60–100 ppm hydrogen cyanide, increased delta activity was observed in electroencephalograms of cynomolgus monkeys, but those exposed at the higher-level experienced semiconsciousness within 20 minutes (Purser 1984; Purser et al. 1984). Cyanide exposure levels in most acute-duration studies were relatively high and usually caused death in some animals. Exposure of dogs to 45 ppm hydrogen cyanide for 28–96 days also caused tremors, convulsions, and coma (Valade 1952). Vascular and cellular lesions were found in the CNS.

CNS effects were also noted in animals following exposure to cyanogen. Following acute-duration inhalation exposure, neurological effects before death included restless and panic movements, poor coordination, tremor, and lethargy in rats exposed to 250 ppm cyanogen for 1.5–120 minutes (McNerney and Schrenk 1960). Only transitory behavioral changes were reported in monkeys intermittently exposed to 25 ppm cyanogen for 6 months (Lewis et al. 1984). No effects were found at 11 ppm cyanogen. As discussed in Section 2.1, these studies are not included in the LSE table due to difficulty in estimation of cyanide levels in body tissues after cyanogen exposure.

One study evaluated potential auditory effects in rats following inhalation exposure to hydrogen cyanide. In rats exposed to 10–50 ppm hydrogen cyanide for 3.5 hours, there was no adverse effect on hearing or the histology of cochlear hair cells when evaluated 4 weeks post exposure (Fechter et al. 2002).

However, co-administration for 2 hours to a single 100 dB broadband noise (13.6 kHz) treatment after and during exposures to hydrogen cyanide at concentrations ≥30 ppm caused exacerbation of noise-induced hearing deficits as measured by increases in auditory compound action potential thresholds, compared to noise-only controls. In rats exposed to hydrogen cyanide with noise, this finding was associated with a loss of outer hair cells in the base of the cochlea. Findings from a 3-day intraperitoneal injection study of potassium cyanide in rats support that the cochlea is a target of cyanide toxicity (Tawackoli et al. 2001). Rats specifically showed hearing deficits in high-frequency tones due to dysfunction of the stria vascularis region of the cochlea.

In humans, neurologic toxicity following cyanide ingestion differs depending on length of exposure and the rate at which treatment is administered. Neurological effects of cyanide poisoning in humans may correlate with the amount ingested; however, the exact doses consumed by the victims are usually unknown. Tremors were reported in a patient who accidentally ingested an unknown amount of fluid containing 2.3% silver cyanide and 6.9% sodium cyanide (Chen and Rose 1952). Children who ingested a large number of apricot pits experienced various neurological effects ranging in severity from headaches to coma (Lasch and El Shawa 1981). The severity of effects corresponded with the number of ingested pits. Comatose patients were admitted to a hospital after ingesting potassium cyanide doses of 5.7–229 mg CN^-/kg (Goodhart 1994; Kasamo et al. 1993; Liebowitz and Schwartz 1948; Valenzuela et al. 1992). A cancer patient who ingested 3,000 mg of amygdalin soon became comatose and had two general tonic-clonic seizures (Bromley et al. 2005). Although the dose is generally nontoxic, hydrolysis would potentially release 180 mg of cyanide. It was suggested that the patient's high daily intake of ascorbic acid (4,800 mg/day) may have elevated the rate of hydrolysis in the gut, resulting in increased release of cyanide. Histopathological effects in the brain were noted in an individual who died 4 days after being poisoned with an unknown dose of potassium cyanide (Riudavets et al. 2005). Effects included autolysis in several regions of the brain (basal ganglia, thalamus, hypothalamus, and cerebellum), acute hypoxic/ischemic changes (neuronal necrosis) in the cerebellum (Purkinje and granule cells), basal ganglia, hypothalamus, and deep cortical layers (manifest as pseudolaminar necrosis), and apoptosis of glial cells in the white matter.

Several case studies report development of Parkinsonism-like signs in patients that survived acute cyanide poisoning (Carella et al. 1988; Chin and Calderon 2000; Feldman and Feldman 1990; Grandas et al. 1989; Rachinger et al. 2002; Rosenberg et al. 1989; Rosenow et al. 1995; Uitti et al. 1985; Zaknun et al. 2005). The dose (and sometime form) of cyanide was unknown in several cases, but when known doses ranged from 4.5 to 8.57 mg CN^-/kg as potassium cyanide (assuming 70 kg weight in some cases). Common

clinical signs in these patients developing weeks after exposure included drooling, marked micrographia, masked faces, mild intention tremor, bradykinesia, and cogwheel rigidity or stiffness. In some cases, symptoms continued to progress over the next several years, with progressive Parkinsonism, speech and balance impairments, dystonia, and apraxia of eye opening (Carella et al. 1988; Grandas et al. 1989). Numerous abnormalities were observed in the brain using computed tomography and magnetic resonance image in these cases, most often in the basal ganglia (putamen and globus pallidus) and substantia nigra, but also throughout the cerebral and cerebellar hemispheres. Reduced uptake of labeled dopamine in the putamen and caudate and in glucose metabolism in the temporo-parieto-occipital cortex, cerebellum, and posterior putamen were detected by positron emission tomography in a patient that ingested 7.4 mg CN^-/kg as potassium cyanide (Rosenow et al. 1995). It must be noted that these studies do not necessarily demonstrate a true cause-and-effect relationship between cyanide exposure and Parkinsonism. However, these nine reports of such a relationship are indicative of the need for further research on the subject. In addition, other chemicals, such as manganese and carbon monoxide, and therapy with certain drugs may result in Parkinsonism.

Memory impairment has been reported as a delayed effect in individuals who survived a cyanide poisoning incident with antidotal treatment. A female developed difficulties with short-term memory 5 months after ingesting an unknown amount of an unspecified cyanide compound (Chin and Calderon 2000).

Outbreaks of adverse neurological effects have been reported in regions of Africa with populations that consume a high level of cassava roots (Howlett et al. 1990; Ministry of Health, Mozambique 1984; Monekosso and Wilson 1966; Tylleskar et al. 1994). Due to this, a limited number of population-based studies of adverse neurological effects were conducted in these regions; however, dietary cyanide intake was not quantified and biomarkers of exposure (urinary thiocyanate levels) were measured at the time of (or after) diagnosis of neurological disease (Money 1958; Osuntokun 1968, 1972; Osuntokun et al. 1969). In some cases, the diet consisted almost exclusively of cassava roots, due to failure of other food crops (Howlett et al. 1990). A variety of neuropathies have been observed in these regions and the findings correlated with increased blood thiocyanate levels, all collectively termed “tropical ataxic neuropathy,” as reviewed by Osuntokun (1973). Symmetrical hyperreflexia of the upper limbs, symmetrical spastic paraparesis of the lower limbs, spastic dysarthria, diminished visual acuity, peripheral neuropathy, cerebellar signs, and deafness were among the clinical findings (Ministry of Health, Mozambique 1984). Decreased plasma vitamin B_{12} levels were also detected in affected individuals (Monekosso and Wilson 1966). Konzo, a distinct upper motor neuron disease characterized by the sudden onset of varying

degrees of symmetric, isolated, nonprogressive spastic paraparesis, has occurred in rural areas of Africa and has been associated with high dietary cyanide exposure from the consumption of insufficiently processed bitter cassava (Tylleskar et al. 1994). However, scopoletin, a potent hypotensive and spasmolytic agent, has also been isolated from cassava roots (Obidoa and Obasi 1991). This substance, which remains in cassava during processing, rather than cyanide, was suggested to be the etiological agent in the tropical ataxic neuropathy observed among cassava eaters (Obidoa and Obasi 1991). In addition, protein and vitamin deficiencies may subject people in the tropics who eat cassava to increased risks of tropical neuropathies (Makene and Wilson 1972; Osuntokun 1972; Osuntokun et al. 1969). Until it can be shown that scopoletin is the etiological agent, cyanide must be considered the primary cause of these neuropathies.

A case-series reported optic atrophy associated with macular degeneration in 20 West Africans who were presumably exposed to elevated cyanide levels over an unspecified period during a drought in which the only food available was bitter cassava (van Heijst et al. 1994). Of these patients, 14 were evaluated for neurological deficits. Six of the 14 cases demonstrated neurological signs and symptoms (e.g., including tinnitus, hearing loss, paresthesia, and impaired sensory discrimination). Of the nine individuals with self-reported hearing loss, three showed severe deafness in a pure-tone audiogram (30–100 dB loss in the low tones of 250–1,000 Hz). It is noted that mean levels of thiocyanate and cyanide in these patients were elevated but were not statistically different from controls (hospital staff). However, two of the three patients with severe hearing loss did have markedly higher thiocyanate levels (45.6 and 54.9 µmol/L) compared to controls (18.85 µmol/L); the sample of the third patient with hearing loss was not tested. Controls were used for blood level comparisons only; neurological testing was not conducted in controls.

The CNS is also a primary target of orally administered cyanide in animals. As observed in human studies, neurologic toxicity following cyanide ingestion differs depending on the rate at which treatment is administered, with toxicity occurring at lower doses following bolus administration. This observation is likely because bolus administration may overwhelm detoxification processes.

Evidence for damage to the CNS was reported in some drinking water and dietary studies in animals; however, there were some inconsistencies between studies. Neurobehavioral assays were not conducted in any of the drinking water or dietary studies. Acute-duration oral exposure to potassium thiocyanate at doses ≥0.6 mg CN^-/kg/day in drinking water on GDs 6–20 resulted in mild-to-moderate brain gliosis in 100% of examined dams; mild CNS congestion and neuronophagia (phagocytosis of dead neurons) was

observed at 6.4 mg CN^-/kg/day (de Sousa et al. 2007). These effects persisted in dams similarly treated on GDs 6–20 but sacrificed on PND 22 after a 3-week recovery period. Although incidences were not reported in this study, the study authors only reported severity scores when all animals analyzed showed the same alteration. Therefore, it is unclear if neurological lesions were observed in some animals (but not all) at the lowest dose tested (0.2 mg CN^-/kg/day); thus, a NOAEL for neurological effects could not be established for this study. All dams similarly exposed to 12 mg CN^-/kg/day as potassium cyanide also exhibited mild-to-moderate congestion, gliosis, necrosis, and neuronophagia along with hemorrhagic areas in the brain at GD 20 and PND 22; again, it is unclear if brain lesions were observed in some (but not all) of the dams exposed to lower potassium cyanide doses (0.4 or 1.2 mg CN^-/kg/day). In contrast to findings from this acute-duration drinking water study, intermediate-duration studies did not report exposure-related changes in brain weight or histology in rats or mice following exposure to sodium cyanide at drinking water concentrations up to 12.5 or 28.8 mg CN^-/kg/day for 13 weeks (NTP 1993).

Dietary studies reported modest myelin degeneration in the spinal cord of rats exposed to 47 mg CN^-/kg/day as potassium thiocyanate or 53 mg CN^-/kg/day as potassium cyanide for 11.5 months (Philbrick et al. 1979). The study authors mentioned that tissues from exposed animals were more subject to autolysis, so the strength of the association between neurological histopathology and cyanide exposure in this study is uncertain; however, vitamin B12 deficiency was ruled out as an etiological factor in this study. No neurological effects were reported in rats fed an estimated dose of 10.4 mg CN^-/kg/day as hydrogen cyanide in their feed for 2 years (Howard and Hanzal 1955). The actual dose, however, may have been considerably lower than 10.4 mg/kg/day due to evaporation of hydrogen cyanide from the food. Due to uncertainty in dose estimation, this study is not included in the LSE table. Degenerative changes in ganglion cells were reported in three dogs that were exposed to 0.27–1.68 mg CN^-/kg/day as sodium cyanide in capsules for 14.5 months (Hertting et al. 1960). However, this study is omitted from the LSE table due to lack of concurrent control and use of only one animal per dose group.

Several gavage or other oral administration (e.g., cheek bolus) studies evaluated neurobehavioral endpoints after cyanide exposure, reporting a variety of neurocognitive and sensorimotor deficits in rodents following acute- and intermediate-duration exposure.

In acute-duration studies, reduced motor activity along with altered gait, decreased sensorimotor reflexes, tremor, and clinical signs of CNS depression were observed in adult and juvenile mice 30 minutes after a single exposure to 3.2 mg CN^-/kg as potassium cyanide (Hawk et al. 2016). These neurobehavioral findings in mice did not persist 24 hours post-exposure. Decreased motor strength and activity were also

observed following a 14-day oral exposure to 0.6 mg CN^-/kg/day as potassium cyanide (Ishaku et al. 2018). In rats, decreased locomotor activity and impaired spatial memory and object recognition were observed in rats after exposure to 12 mg CN^-/kg/day (the only dose tested) as potassium cyanide for 10 days (Ogundele et al. 2014b). Neurobehavioral impairments were no longer observed following a 10-day recovery period. Clinical signs of neurotoxicity were reported in a dose-range finding study in adult and juvenile mice exposed to potassium cyanide via gavage, including decreased activity at ≥1.6 mg CN^-/kg and convulsions and tremors at ≥3.2 mg CN^-/kg (Sabourin et al. 2016). Overt clinical signs of neurotoxicity, including lethargy and convulsions, were also observed in rats following a single exposure to doses ≥8.5 and ≥17 mg CN^-/kg as sodium cyanide (Rice et al. 2018). This study also reported impaired operant conditioning in exposed animals; however, due to a lack of a concurrent unexposed control group, findings from the operant conditioning assay cannot be adequately interpreted.

In intermediate-duration studies, decreased motor strength and activity were observed in mice orally exposed to 0.6 mg CN^-/kg/day as potassium cyanide for 28 days and decreased motor coordination was observed in rats exposed to 0.56 mg CN^-/kg/day as potassium cyanide for 90 days (Ishaku et al. 2018; Mathangi et al. 2011). Motor impairments were associated with elevated dopamine levels in the corpus striatum and cerebral cortex (Mathangi et al. 2011).

Histopathological lesions of the spinal cord (axonal "spheroids" or swellings), hippocampus (neuronal loss), and cerebellum (damage to Purkinje cells and loss of white matter) were qualitatively reported in male rats receiving 0.24 mg CN^-/kg/day as potassium cyanide by gavage for 3 months (Soto-Blanco et al. 2002). However, since incidence data were not reported, the significance of these findings cannot be determined; therefore, these data are omitted from the LSE table. In contrast, no exposure-related changes in brain weight or histology were observed in mice exposed once to gavage doses up to 4.6 mg CN^-/kg as potassium cyanide (Hawk et al. 2016; Sabourin et al. 2016) or in rats exposed to 0.56 mg CN^-/kg/day as potassium cyanide via gavage for 90 days (Mathangi et al. 2011).

Tremors, convulsions, recumbency, and lethargy were observed in rats exposed to 7.8 mg CN^-/kg/day as potassium silver cyanide for 90 days by gavage (Gerhart 1987). Since 28 of 40 rats died at this dose level, some of the effects described may represent nonspecific signs that precede death. Hypoactivity was observed in all exposed groups starting at a dose of 0.8 mg CN^-/kg/day. Similarly, hypoactivity was reported in rats exposed to ≥0.14 mg CN^-/kg/day as copper cyanide for 90 days by gavage (Gerhart 1986). At 4.35 mg CN^-/kg/day, fixed posture occurred, while pronounced lethargy was noted at 14.5 mg CN^-/kg/day. Decreased brain weight was reported at 14.5 mg CN^-/kg/day cyanide (Gerhart 1987). The

severity of effects increased as the dose increased in both of these studies and males seemed to be more sensitive to cyanide toxicity than females. Due to potential confounding effects of silver and copper on observe dose-response; these studies are not included in the LSE table.

Deep coma developed in two persons who accidentally fell into cisterns containing copper cyanide (Dodds and McKnight 1985) and potassium cyanide (Trapp 1970). Similarly, a worker, whose hand was exposed to liquid hydrogen cyanide, fell into a coma, lost deep reflexes, and showed dilated pupils within 5 minutes (Potter 1950). Men working in an atmosphere containing 20,000 ppm hydrogen cyanide for 8–10 minutes experienced dizziness, weakness, and headaches (Drinker 1932). The workers wore masks that were reported to give excellent respiratory protection. However, exposure to such high concentrations is not safe because the gas is absorbed through the unprotected skin. The effects seen in these men may have been due to dermal exposure.

Weakness and ataxic movements, convulsions, and coma developed in rabbits that received 0.9 mg CN^-/kg as hydrogen cyanide, 2.1 mg CN^-/kg as sodium cyanide, and 2.5 mg CN^-/kg as potassium cyanide into their conjunctival sacs (Ballantyne 1983b). In dermal acute lethality studies, overt signs of neurotoxicity preceded death in rabbits exposed to lethal concentrations of hydrogen cyanide, potassium cyanide, or sodium cyanide (Ballantyne 1994). Similarly, convulsions and coma preceded death in guinea pigs exposed dermally to unknown doses of hydrogen cyanide (Fairley et al. 1934; Walton and Witherspoon 1926).

Mechanisms of Neurotoxicity. Acute effects of cyanide on the CNS are probably due to rapid biochemical changes in the brain, such as changes in ion flux, neurotransmitter release, and possibly peroxide formation (Johnson and Isom 1985; Kanthasamy et al. 1991a, 1994; Persson et al. 1985). In both *in vivo* and *in vitro* studies using brain tissue, the sensitivity of mitochondrial cytochrome c oxidase activity to inhibition by cyanide was greater than the inhibition of mitochondrial respiratory activity. Only after cytochrome c oxidase activity was depressed by >50% was a large decrease in respiratory activity detected, suggesting that a large portion of cytochrome c oxidase may serve as a functional reserve. Tawackoli et al. (2001) proposed that observed ototoxicity in some studies is attributable to disruption of the electron transport chain in the metabolically active cochlea, specifically the stria vascularis.

Cyanide poisoning likely involves mechanisms in addition to inhibition of cytochrome c oxidase activity (Pettersen and Cohen 1993). Cyanide is a strong nucleophile with multiple effects including release of

secondary neurotransmitters, release of catecholamines from adrenal glands and adrenergic nerves, and inhibition of antioxidant enzymes in the brain (Smith 1996). However, the extremely low concentration of cyanide required to inhibit the oxidase, the rapid interaction of hydrogen cyanide with the enzyme, and the key role of cytochrome c oxidase in aerobic metabolism all combine to make cyanide inhibition of the terminal step of electron transport (Chance and Erecinska 1971; Gibson and Greenwood 1963), the key molecular target in cyanide poisoning. Real-time measurements during sublethal cyanide exposure showed decreased cerebral metabolic activity in rats exposed to 2 mg/kg/hour for 90 minutes via a femoral line, compared to saline-exposed controls (Alomaja et al. 2023). Metabolic activity was measured via lactate, pyruvate, glycerol, and glucose levels measured in cerebral microdialysis samples taken at 30-minute intervals. The lactate-to-pyruvate ratio was decreased in exposed rats at each timepoint, compared to controls. Consistent with these findings, isolated cerebellar mitochondria obtained post-exposure showed decreased *ex vivo* mitochondrial respiration and ATP concentrations (Alomaja et al. 2023).

Inhalation and oral studies in animals have shown that acute- or chronic-duration cyanide exposure leads to encephalopathy in both white and gray matter. In particular, damage has been observed in regions such as the deep cerebral white matter, corpus callosum, hippocampus, corpora striata, pallidum, and substantia nigra. White matter may be more sensitive because of its relatively low cytochrome c oxidase content. Rats injected subcutaneously with daily maximal doses between >3.7 and 9.2 mg CN^-/kg/day (not averaged) 3 days/week for 3 months developed necrotic lesions of the corpus callosum and optic nerve, but there was not a consistent dose-response (Lessell 1971); this may reflect variability in diffusion of cyanide into the systemic circulation by the subcutaneous injection route. High mortality was observed among exposed animals. These effects have been observed following acute-duration exposures (Levine and Stypulkowski 1959) and chronic-duration exposures (Hertting et al. 1960). Necrosis is a prevalent CNS effect following acute-duration exposure to high concentrations of cyanide, whereas demyelination is observed in animals that survive repeated exposure protocols (Bass 1968; Ibrahim et al. 1963). The mechanism of cyanide-induced demyelination is not completely understood, but the evidence suggests that a direct effect of cyanide on white matter may not be necessary. It has been suggested that local edema affecting the oligodendrocytes and caused by vascular changes triggered by cyanide represent a primary event in demyelination (Bass 1968; Ibrahim et al. 1963). Aitken and Braitman (1989) determined that cyanide has a direct effect on neurons not mediated by its inhibition of metabolism. Consistent with the view that cyanide toxicity is due to the inability of tissue to utilize oxygen is a report that in cyanide-intoxicated rats, arterial pO_2 levels rose, while carbon dioxide levels fell (Brierley et al. 1976). The study authors suggested that the low levels of carbon dioxide may have led to

vasoconstriction and reduction in brain blood flow; therefore, brain damage may have been due to both histotoxic and anoxic effects. Partial remyelination after cessation of exposure has been reported, but it is apparent that this process, unlike that in the peripheral nervous system, is slow and incomplete (Hirano et al. 1968). The topographic selectivity of cyanide-induced encephalopathy may be related to the depth of acute intoxication and distribution of blood flow, which may result in selected regions of vascular insufficiency (Levine 1969).

Several studies have suggested that a disruption in neuronal calcium regulation may be an important factor in the manifestation of cyanide-induced neurotoxic events following acute-duration exposure. The predominance of anaerobic metabolism in a cyanide-poisoned cell decreases the ATP/ADP ratio, or energy charge (Isom et al. 1975), and thus alters energy-dependent processes such as cellular calcium homeostasis (Johnson et al. 1986). Elevated levels of intracellular calcium in a cyanide-exposed, presynaptic squid neuron were observed in an *in vitro* study (Adams et al. 1985). Elevated levels of neuronal calcium may initiate release of neurotransmitters from the presynaptic terminal, which can activate the nervous system (Maduh et al. 1990a). Levels of whole-brain calcium increased when potassium cyanide was administered subcutaneously to mice. These increases were correlated with cyanide-induced tremors (Johnson et al. 1986). Brain injury may be associated with cyanide-induced endogenous glutamate release, mediated by both calcium dependent and independent mechanisms, which in turn produce excitotoxic responses in select brain areas (Patel et al. 1991, 1992, 1993). In examining receptor subtypes involved in mediating cyanide-induced toxicity, sodium cyanide-induced cytotoxicity was found to be mediated primarily by activation of the N-methyl-D aspartate (excitatory amino acid) receptor. Sturm et al. (1993) examined the ability of adenosine to attenuate the excitotoxicity secondary to glutamate receptor activation following potassium cyanide exposure in hippocampal neuronal cell cultures. The study authors concluded that neuronal cell death was mediated at least in part by glutamate and that the cell death was attenuated by adenosine via the A_1-specific receptor. Increases in intracellular calcium have also been associated with cyanide-induced effects on vascular smooth muscle and cardiac muscle, possibly inducing cell damage (Allen and Smith 1985; Robinson et al. 1985a). These effects may result from ischemia-induced increases in extracellular potassium, which in turn enhance cellular permeabilities to calcium (Robinson et al. 1985b). Furthermore, changes in cytosolic pH and dysfunction of hydrogen ion handling mechanisms were observed in neuronal cells exposed *in vitro* to cyanide (Maduh et al. 1990b). Pazdernik et al. (1994) reported an increase of local cerebral glucose utilization (LCGU) in many regions of the brain within a minute after sublethal exposure to 2.7–5 mg CN^-/kg as sodium cyanide by controlled intravenous infusion over 1 hour. However, by 1 hour, there was a global increase in LCGU in almost every region of the brain. LCGU values returned to normal in all regions

except the choroid plexus by 6 hours and in that region as well by 24 hours. These results support the expectation that cyanide causes a shift from aerobic to anaerobic metabolism, as illustrated by increases in extracellular lactate and pyruvate and in LCGU.

When cyanide blocks oxidative metabolism in mitochondria, cells shift their metabolism and enhanced glucose utilization occurs. One consequence of this altered metabolic pattern is accumulation of nicotinamide adenine dinucleotide (NADH), which is a powerful stimulant of calcium mobilization from cell stores through "inositol triphosphate receptors." Elevated calcium damages cells. Increases in cellular NADH, therefore, are important events in the toxic action of cyanide (Kaplin et al. 1996).

Studies have shown that cyanide releases catecholamines from rat pheochromocytoma cells and brain slices (Kanthasamy et al. 1991b), from isolated bovine adrenal glands (Borowitz et al. 1988), and from adrenals of mice following subcutaneous injection of high doses of potassium cyanide (Kanthasamy et al. 1991b). Thus, it was proposed that the cardiac and peripheral autonomic responses to cyanide are partially mediated by an elevation of plasma catecholamines (Kanthasamy et al. 1991b). Dopamine levels in potassium cyanide-treated animals were significantly decreased in the striatum and hippocampus, and somewhat decreased in cerebral cortex of mice (Kanthasamy et al. 1994), while extracellular levels of dopamine and homovanillic acid were increased in the brain of rats treated with sodium cyanide (Cassel et al. 1995). Kiuchi et al. (1992) suggested that suppression of ATP production by sodium cyanide induces an abrupt and remarkable increase in dopamine release from the nerve terminal in the striatum. Kanthasamy et al. (1994) also observed that in striatal and hippocampal tissues, but not in cerebral cortex tissues, malondialdehyde levels increased indicating the occurrence of lipid peroxidation in these brain regions. In addition, reduced numbers of tyrosine hydroxylase (TH) positive cells indicated a loss of dopaminergic neurons (Kanthasamy et al. 1994). Behavioral effects seen in the mice were reversed by administration of l-DOPA (treatment for dopamine-deficiency). Ardelt et al. (1994) also evaluated hydroperoxide generation as a potential mechanism of cyanide neurotoxicity. Increased lipid peroxidation was observed in brain and kidney, but not in liver or heart. It was also determined that calcium plays a critical role in lipid peroxidation in neuronal cells. Subcellular fractionation of brain tissue showed an increase in lipid peroxidation in the microsomal but not mitochondrial fraction. Matsumoto et al. (1993) evaluated the involvement of extracellular calcium in dopamine release from rat striatum resulting from cyanide exposure. A gradual increase in intracellular calcium was observed during incubation of sodium cyanide with striatal slices. The excessive influx of extracellular calcium during sodium cyanide perfusion may contribute to the changes in dopamine levels in the striatum and to the observed suppression of dopamine release in response to high potassium stimulation. Release of

dopamine was not suppressed by perfusion with a calcium-free solution; thus, additional mechanisms other than the opening of calcium channels must also be involved in dopamine release by cyanide. Decreased dopamine uptake has been suggested as an explanation for this increase in dopamine, since dopamine uptake is driven by a sodium gradient that is maintained by the Na/K ATPase and could be reduced if ATP is depleted. Cyanide did not affect monoamine oxidase or catechol-o-methyl transferase, suggesting that a disturbance in dopamine metabolism did not lead to extracellular dopamine elevation (Matsumoto et al. 1993).

Mills et al. (1999) reported that there is more than one mode of cell death operating in the brains of mice injected with potassium cyanide. Extensive deoxyribonucleic acid (DNA) fragmentation, pyknosis, and chromosome condensation, all characteristics of apoptosis, were observed in the parietal and suprarhinal regions of the motor cortex of treated mice. However, necrotic lesions with astrocytic gliosis were found in the substantia nigra. Pretreatment with the antioxidant, alpha-phenyl-tert-butyl nitrone, reduced cortical DNA fragmentation but had no effect on the necrotic lesions produced in the substantia nigra.

Prabhakaran et al. (2002) similarly reported different modes of death induced by cyanide in primary cultures of rat cortical or mesencephalic neurons; the mode of cell death and the reactive oxygen species generated differed in the two kinds of cells. Cortical neurons exhibited apoptosis, with increases in hydrogen peroxide and superoxide, and a moderate change in mitochondrial membrane potential, leading to release of cytochrome c and activation of caspase-3-like protease (a cysteine protease associated with apoptosis). Mesencephalic neurons exhibited necrosis involving excess nitric oxide and superoxide, with a more pronounced reduction in mitochondrial membrane potential. Additional studies demonstrated that necrosis of exposed mesencephalic cells or cortical neurons exposed to 0.5–0.6 mM KCN was induced by the upregulation of uncoupling protein 2 (UCP-2), a protein of the inner mitochondrial membrane (Li et al. 2005; Prabhakaran et al. 2005). UCP-2 increases proton leak across the inner mitochondrial membrane, dissociating respiration from ATP synthesis. In experiments preventing the expression of UCP-2, the necrotic death of cultured mesencephalic cells exposed to cyanide was also prevented (Prabhakaran et al. 2005).

The mediation of cyanide-induced apoptosis has been studied in cultured cortical neurons exposed to 0.3 mM cyanide (Shou et al. 2002, 2003). Treatment with cyanide activated p38 mitogen-activated protein (MAP) kinase within 30 minutes, an upstream event necessary for the translocation of Bax protein from the cytosol to mitochondria 2.5 hours later (Shou et al. 2003). Translocation of Bax protein to mitochondria is a required step in the release of cytochrome c from mitochondria as well as the caspase

cascade that regulates apoptosis. Cyanide treatment of cortical neurons also results in the activation of the redox-sensitive transcription factor NF-κB, and its translocation to the nucleus, where it upregulates expression of the pro-apoptotic proteins Bax and Bcl-X_S (Shou et al. 2002). Increased cytosolic calcium levels also contribute to apoptosis of cyanide-treated cortical neurons (Shou et al. 2004). Increased calcium activates cellular calcineurin, which stimulates the activation of the protein known as BAD (Bcl-2/Bcl-X_L-antagonist, causing cell death) and its translocation to mitochondria within 1 hour of treatment with cyanide. The net effect of BAD is to selectively inhibit proteins (Bcl-1/Bcl-X_L) that are antagonists to apoptosis (Shou et al. 2004). A series of RNAi knock-down studies in cultured rat N27 dopaminergic mesencephalic cells confirmed that down regulation of Bcl-2 expression following cyanide exposure mediates cyanide-associated neuronal cell death (Zhang et al. 2009).

It has been noted that survivors of cyanide poisoning incidents may develop Parkinsonian-like signs, with lesions in the substantia nigra, a dopaminergic center, confirmed by magnetic resonance imaging (MRI) (Carella et al. 1988; Chin and Calderon 2000; Grandas et al. 1989; Feldman and Feldman 1990; Rachinger et al. 2002; Rosenberg et al. 1989; Rosenow et al. 1995; Uitti et al. 1985; Zaknun et al. 2005). Osmotic imbalance and generation of reactive oxygen species with lipid peroxidation was associated with cellular degeneration in the cortex and cerebellum of rats exposed to ≥4 mg CN^-/kg/day as potassium cyanide for 15 days (Ogundele et al. 2013). Jones et al. (2000, 2003) presented evidence based on experiments on PC12 cells (a pheochromocytoma cell line that can be induced to differentiate as neurons) and fetal rat mesencephalic cells indicating that cyanide toxicity is exacerbated by the oxidation of dopamine. Increases in apoptosis and reactive oxygen species occurred at higher levels in PC12 cells incubated in dopamine plus potassium cyanide compared to those incubated in either chemical separately; concentrations of potassium cyanide that had no effect on fetal rat midbrain cells significantly increased the adverse effects of added dopamine. Toxicity in one or both systems was reduced by preincubation with antioxidants (superoxide dismutase, glutathione catalase), an inhibitor to nitric oxide synthase (N^{omega}-nitro-L-arginine methyl ester), and the peroxynitrite scavenger, uric acid. The study authors suggested that the inactivation of antioxidant enzymes by cyanide as described by Ardelt et al. (1989) may render neurons more vulnerable to the adverse effects of dopamine oxidation. Dopaminergic brain centers would therefore be more sensitive to cyanide neurotoxicity. In cultured cerebellar granule cells taken from 8-day-old rat pups, cyanide treatment generated nitric oxide and reactive oxygen species concurrently, resulting in lipid peroxidation (Gunasekar et al. 1996).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans following exposure to cyanide via any route. Studies evaluating reproductive effects in animals are limited to the oral route and are primarily focused on potential adverse effects on the male reproductive system. Animal studies identify the male reproductive system as a potentially sensitive target of cyanide toxicity following gavage exposure; however, findings from drinking water studies are mixed. Based on systematic review, male reproductive effects are suspected human health effects following oral exposure based on no human data and a moderate level of evidence in animals (see Appendix C).

A number of exposure-related changes in the male reproductive system were reported following exposure of rats and mice to sodium cyanide in the drinking water for 13 weeks (NTP 1993). In male rats, exposure-related effects on male reproductive organs observed included decreased absolute left epididymis weight, decreased absolute left cauda epididymis weight, and decreased absolute left testis weight at the highest administered dose (12.5 mg CN^-/kg/day); mild decreases in decreased absolute left cauda epididymis weight were also observed at 1.7 and 4.9 mg CN^-/kg/day. Relative organ weights were not reported for the left testis or epididymis weights, and no changes in the absolute or relative right testis weights were observed at any dose. Sperm analysis showed a decreased number of spermatid heads per testis (but not number of spermatid heads per gram testis) and decreased total spermatid counts at 12.5 mg CN^-/kg/day. Sperm motility was slightly decreased at ≥1.7 mg CN^-/kg/day but findings were not dose related; no changes in spermatozoa concentration were observed. In similarly exposed and evaluated male mice, findings were limited to a significant decrease in the absolute left epididymal and caudal epididymal weights at the highest dose of 24.3 mg CN^-/kg/day (NTP 1993). No histopathological changes in male reproductive organs were found in male rats or mice at doses up to 12.5 or 24.3 mg CN^-/kg/day, respectively (NTP 1993).

The male rat reproductive findings from the NTP (1993) study have been questioned due to decreased water intake observed in the highest dose group. To re-evaluate findings controlling for this potential confounding factor, Tyner and Greeley (2023) repeated the NTP (1993) study in male F344 rats utilizing a water-intake-matched control for the highest dose group (300 ppm; calculated at 11.50 mg CN^-/kg/day based on measured body weight and water intake for this study). In contrast to the NTP (1993) study, no exposure-related changes in absolute or relative testes, epididymis, or cauda epididymis weights were observed compared to either the standard or water-restricted controls. While some dose-related trends were observed for decreased sperm motility compared to standard control, this trend disappeared when

compared to the water-intake-matched control. Consistent with the NTP (1993) study, no histopathological changes in male reproductive organs were found in male rats at doses up to 11.50 mg CN^-/kg/day.

In general, animal studies that employed bolus dosing (i.e., gavage, buccal bolus) reported male reproductive effects at doses below those associated with effects in the drinking water study by NTP (1993). This observation is likely because bolus administration may overwhelm detoxification processes.

Acute-duration bolus administration studies in animals do not report exposure-related changes in the weight or histology of the cauda epididymis in mice exposed once to doses up to 4.6 mg CN^-/kg as potassium cyanide (Hawk et al. 2016; Sabourin et al. 2016). In a series of intermediate-duration studies in rats, exposure to sodium cyanide at gavage doses ≥0.5 mg CN^-/kg/day for 30 or 56 days resulted in alterations in serum reproductive hormones (decreased serum testosterone, follicle stimulating hormone, and luteinizing hormone; increase serum prolactin), sperm alterations (decreased total sperm count, percent motility, and percent normal sperm), and morphological changes in the testes (decreased diameter of seminiferous tubules, decreased epithelial cell height; increased Leydig cell area, and decreased nuclear volume of Sertoli cells) (Oyewopo et al. 2021a, 2021b). Absolute testicular weights were also decreased; however, findings were confounded by concurrent decreases in body weight gain and lack of reported relative organ weights (Oyewopo et al. 2021a). Altered serum hormones (decreased serum testosterone and luteinizing hormone), sperm alterations (decreased sperm count and motility), decreased prostate and testes weights, and histopathological changes (mild atrophy and degeneration of seminiferous tubules; mild vacuolation in the epididymis) were also reported in rats exposed to sodium cyanide via gavage at doses ≥0.64 mg CN^-/kg/day for 90 days (Shivanoor and David 2015). Additional effects noted at 1.70 mg CN^-/kg/day include decreased epididymal weights, increased sperm abnormalities, and desquamation of the glandular epithelium in the prostate.

Increased gonadal weight was observed in male rats exposed for 90 days by gavage to 14.5 mg CN^-/kg/day as copper cyanide (Gerhart 1986) or 2.6 mg CN^-/kg/day as potassium silver cyanide (Gerhart 1987). The NOAEL values were 4.35 mg CN^-/kg/day (Gerhart 1986) and 0.8 mg CN^-/kg/day (Gerhart 1987), respectively. Due to potential confounding effects of silver and copper on the observed dose-response, these studies are not included in the LSE table.

As discussed in Section 2.1, animal studies on cassava are discussed in this profile, but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Dogs fed a diet

of cassava ingested an estimated 1.04 mg CN^-/kg/day for 14 weeks showed a reduction in the spermatogenic cycle, testicular germ cell sloughing and degeneration, and occasional abnormal cells (Kamalu 1993). Dogs similarly fed rice to which 1.04 mg CN^-/kg food was added (sodium cyanide was added to release hydrogen cyanide during the cooking process) showed similar effects (Kamalu 1993). However, findings in this study were potentially confounded by concurrent diseases in study animals requiring pharmaceutical intervention (Kamalu 1991, 1993); therefore, the sodium cyanide study is also omitted from the LSE table.

Data pertaining to potential adverse effects in the female reproductive system are limited. In female rats exposed to sodium cyanide in drinking water for 13 weeks, significantly more time was spent in proestrus and diestrus stages, and less time was spent in estrus and metestrus stages in the 4.9 and 12.5 mg CN^-/kg/day groups; however, these effects were not considered to be adverse since overall length of estrus was unaffected (NTP 1993). No changes were noted on the estrus cycle in female mice similarly exposed to doses up to 28.8 mg CN^-/kg/day (NTP 1993). No exposure-related changes in female reproductive organ weight or histology were observed in rats or mice at doses up to 12.5 or 28.8 mg CN^-/kg/day, respectively (NTP 1993). No exposure-related changes in female reproductive organs were observed in rats in 90-day gavage studies at doses up to 14.5 mg CN^-/kg/day as copper cyanide (Gerhart 1986) or 15.6 mg CN^-/kg/day as potassium silver cyanide (Gerhart 1986).

Mechanisms of Male Reproductive Toxicity. No studies specifically evaluating mechanisms of male reproductive toxicity were identified. EPA (2010) proposed that cyanide-associated hypothyroidism could potentially underlie male reproductive effects observed in some studies. In support, male infertility, altered sperm parameters, and/or altered reproductive hormone levels have been associated with thyroid disease in humans, either hypo- or hyperthyroidism (Krajewska-Kulak and Sengupta 2013; Krassas and Pontikides 2004; Trokoudes et al. 2006). However, a review by Williams and DeSesso (2023) challenged this proposal, pointing out that the perchlorate anion has a much higher affinity for the sodium iodide symporter compared to thiocyanide (see *Mechanisms of Thyroid Toxicity* in Section 2.13 for details), but shows no evidence of adverse effects on the male reproductive system.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans following exposure to cyanide via any route. Studies evaluating developmental effects in animals are very limited and are restricted to the oral route.

The overall number of fetuses with any visceral abnormality was increased when rat dams were exposed to 12 mg CN^-/kg/day as potassium cyanide in the drinking water on GDs 6–20, compared to control (de Sousa et al. 2007). However, no single abnormality was increased compared with the control, and the overall number of affected litters was comparable to control (de Sousa et al. 2007). No exposure-related external or skeletal malformations were observed at maternal doses up to 12 mg CN^-/kg/day potassium cyanide. No exposure-related external, visceral, or skeletal malformations were observed in the fetuses of rat dams similarly exposed to 6.4 mg CN^-/kg/day as potassium thiocyanate in the drinking water on GDs 6–20 (de Sousa et al. 2007). In the same study, additional groups of dams were similarly exposed on GDs 6–20 and allowed to deliver, and pups were examined on PND 22. Effects observed in pups born to dams exposed to 12 mg CN^-/kg/day as potassium cyanide or 6.4 mg CN^-/kg/day as potassium thiocyanate during gestation included brain lesions (CNS gliosis, mild-to-moderate necrosis, mild-to-moderate neuronophagia, congestion) and hepatic lesions (mild-to-moderate vacuolation and congestion, mild bile duct proliferation). Although incidences were not reported in this study, the study authors only reported severity scores when all pups analyzed showed the same alteration. Therefore, it is unclear if brain and/or hepatic lesions were observed in some pups (but not all) at the lower maternal doses; thus, a NOAEL for developmental effects in PND 22 pups could not be established for this study.

As discussed in Section 2.1, animal studies on cassava and natural cyanogenic glycosides are discussed in this profile but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Increased early embryonic deaths and increased developmental abnormalities (microcephaly with open eyes, limb defects, and growth retardation) were observed in fetuses of rats fed a diet containing 80% cassava powder during gestation, but no developmental effects were found in a group fed with 50% cassava powder (Singh 1981). Fetotoxicity (reduced fetal weight and ossification) were found in the offspring of hamsters fed a cassava diet providing 1.0 mg CN^-/kg/day during pregnancy (Frakes et al. 1986) or to the cyanogenic glucoside, linamarin, at 120 or 140 mg/kg (Frakes et al. 1985). Teratogenic effects (encephalocele and rib abnormalities) were also reported in hamsters exposed to a single oral dose of the cyanogenic glucoside, amygdalin, during gestation, but these changes were found only at maternally toxic doses (Willhite 1982). In contrast, no major developmental effects were observed in rats that were fed a basal cassava diet providing ≈1.2 mg CN^-/kg/day or in rats whose cassava feed was supplemented with potassium cyanide bringing the total dose to 51 mg CN^-/kg/day (assuming young growing rats and pregnant rats consume food each day equivalent to 10% of their body weight) (Tewe and Maner 1981a). The rats were exposed to cyanide during GDs 16–20 and then for 21 days during lactation. When their offspring were exposed to similar diets providing doses of ≈1.2 and 51 mg

CN^-/kg/day, decreased growth was observed in the higher-dosed weanlings regardless of the exposure *in utero*.

2.18 OTHER NONCANCER

Yen et al. (1995) reported metabolic acidosis in 67% of patients acutely poisoned by unknown concentrations of cyanide. Metabolic acidosis was observed in a woman who received an estimated dose of cyanide between 0.026 and 0.234 mg CN^-/kg from ingesting 30 apricot kernels (approximately 15 g) (Suchard et al. 1998). An apparent attempted homicide victim developed metabolic acidosis after ingesting an unknown quantity of cyanide (Chin and Calderon 2000). Metabolic acidosis also developed in six of eight individuals who entered a 27-m^3 well that contained pickled bamboo shoots; four recovered with supportive care (Sang-a-Gad et al. 2011). Model simulations estimated air levels of 10 ppm hydrogen cyanide (as well as 7.5 ppm sulphur dioxide).

Cyanide exposure may alter glucose homeostasis. In an acute-duration oral study, rat dams exposed to 12 mg CN^-/kg/day as potassium cyanide in drinking water on GDs 6–20 had a 38% increase in serum glucose levels (de Sousa et al. 2007). This is consistent with pancreatic islet cell vacuolation observed at the same dose (see Section 2.13 for more details). Findings did not persist after a 3-week recovery period. In contrast, no effects on serum glucose were observed in rat dams similarly exposed to drinking water doses up to 6.4 mg CN^-/kg/day as potassium thiocyanate on GDs 6–20 (de Sousa et al. 2007) and there were no changes in serum levels of glucose in rabbits fed 20 mg CN^-/kg/day as potassium cyanide for 40 weeks (Okolie and Osagie 2000). In humans, blood glucose levels were comparable between 20 workers exposed to unspecified levels of hydrogen cyanide in the cassava processing industry and 20 age-matched referents (Janagam et al. 2008).

As discussed in Section 2.1, animal studies on cassava are discussed in this profile, but are not included in the LSE table due to concurrent exposure to additional compounds found in cassava root. Serum levels of glucose were elevated in rats exposed to ≥5.50 mg CN^-/kg/day as cassava in the diet for 28 days (Okafor et al. 2006; Udeme et al. 2015). No exposure-related changes in serum glucose were noted in rats exposed to 6.27 mg CN^-/kg/day as cassava in the diet for 7 days (Okafor et al. 2006). Yessoufou et al. (2006) examined the potential role of cassava (in general) versus cyanide in glucose homeostasis in a diabetic rat model (streptozotocin-induced model of diabetes, or STZ-rats). In this study, healthy and STZ-rats were fed standard diets, diets containing cassava flour that was certified as cyanide-free (CFC diets), or CFC diets supplemented with 1.9 g/kg of potassium cyanide (delivering a cyanide dose of

72 CN^-/mg /kg/day) for 28 days. CFC diets did not induce hyperglycemia in healthy rats; however, CFC diets (with or without potassium cyanide) exacerbated measures of hyperglycemia (elevated blood glucose, decreased serum insulin) in STZ-rats, compared to STZ-rats fed standard diets.

2.19 CANCER

The EPA (IRIS 2010) determined that there is inadequate information to assess the carcinogenic potential of hydrogen cyanide and cyanide salts. IARC (2023) and NTP (2021) have not evaluated the potential for cyanide or cyanide compounds to cause carcinogenicity in humans.

No studies were located regarding cancer effects in humans or animals after exposure to cyanide. Some populations with high intake of cassava have shown decreased risk for thyroid cancer (Cléro et al. 2012) or breast cancer (Jayalekshmi et al. 2009).

2.20 GENOTOXICITY

A limited number of studies evaluating *in vivo* genotoxicity studies were identified (Table 2-7).

One cross-sectional study evaluated clastogenicity in 17 male automotive painting workers exposed to inorganic cyanide compounds for 5–20 years, compared to 5 unexposed male referents (Haleem and Hussein 2024). Details on the referents were limited to the information on sex and age; mean ages were 33.11 years for exposed workers and 33.4 years for referents. The mean hydrogen cyanide level in workplace air was 2.8 ppm and mean plasma thiocyanate levels were 0.54 µM in referents, 1.78 µM in workers aged 22–33 years, and 1.99 µM in workers aged 33–44 years. Both total chromosomal aberrations and micronuclei were increased in exposed workers, compared to referents. However, it is noted that automotive painting workers are exposed to numerous chemicals and no analysis was conducted to determine if the observed effects were associated with plasma thiocyanate levels.

A single oral dose of 1 mg CN^-/kg as potassium cyanide did not inhibit testicular DNA synthesis in mice (Friedman and Staub 1976). Increased DNA fragmentation was observed in mice in two studies. Increased DNA fragmentation was observed by electrophoresis in isolated brain mitochondria of male ddy mice that had received a single subcutaneous injection of 2.8 mg CN^-/kg/day as potassium cyanide (Yamamoto and Mohanan 2002). DNA fragmentation was also detected by *in situ* terminal deoxynucleotide transferase nick-end labeling (TUNEL) in the parietal and suprarhinal regions of the

motor cortex in mice injected with 2.4 mg CN/kg/day as potassium cyanide for 1–12 days (Mills et al. 1999).

Table 2-7. Genotoxicity of Cyanide *In Vivo*

Species (exposure route)	Endpoint	Results	Reference	Form
Human (inhalation)	Chromosomal aberrations	+	Haleem and Hussein 2024	HCN
Human (inhalation)	Micronuclei	+	Haleem and Hussein 2024	HCN
Mouse (oral)	DNA damage	-	Friedman and Staub 1976	KCN
Mouse (i.p.)	DNA damage	+	Yamamoto and Mohanan 2002	KCN
Mouse (i.p.)	DNA damage	+	Mills et al. 1999	KCN

– = negative result; + = positive result; i.p. = intraperitoneal; DNA = deoxyribonucleic acid; HCN = hydrogen cyanide; KCN = potassium cyanide

The *in vitro* genotoxicity of cyanide has been examined in prokaryotic organisms and mammalian cell systems, and studies are summarized in Table 2-8. In prokaryotic cells, the overall evidence indicates that cyanide is not mutagenic. Cyanide in the form of potassium cyanide tested negative in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 (De Flora 1981), TA97, and TA102 (De Flora et al. 1984). Potassium cyanide tested at 0.01 or 1.0 mM failed to induce reverse mutations in *S. typhimurium* strains TA98 or TA100 with or without metabolic activation (Kubo et al. 2002). Cyanide in the form of sodium cyanide tested negative in *S. typhimurium* strains TA97, TA98, TA100, and TA1535, with and without metabolic activation (NTP 1993). A positive mutagenic response was reported for hydrogen cyanide in strain TA100 without metabolic activation (Kushi et al. 1983). Adding S9 mix to the culture decreased the induction of reverse mutations by cyanide to 40% of the nonactivated reaction. *S. typhimurium* strain TA98 was negative for mutagenicity (Kushi et al. 1983). Negative results were also obtained in the DNA repair test in *Escherichia coli* WP67, CM871, and WP2 with potassium cyanide (De Flora et al. 1984). Sodium cyanide tested at concentrations up to 0.8 mM without metabolic activation yielded negative results for DNA damage in a screening assay (vitotox test) (Meriläinen and Lampinen 2004)

Table 2-8. Genotoxicity of Cyanide *In Vitro*

Species (test system)	Endpoint	Results		Reference	Form
		With activation	Without activation		
Prokaryotic organisms					
Salmonella typhimurium TA82, TA102	Reverse mutation	–	Not tested	De Flora et al. 1984	KCN
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	De Flora 1981	KCN
S. typhimurium TA98	Reverse mutation	–	–	Kushi et al. 1983	HCN
S. typhimurium TA100	Reverse mutation	(+)	+	Kushi et al. 1983	HCN
S. typhimurium TA98, TA100	Reverse mutation	–	–	Kubo et al. 2002	KCN
S. typhimurium TA97, TA98, TA 100, TA 1535	Reverse mutation	–	–	NTP 1993	NaCN
Escherichia coli WP67, CM871, WP2	DNA repair test	–	–	De Flora et al. 1984	KCN
Eukaryotic organisms					
HeLa cells	DNA synthesis inhibition	–	–	Painter and Howard 1982	KCN
Human A549 lung carcinoma cells	DNA breakage		$+^{cyt}$	Vock et al. 1998	KCN
Human TK6 lymphoblastoma cells	DNA breakage		$+^{cyt}$	Henderson et al. 1998	KCN
Rat thymocytes	DNA breakage		$+^{cyt}$	Bhattacharya and Laskshmana Rao 1997	KCN
Hamster BHK-21 cells	DNA breakage		$+^{cyt}$	Bhattacharya and Laskshmana Rao 1997	KCN
Rat hepatocytes (primary)	DNA breakage		$+^{cyt}$	Storer et al. 1996	KCN
Primary brain cells, mitochondrial fraction (male ddy mice)	DNA breakage (mitochondria)		+	Yamamoto and Mohanan 2002	KCN

– = negative result; + = positive result; (+) = weakly positive result; $+^{cyt}$ = DNA breakage associated with cytotoxicity; DNA = deoxyribonucleic acid; HCN = hydrogen cyanide; KCN = potassium cyanide; NaCN = sodium cyanide

In mammalian cell systems, DNA fragmentation was observed with cytotoxicity in numerous studies. In cultured A549 human epithelial-like lung carcinoma cells, potassium cyanide induced dose-related

reductions in cell viability by 8 hours and increases in double-strand DNA breaks by 24 hours (Vock et al. 1998). Based on the temporal relationship and the small size of DNA fragments (<0.5 Mbp), the study authors concluded that the effect of cyanide on DNA was indirect and a result of the activation of endonucleases by calcium entering the damaged cells. Dose-related increases in DNA breaks were induced in rat thymocytes and baby hamster kidney (BHK-21) cells exposed to potassium cyanide (Bhattacharya and Laskshmana Rao 1997). Incubation of cells in calcium-free medium significantly reduced the level of DNA damage, supporting the hypothesis that a cytotoxic-related calcium influx contributes to this fragmentation of DNA. Storer et al. (1996) evaluated 81 chemicals including potassium cyanide for DNA strand breaks in an alkaline elution assay in primary cultures of rat hepatocytes. The study included a battery of assays for cytotoxicity including tetrazolium dye reduction, trypan blue dye exclusion, ATP content, K+ content, and cell blebbing to distinguish between genotoxicity and false-positive results resulting from the loss of membrane integrity in damaged cells. Following treatment with potassium cyanide, DNA strand breakage was determined to be associated with the induction of endonucleolytic DNA degradation caused by cytotoxicity (ATP content ≤5% of control, increased cell blebbing). Henderson et al. (1998) detected significant DNA breakage, characterized by DNA migration, in TK6 human lymphoblastoma cells treated with potassium cyanide at concentrations that also reduced cell survival (as measured by trypan blue exclusion). Exposure to potassium cyanide resulted in dose-related increases in DNA breaks in the mitochondrial fraction of primary cultures of brain cells from male ddy mice (Yamamoto and Mohanan 2002).

Potassium cyanide did not inhibit DNA synthesis in cultured HeLa cells (Painter and Howard 1982).

In conclusion, the overall evidence indicates that cyanide is probably not a direct genotoxic agent, as cyanide-induced DNA fragmentation is secondary to cytotoxicity. *In vivo* studies on the genotoxicity of cyanide were limited. Available human data are limited by small sample size, lack of appropriate statistical analysis, and lack of control for known co-exposures. No DNA damage was found in mice exposed orally to potassium cyanide; however, DNA fragmentation has been detected in the brains of mice injected with potassium cyanide. A number of *in vitro* studies on mammalian cells reported DNA fragmentation following exposure to potassium cyanide. These studies indicate that the DNA fragmentation is secondary to the cytotoxicity of cyanide, which results in the release of endonucleases by the dying cells. *In vitro* studies in prokaryotes with cyanide in the form of potassium or sodium cyanide did not show any mutagenic activity in *S. typhimurium* or *E. coli*. One study in *S. typhimurium* TA100 suggested that hydrogen cyanide may be mutagenic in the absence of metabolic activation; however, no

additional studies were available to support this result, possibly due to the volatility of hydrogen cyanide. Additionally, there are no structural reasons to suggest that cyanide may be genotoxic.

2.21 MECHANISMS OF ACTION

This section discusses the general toxic mechanism of cyanide that can occur throughout the body following exposure. Specific information on target organ toxicity is discussed in the preceding sections.

Cyanide (as hydrogen cyanide), originating *in vivo* by dissociation of potassium cyanide, sodium cyanide, and other cyanogenic compounds or arising from catabolism of cyanogenic glycosides, exerts its acute toxic effects by complexing with the ferric iron atom in metalloenzymes, resulting in histotoxic anoxia through inhibition of cytochrome c oxidase (Rieders 1971; Way 1984), metalloenzymes that function as the terminal oxidase of the inner mitochondrial membrane respiratory chain. A two-step process has been proposed. Cyanide as hydrogen cyanide first penetrates a protein crevice of cytochrome c oxidase and binds to the protein (Stannard and Horecker 1948). Hydrogen cyanide then binds to the trivalent iron ion of the enzyme, forming a relatively stable (but reversible) coordination complex. One mole of hydrogen cyanide is bound to one mole of cytochrome c oxidase (Van Buuren et al. 1972). As a result, the enzyme becomes unable to catalyze the reactions in which electrons would be transferred from reduced cytochrome to oxygen. Cellular oxygen utilization is thus impaired, with resultant reduction in, or cessation of, aerobic metabolism (Rieders 1971; Way 1984). Glucose catabolism then shifts from the aerobic pathway to anaerobic metabolism including the pentose phosphate pathway, resulting in increased blood glucose, pyruvic acid, lactic acid, and nicotinamide adenine dinucleotide (NADPH) levels, and a decrease in the ATP/adenosine diphosphate (ADP) ratio (Rieders 1971; Way 1984). Wilson et al. (1994) suggested that it is the binding of cyanide to oxidized Cu_B, the copper ion that is part of the dioxygen binding-site, that leads to the inhibition of cytochrome c oxidase. As reviewed by Zuhran and Szabo (2022), studies *in vitro* have described that cyanide-mediated inhibition of complex IV of the mitochondrial electron transport chain via cytochrome c oxidase results in impaired ATP generation. This has been confirmed in *ex vivo* studies in numerous tissues, especially those with high energy needs. Energy deficiency in tissues with high pools of phosphocreatine present with phosphocreatine depletion, rather than decreased ATP.

The inhibition of oxygen use by cells (termed histotoxic hypoxia) causes oxygen tensions to rise in peripheral tissues (Smith 1996). This results in a decrease in the unloading gradient for oxyhemoglobin; thus, oxyhemoglobin is carried in the venous blood (Rieders 1971). Inhibition of oxygen utilization is

thought to occur rapidly after cyanide exposure. Tadic (1992) determined that inhibition of cytochrome c oxidase activity in rat brains was most pronounced between 15 and 20 minutes after administration of sodium cyanide (12 mg/kg or 1.3xLD_{50}). In addition to binding to cytochrome c oxidase, cyanide also binds to catalase, peroxidase, methemoglobin, hydroxocobalamin, phosphatase, tyrosinase, ascorbic acid oxidase, xanthine oxidase, and succinic dehydrogenase. These reactions may also contribute to the classic signs of cyanide toxicity (Ardelt et al. 1989; Rieders 1971).