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

3.1 TOXICOKINETICS

- Absorption of cyanide gas and salts such as sodium or potassium cyanide is rapid through the lungs and gastrointestinal tract. Absorption of cyanide gas and salts through the skin is slower.
- Following inhalation, cyanide is rapidly distributed throughout the body, with measurable levels detected in all organs studied to date. Following oral exposure, the highest levels have been detected in the lungs and blood. Animal studies have shown that cyanide does not accumulate in the blood and tissues following chronic-duration oral exposure.
- The predominant metabolic pathway for cyanide is conversion to thiocyanate by a sulfur donor (e.g., rhodanese). Minor metabolic pathways include conversion to 2-amino-2-thiazoline-4-carboxylic acid (ATCA) via reaction with cysteine, incorporation into the 1 carbon metabolic pool, and formation of cyanocobalamin via reaction with hydroxocobalamin. Cyanide has a plasma half-life of 20 minutes to 1 hour.
- Cyanide metabolites are excreted primarily in the urine (approximately 80% as thiocyanate), with small amounts excreted through the lungs.
- Two types of physiologically based pharmacokinetic (PBPK) models are currently available. The first extrapolates internal cyanide doses from oral or inhalation exposure levels. The second extrapolates inhaled cyanide levels based on biomarker levels. PBPK models for interspecies and route-to-route dosimetry extrapolation have not been developed.

3.1.1 Absorption

Cyanide as hydrogen cyanide is rapidly absorbed (within seconds) following inhalation exposure. Humans retained 58% of hydrogen cyanide in the lungs after inhaling the gas through normal breathing (Landahl and Herrmann 1950).

Quantitative data on the absorption of hydrogen cyanide by inhalation were reported in dogs (Gettler and Baine 1938). During exposure to an unknown concentration of hydrogen cyanide, one dog reportedly absorbed 16.0 mg (1.55 mg/kg); the other dog absorbed 10.1 mg (1.11 mg/kg). These doses were fatal to the dogs in 15 and 10 minutes, respectively. More recent quantitative data were not available.

Information regarding the rapid lethal effects following oral intake of cyanide as soluble cyanide salts in humans indicates that cyanide is rapidly absorbed from the gastrointestinal tract. In a case study, an 80-kg male ingested an estimated 15–25 mg CN^-/kg as potassium cyanide in a suicide attempt (Liebowitz

and Schwartz 1948). Based on a concentration of 200 mg hydrogen cyanide/L in the blood 2 hours after ingestion, it was estimated that the patient had 1.2 g hydrogen cyanide in the blood, with ≈2.3 g CN^- in the body, after 2 hours.

The gastrointestinal absorption of cyanide following ingestion of certain complex iron-containing cyanide compounds is low because cyanide binds with high affinity to iron. In three volunteers (study authors), each of whom ingested a capsule containing 500 mg labeled potassium ferric hexacyanoferrate ($KFe[Fe(CN)_6]$), equivalent to a lethal dose of 3.14–3.64 mg CN^-/kg, only 0.03 mg of free CN^-/kg were absorbed (Nielsen et al. 1990). From the mild toxicological effects, minimal absorption of free cyanide was suspected to have occurred in a woman who attempted suicide by ingesting a coffee spoonful of potassium ferricyanide ($K_3Fe(CN)_6$ or Prussian red) (Hantson et al. 1996). Low bioavailability of cyanide was deduced in the case of a man who attempted suicide by ingesting an unknown amount of cyanide in the form of potassium ferrocyanide (Laforge et al. 1999). Despite an initial toxic blood cyanide concentration of 0.3 mg/100 mL, there were no clinical signs of toxicity and blood chemistry was otherwise normal. As discussed in Section 2.1, since free cyanide absorption and toxicity is unusually low for these iron compounds, the data are not discussed in Chapter 2.

Three dogs were given lethal doses of hydrogen cyanide by gavage. The amount of cyanide absorbed was determined by the difference between the cyanide given and the cyanide left in the stomach and intestines (Gettler and Baine 1938). The dogs dosed with 8.4, 4.4, or 1.6 mg HCN/kg, died 8, 21, and 155 minutes after treatment and had absorbed 17, 24, and 72%, respectively, of the dose given. Rats excreted 47% of a dose of radioactivity in the urine during 24 hours following gavage treatment with 2 mg CN^-/kg as radiolabeled potassium cyanide (Farooqui and Ahmed 1982), indicating that at least 53% of the cyanide was absorbed in 24 hours.

Sousa et al. (2003) compared the absorption of cyanide in male Wistar rats and Landrace-Large White pigs that were given a single dose of 1.2 mg CN^-/kg as potassium cyanide by aqueous gavage. The peak blood concentration (Cmax) of cyanide was reached within 15 minutes in rats and by 30 minutes in pigs. The peak plasma cyanide concentrations were 0.23 and 0.15 mg/100 mL for rats and pigs, respectively. In this study, the peak blood concentration of thiocyanate was reached within 6 hours in rats and pigs. The peak plasma thiocyanate concentrations were 42.8 and 58.1 µmol/L for pigs and rats, respectively.

Absorption of cyanide across the gastrointestinal mucosa depends on the pH of the gut and the pKa and lipid solubility of the particular cyanide compound. Hydrogen cyanide is a weak acid with a pKa of 9.2 at

25 °C. The acidic environment in the stomach favors the non-ionized form of hydrogen cyanide and facilitates absorption. Information regarding the rapid lethal effects following oral intake of cyanide in humans (Gosselin et al. 1984) indicates that cyanide is rapidly absorbed from the gastrointestinal tract.

Oral bioavailability of cyanide from foods containing naturally occurring cyanogenic glycosides such as cassava, linseed, and bitter apricot kernels, is lower than ingestion of the same administered dose as free cyanide due to a variety of factors including delayed and/or incomplete release of cyanide from the cyanogenic glycosides, (Abraham et al. 2016). Cyanide release is greatly reduced in processed food sources (e.g., cassava flour) compared to unprocessed or raw sources containing intact ß-glucosidase. In volunteers given foods containing the same "dose" of naturally occurring cyanide (6.8 mg), blood cyanide levels were highest after consumption of cassava, followed by bitter apricot kernels, then linseed, with very low levels detected after ingestion of persipan paste (Abraham et al. 2016). Peak blood cyanide levels occurred after 37.5 minutes for cyanide (15.4 µM), 20 minutes for bitter apricot kernels (14.3 µM), 40 minutes for linseed (5.7 µM) and 105 minutes for persipan paste (1.3 µM).

No studies were located regarding quantitative absorption in humans after dermal exposure to cyanide gases or common inorganic salts. Evidence that cyanide can be absorbed through the skin of humans is provided in case reports of toxic effects in humans after accidental dermal contact with cyanide (Drinker 1932; Rieders 1971). Hydrogen cyanide is moderately lipid-soluble, which, along with its small size, allows it to rapidly cross mucous membranes; however, penetration across the epidermis is less rapid (Ballantyne 1983a, 1983b, 1988; Walton and Witherspoon 1926). In addition, some cyanide compounds, such as potassium cyanide, have a corrosive effect on the skin that can increase the rate of percutaneous absorption (NIOSH 1976). *In vitro* studies indicate minimal penetration of hydrogen cyanide vapor through human skin samples at concentrations up to 800 ppm; absorption was not significantly impacted by clothing or presence of sunscreen (Gaskin et al. 2013).

Information regarding dermal absorption of cyanide in animals was provided in studies of guinea pigs and dogs (Walton and Witherspoon 1926). When a small area of the shaved abdomen of guinea pigs was exposed to hydrogen cyanide vapor for 30–60 minutes, signs of cyanide toxicity observed included rapid respiration followed by general twitching of muscles, convulsions, and death. In a similar experiment, shaved and unshaved dogs were placed in a chamber in which their bodies, with the exception of the head and neck, were exposed to hydrogen cyanide vapor. No signs of toxicity were reported after exposure to 4,975 ppm hydrogen cyanide for 180 minutes. Deaths occurred after exposure to 13,400 ppm hydrogen cyanide for 47 minutes and suggested dermal absorption.

3.1.2 Distribution

Once cyanide is absorbed, it is rapidly distributed by the blood throughout the body. Tissue levels of hydrogen cyanide were 0.75, 0.42, 0.41, 0.33, and 0.32 mg/100 g of tissue in the lung, heart, blood, kidney, and brain, respectively, in a man who died following inhalation exposure to hydrogen cyanide gas (Gettler and Baine 1938). In one case, tissue cyanide levels from a man who died from inhalation of hydrogen cyanide were reported as 0.5 mg per 100 mL of blood and 0.11, 0.07, and 0.03 mg/100 g in the kidney, brain, and liver, respectively (Finck 1969). Following chronic-duration occupational exposure to 0.19–0.75 ppm hydrogen cyanide, 56.0 and 18.3 µg CN^-/100 mL were found in the blood of smokers and nonsmokers, respectively (Chandra et al. 1980). The cyanide levels in control groups were 4.8 µg/mL for smokers and 3.2 µg/mL for nonsmokers. In a case of death due to oral cyanide exposure, it was estimated that 30 mg of hydrogen cyanide had been ingested and that 3 hours had elapsed before death (Gettler and Baine 1938). Urinary cyanide levels were reported as 0.2 mg/100 mL, and 0.03 mg/100 g were found in the gastric contents (Finck 1969). In a review of 21 oral cyanide-related fatalities, distribution of cyanide in the heart blood, peripheral blood, and gastric contents were 0.1–248.6 mg/L, 0.3–212.4 mg/L, and 2.0–6398.0 mg/kg, respectively (Rhee et al. 2011). A study of tissue distributions of cyanide in five victims of acute cyanide poisoning found that cyanide concentrations are highest in blood (Zhang et al. 2005). Blood had the highest concentration of cyanide in all the victims, ranging between 0.65 and 30.6 µg/mL. Normalizing cyanide concentrations in liver, kidney, brain, and urine samples to cyanide concentrations in blood, it was found that liver had the next highest concentrations of cyanide, with sample/blood coefficients ranging from 0.24 to 0.35.

In two dogs exposed to unspecified fatal concentrations of hydrogen cyanide, the highest cyanide levels were found in the lungs, blood, and heart (Gettler and Baine 1938). Rats exposed to hydrogen cyanide gas at 356 or 1,180 ppm died within 10 and 5 minutes, respectively (Yamamoto et al. 1982). Samples taken immediately after respiration stopped showed that the pattern of tissue distribution of cyanide did not vary with the concentration used. In averaging data for both dose groups, tissue concentrations, reported as µg/g wet weight (ww), were 4.4 in the lungs, 3.0 in the blood, 2.15 in the liver, 1.4 in the brain, and 0.68 in the spleen. Thus, the highest cyanide concentrations were observed in the lung. Rabbits exposed to hydrogen cyanide at 2,714 ppm for 5 minutes had cyanide levels of 170 µg /100 mL in blood and 48 µg/100 mL in plasma, and tissue levels (in units of µg/100 g) of 0 in the liver, 6 in the kidney, 50 in the brain, 62 in the heart, 54 in the lung, and 6 in the spleen (Ballantyne 1983a).

Small but significant levels of cyanide are present in normal blood plasma at concentrations of 0–14 µg % in humans (Feldstein and Klendshoj 1954). Vitamin B_{12} contains cyanide, with the source of cyanide attributed to breakdown of cyanogenic foods by bacteria in the gut.

Cyanide levels in a woman who died 30 minutes after ingesting ≈1,325 mg cyanide as sodium cyanide were, in mg %: stomach contents, 3.2; brain, 0.7; urine, 0.5; blood, 0.4; kidney, 0.2; stomach wall, 0.2; and liver, 0.1 (Ansell and Lewis 1970). The mean organ levels of cyanide ion in cases of fatal poisoning in 17–58 cases were, in mg %: stomach contents, 160; spleen, 3.77; blood, 2.39; liver, 1.62; brain, 1.2; kidney, 0.61; and urine, 0.06 (Ansell and Lewis 1970). Brain cyanide levels were 0.06–1.37 mg hydrogen cyanide/100 g of tissue in four humans who ingested fatal doses of cyanide (Gettler and Baine 1938). Cyanide levels in the livers of six humans were 0.22–0.91 mg hydrogen cyanide/100 g of tissue. In two cases in which men died from ingestion of unknown quantities of unspecified cyanide salts, cyanide levels were highest in the gastric contents, and next highest in the lungs and blood (Finck 1969).

Combined data from 9 to 10 rats that died 3.3 and 10.3 minutes after gavage doses of 7 or 21 mg CN^-/kg as sodium cyanide showed average tissue concentrations of cyanide in µg/g of: liver, 8.9; lung, 5.8; blood, 4.9; spleen, 2.1; and brain, 1.5 (Yamamoto et al. 1982). The pattern of distribution did not vary with administered concentration. When six rats were treated with 4 mg CN^-/kg as potassium cyanide, signs of CNS toxicity were observed (Ahmed and Farooqui 1982), and cyanide levels 1 hour after exposure were 3,380 µg/g in liver, 748 µg/g in brain, and 550 µg/g in kidney. Forty minutes after male Wistar rats received an oral dose of 1.2 mg CN^-/kg as potassium cyanide, the tissue levels of cyanide were 1.04 µg/mL in blood, 0.54 µg/g in liver, 0.20 µg/g in brain, 0.29 µg/g in kidney, and 0.07 µg/g in stomach (Saito et al. 2000). Two-fold increases in the administered dose (2.4 or 4.8 mg CN^-/kg) resulted in approximate 2-fold increases in the cyanide content of these tissues, except for the liver, which showed 3-fold increases. In a study using orally administered radioactively labeled potassium cyanide, the radioactivity detected in whole blood or plasma decreased rapidly within 6 hours. Of the low levels of radioactivity detected in red blood cells, about 94% of the radioactivity recovered was found in the hemolysate, of which 70, 14–25, and 5–10% was detected in the heme fraction, globin, and cell membranes, respectively (Farooqui and Ahmed 1982). Rabbits treated by gavage with 11.9–20.3 mg CN^-/kg as hydrogen cyanide had cyanide levels of 480 µg/100 mL in blood, 252 µg/100 mL in serum, and tissue levels (µg/100 g wet tissue) of 512 in liver, 83 in kidney, 95 in brain, 105 in the heart, 107 in the lung, and 72 in the spleen at necropsy (Ballantyne 1983a).

Cyanide has not been shown to accumulate in the blood and tissues following chronic-duration oral exposure to inorganic cyanides. Following the treatment of groups of 10 male and 10 female rats with hydrogen cyanide in the diet at ≤10.4 mg CN^-/kg/day for 2 years, virtually no cyanide was found in plasma or kidneys (Howard and Hanzal 1955). Low levels were found in erythrocytes (mean of 1.9 µg/100 g). Levels of thiocyanate, the less toxic primary metabolite of cyanide, increased 3.5-fold in plasma, 3.3-fold in erythrocytes, 1.3-fold in liver, and 2.5-fold in kidney. Evaporation of hydrogen cyanide from the feed was thought to have occurred in this study, resulting in lower exposure levels than stated.

No studies were located regarding distribution in humans after dermal exposure to cyanide.

Six rabbits exposed dermally (area not reported) to 33.75 mg CN^-/kg as hydrogen cyanide had blood and serum cyanide levels of 310 and 144 µg/dL, respectively, and tissue levels (µg/100 g) of 26 in liver, 66 in kidney, 97 in brain, 110 in heart, 120 in lungs, and 21 in the spleen (Ballantyne 1983a). Rabbits were administered 5.25 mg CN^-/kg as hydrogen cyanide, sodium cyanide, or potassium cyanide to their conjunctival sac (Ballantyne 1983b). Cyanide concentrations in the tissues were measured immediately after death, which occurred 3–12 minutes after administration. Higher cyanide levels were observed in whole blood than in serum in all three groups. However, blood and serum cyanide levels were significantly lower in sodium cyanide and potassium cyanide groups than in the hydrogen cyanide group. Hydrogen cyanide-treated rabbits also had higher concentrations of cyanide in myocardium, lungs, and brain than rabbits from the other two groups. In all groups, the least amount of cyanide was found in the liver and kidney.

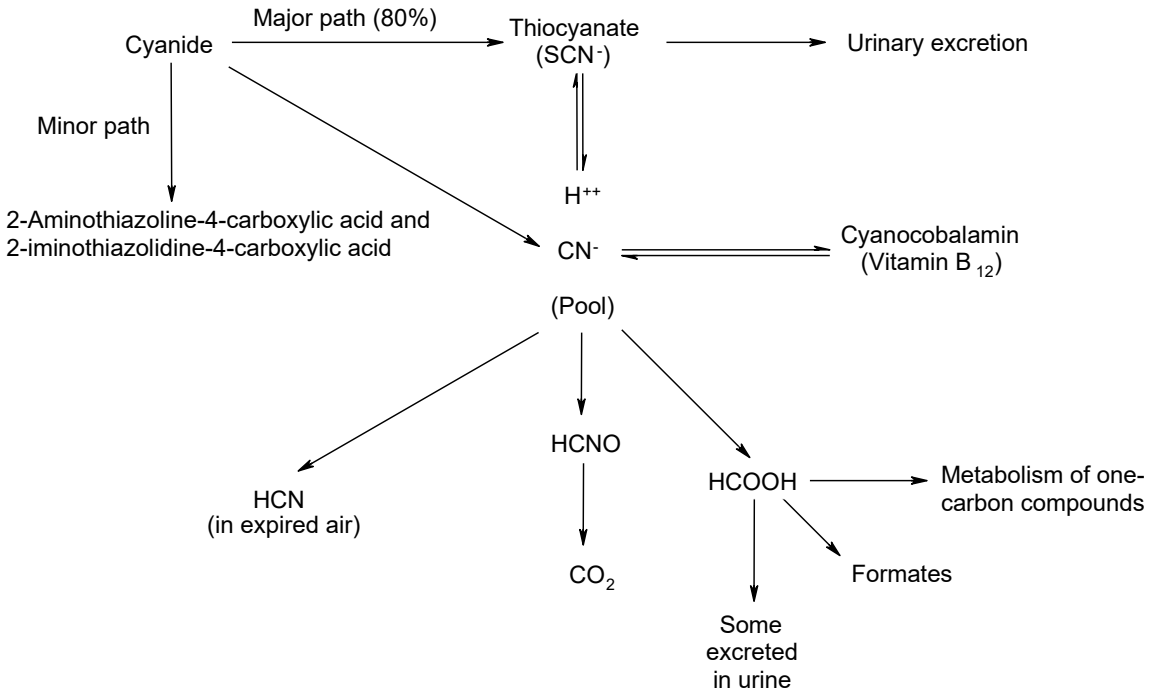
3.1.3 Metabolism

Reports of ingestion of cyanides by humans and reports of occupational exposure indicate that cyanide is transformed into thiocyanate. A plasma half-life of 20 minutes to 1 hour has been estimated for cyanides in humans after nonlethal exposures (Hartung 1982). In rats and pigs, peak plasma thiocyanate concentrations were reached within 6 hours following gavage exposure to a single dose of 1.2 mg CN^-/kg as potassium cyanide (Sousa et al. 2003).

The metabolism of cyanide has been well-studied in animals and major metabolic pathway (conversion of cyanide to thiocyanate) was first demonstrated in 1894. All proposed metabolic pathways are shown in Figure 3-1, including (1) the major pathway, conversion to thiocyanate by either rhodanese or

3-mercaptopyruvate sulfur transferase; (2) conversion to ACTA (or its tautomeric form 2-iminothiazolidine-4-carboxylic acid, ICTA) (Logue et al. 2010; Wood and Cooley 1956); (3) incorporation into a 1-carbon metabolic pool (Boxer and Rickards 1952); and (4) combining with hydroxocobalamin to form cyanocobalamin (vitamin B_{12}) (Ansell and Lewis 1970). Thiocyanate has been shown to account for up to 80% of an administered cyanide dose (Blakley and Coop 1949; Wood and Cooley 1956) while ACTA acid accounts for about 15% of the dose (Wood and Cooley 1956). It is possible that the formation of ACTA could occur over thiocyanate in conditions in which sulfur donors such as rhodanese become depleted or have low initial levels (Logue et al. 2010). Under acidic conditions, thiocyanate can be converted back into cyanide (Seto 1995).

Figure 3-1. Basic Processes Involved in the Metabolism of Cyanide



Source: Ansell and Lewis 1970

Conversion of cyanide to thiocyanate is enhanced when cyanide poisoning is treated by intravenous administration of a sulfur donor (Smith 1996; Way 1984). The sulfur donor must have a sulfane sulfur, a sulfur bonded to another sulfur (e.g., sodium thiosulfate). During conversion by rhodanese, a sulfur atom is transferred from the donor to the enzyme, forming a persulfide intermediate. The persulfide sulfur is then transferred from the enzyme to cyanide, yielding thiocyanate. Thiocyanate is then readily excreted in the urine as the major metabolite.

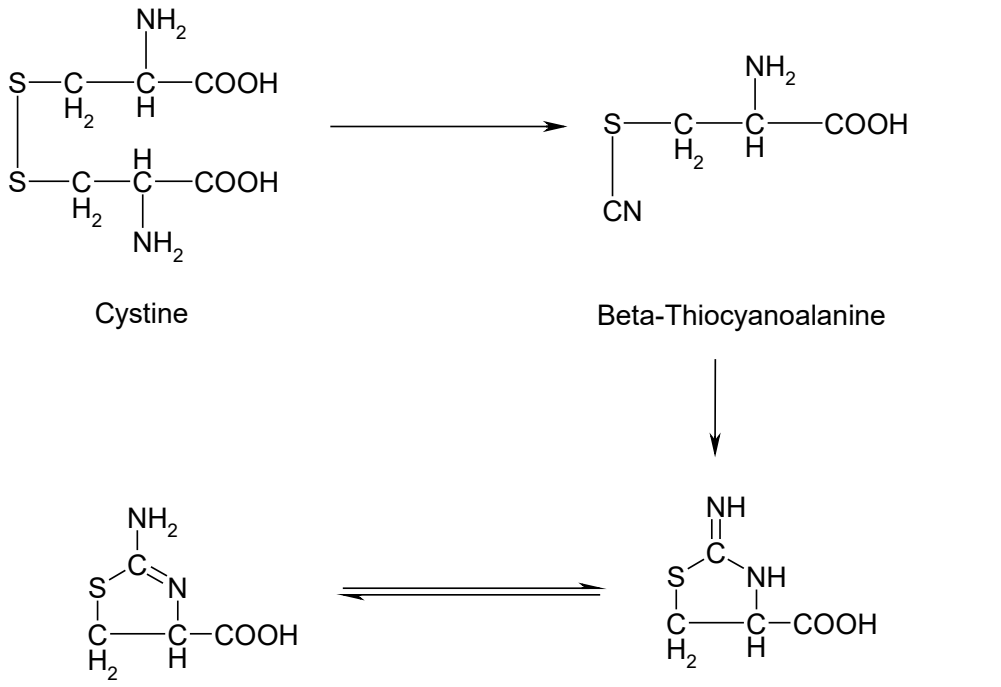
Radioisotopic studies showed that albumin interacts with the sulfane pool and that the serum albumin-sulfane sulfur carrier complex can react with cyanide (Schneider and Westley 1969). Higher hepatic rhodanese and lower serum albumin levels were found in mice fed a protein-free diet for 14 days compared with mice fed a control diet (Rutkowski et al. 1985). Despite the higher rhodanese levels, mortality following an intraperitoneal injection of sodium cyanide was higher in mice fed the protein-free diet both with and without thiosulfate pretreatment. In mice fed the control diet in reduced amounts, serum albumin levels were higher than controls. Mortality in food-deprived mice was also higher compared with controls, but only at high cyanide doses when thiosulfate was also administered. However, the pharmacokinetic studies in dogs, in which thiosulfate administration increased the rate of elimination of cyanide, suggest that the sulfane sulfur pool may play an important role as the central compartment for cyanide detoxification (Sylvester et al. 1983; Way 1984).

The species and tissue distribution of rhodanese is highly variable (Himwich and Saunders 1948). In dogs, the highest activity (conversion of cyanide to thiocyanate) of rhodanese was found in the adrenal gland, ≈2.5 times greater than the activity in the liver. Monkeys, rabbits, and rats had the highest rhodanese activity in the liver and kidney, with relatively low levels in the adrenals. Low levels of rhodanese activity were found for the brain, testes, lungs, spleen, and muscle among various species. It should be noted that rhodanese activity in other species was higher than in dogs, which is consistent with the greater susceptibility of dogs to the acute effects of cyanide (Drawbaugh and Marrs 1987). Rhodanese activities in the liver, expressed as units per g wet organ weight, were as follows: 1,310–1,313 units in rats, 1,104–1,103 units in hamsters, 917–928 units in guinea pigs, 540–722 units in rabbits, 478–502 units in pigeons, 476–516 units in marmoset monkeys, and 453 units in Beagle dogs (Drawbaugh and Marrs 1987). In the kidney, rhodanese activities were as follows: 802–823 units in rats, 734–748 units in guinea pigs, 590–680 units in rabbits, 555–591 units in hamsters, 424–434 units in pigeons, 292–318 units in marmoset monkeys, and 301 units in Beagle dogs (Drawbaugh and Marrs 1987). Dogs also showed the lowest activity levels for 3-mercaptopyruvate sulfur transferase (NIH/NINDS 2016a, 2016b). Mean 3-mercaptopyruvate sulfur transferase activity in human blood was 113.3 and 114.8 units, defined as μmoles of pyruvate generated per minute per 10^{10} red blood cells. In other species, activities were as follows: 15.1–18.2 units in Beagle dogs, 40.8 units in cynomolgus monkeys, 62.5 units in rabbits, 121.8 units in Swiss mice, and 532.2–639.7 in Wistar rats (NIH/NINDS 2016a, 2016b).

In vitro studies with rat tissues indicated that rhodanese activity was ≈7 times higher in the nasal mucosa than in the liver (Dahl 1989). Furthermore, kinetic constants for rhodanese in mitochondria were higher in nasal than in liver tissue.

Figure 3-2 illustrates the minor pathway for metabolism of cyanide in mammalian systems in which cyanide chemically combines with the amino acid cystine. This chemical reaction yields cysteine and β-thiocyanoalanine that is further converted to form ACTA and its tautomer, ITCA (Wood and Cooley 1956). Zottola et al. (2009) propose an alternate oxidative pathway for ACTA formation in which cyanide interacts with an oxidized disulfide to form methyl thiocyanate, which interacts with a sulfur nucleophile from glutathione to form ACTA. However, this theoretical pathway has not been demonstrated *in vitro or in vivo*.

Figure 3-2. Minor Path for the Removal of Cyanide from the Body



Source: Ansell and Lewis 1970

Reactions of cyanide with the salts or esters of some amino acids (e.g., pyruvate, α-ketoglutarate, oxaloacetate) lead to formation of cyanohydrin intermediates and their incorporation into intermediary metabolism (Bhattacharya and Flora 2009).

The ability of cyanide to form complexes with some metallic ions such as cobalt is the basis for the reaction with hydroxocobalamin that yields cyanocobalamin (Bhattacharya and Flora 2009). Cyanocobalamin (vitamin B_{12}), which contains cyanide and cobalt, is essential for the health of mammalian organisms.

3.1.4 Excretion

Following chronic-duration occupational exposure to 0.19–0.75 ppm hydrogen cyanide, 24-hour urinary levels of thiocyanate were 6.23 (smokers) and 5.4 µg/mL (nonsmokers) in exposed workers as compared with 3.2 (smokers) and 2.15 µg/mL (nonsmokers) in the controls (Chandra et al. 1980). This study demonstrates that tobacco smoking contributes to higher thiocyanate levels excreted in the urine. No studies were located regarding excretion of cyanide in animals after inhalation exposure to cyanide.

Cyanide metabolites are normally excreted in urine, with small amounts eliminated through the lungs (Asiah et al. 2014; Logue et al. 2010; Stamyr et al. 2008, 2015). Urinary excretion of thiocyanate was monitored in a man after ingestion of ≈3–5 g potassium cyanide (15–25 mg CN^-/kg) (Liebowitz and Schwartz 1948). The results indicated that the patient excreted 237 mg of thiocyanate over a 72-hour period. This quantity was substantially more than the normal average amount of thiocyanate in urine, which varies between 0.85 and 14 mg/24 hours. Thirty-one children who had consumed flour made from insufficiently processed cassava had mean urinary thiocyanate levels of 757 µmol/L, compared with 50 µmol/L in those children who had consumed sufficiently processed cassava (Tylleskar et al. 1992). In another study (Mlingi et al. 1993), mean urinary thiocyanate was 490 µmol/L in a village affected by Konzo disease (a cyanide-related neurological disease in which upper motor neuron damage results in paralysis) and 350 µmol/L in an unaffected village, with the villages being comparable in all other respects.

When male Sprague-Dawley rats were given an oral dose of 2 mg CN^-/kg [^{14}C] potassium cyanide, urinary excretion of radioactivity reached 47% of the dose within 24 hours following administration (Farooqui and Ahmed 1982). When [^{14}C] sodium cyanide was injected subcutaneously into rats at a level of 8.3 µmol, no difference in radioactivity eliminated was observed between the group pretreated for 6 weeks with a diet containing 0.7 mg CN^-/kg as potassium cyanide and their matching controls (Okoh 1983). Most of the radioactivity was detected in the urine (89% by 24 hours). Thiocyanate was the major metabolite. About 4% of the radioactivity was expired, mostly as carbon dioxide.

Sousa et al. (2003) compared toxicokinetic parameters in male Wistar rats and Landrace-Large White pigs that were given 1.2 mg CN^-/kg as potassium cyanide by aqueous gavage. The half-lives of elimination of cyanide from the blood were 0.54 hours for pigs and 0.64 hours for rats. The half-lives of elimination of thiocyanate from the blood were 4.95 hours in pigs and 5.8 hours in rats. The overall clearance of cyanide from the blood was reported as 0.367, and 0.379 mL/minute per kg for pigs and rats, respectively; the clearance of thiocyanate was reported as 0.135 and 0.061 mL/minute per kg for pigs and rats, respectively.

Following oral administration of potassium cyanide during gestation and lactation, thiocyanate was detected in amniotic fluid and milk of lactating rats, indicating it can cross the placenta and be excreted via breast milk (Soto-Blanco and Gorniak 2004). Orally administered cyanide and its metabolite thiocyanate were also eliminated in the breast milk of lactating goats (Soto-Blanco and Gorniak 2003). The relevance of the goat data to humans is not established.

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

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

Tran et al. (2020a, 2020b) developed PBPK models for oral and inhalation exposure to hydrogen cyanide in humans. Due to limited toxicokinetic data for inhalation exposure, the inhalation model relies heavily on parameters from the oral PBPK model (which was developed first). These models are described in detail below. Stamyr et al. (2015) also developed a preliminary PBPK model to estimate hydrogen cyanide exposure levels and concentration time-course using a four-compartment model (blood, muscle, liver, and other tissue) based on hydrogen cyanide levels in exhaled breath. However, due to critical limitations of this preliminary model, namely validation against only two datasets, this model is not further described.

Tran et al. (2020b) created a model to predict kinetics of hydrogen cyanide in human tissues following oral exposure to either potassium cyanide or cyanogenic glycosides from food. The body was divided into four main compartments: the lungs, kidney, liver, and slowly perfused tissues. Each compartment is connected via the blood circulation, with distinct blood flood rates (Q) for each compartment. Absorption into the circulatory system through the gut lumen was estimated. Metabolism is modeled in the liver compartment, with 80% of absorbed dose metabolized to thiocyanate and 20% metabolized via other pathways (Ansell and Lewis 1970). Model parameters were optimized using data from a studies in which cyanide concentrations were determined in 12 volunteers following ingestion of potassium cyanide or various food sources of cyanogenic glycosides containing the same dose of total cyanide (Abraham et al. 2016) or a single volunteer that ingested various doses of potassium cyanide (Schulz 1984). Unknown parameters (maximum velocity of rhodanese, absorption rate constant, bioavailability) were estimated via parameter optimization using Berkely Madonna software. Model simulations were validated against clinical results from exposure to potassium cyanide or linseed at three doses that differed from the doses used in model optimization. PBPK model estimates of accumulation and elimination of hydrogen cyanide from the blood showed good agreement with experimental data, validating the model.

Tran et al. (2020a) developed a Human Continuous Cyanide Inhalation Predictor (HCCIP) model to predict concentration-time courses of cyanide in inhaled air (based on cyanide levels in the blood) or in the blood and exhaled air (based on cyanide levels in the inhaled air). Due to the paucity of pharmacokinetic data for hydrogen cyanide following inhalation exposure, this model was developed using the data curated for the oral PBPK model described above (Tran et al. 2020b) with the addition of inhalation parameters (inhalation and exhalation concentrations). Similar to that model, the body was divided into four main compartments: the lungs, kidney, liver, and slowly perfused tissues. HCCIP model

estimates of cyanide levels in inhaled air and the blood showed good agreement with experimental data, validating the model.

3.1.6 Animal-to-Human Extrapolations

Biological effects of cyanide in humans have been demonstrated (Smith 1996; Wexler et al. 1947). While there are no studies directly comparing the cytotoxicity between animal and human cells, a difference in species susceptibility to cyanide poisoning was indicated by slightly lower lethal concentrations in rabbits compared to rats (Ballantyne 1983a). Additionally, mortality from cyanides applied dermally varied depending on the cyanide compound used. In the Ballantyne (1983a) study, dermal application resulted in cyanide levels in blood and serum that were lower after topical sodium cyanide and potassium cyanide exposure than from hydrogen cyanide; however, oral exposure in rabbits produced an LD_{50} of 2.3–2.7 mg CN^-/kg/day, regardless of whether the source was hydrocyanic acid, sodium cyanide, or potassium cyanide (Ballantyne 1983a).

Species and tissue distribution of rhodanese (thiosulfate sulfurtransferase), an enzyme important in metabolizing cyanide, is highly variable (Drawbaugh and Marrs 1987; Himwich and Saunders 1948). In dogs, the highest activity of rhodanese was found in the adrenal gland, ≈2.5 times greater than the activity in the liver (Himwich and Saunders 1948). Monkeys, rabbits, and rats had the highest rhodanese activity in liver and kidney, with relatively low levels in adrenals.

It should be noted that activity of the sulfur donors, rhodanese and 3-mercaptopyruvate sulfur transferase, is much lower in dogs compared to other mammalian species (Drawbaugh and Marrs 1987; NIH/NINDS 2016a, 2016b), which is consistent with the greater susceptibility of dogs to the acute effects of cyanide. Thus, dogs may be an inappropriate model from which to extrapolate the toxicity of cyanide to humans. For example, measured rhodanese activities are 1,310–1,313 units/g liver and 802–823 units/g kidney in rats compared to 453 units/g liver and 301 units/g kidney in Beagle dogs (Drawbaugh and Marrs 1987). Details on additional species can be found in Section 3.1.3. For 3-mercaptopyruvate sulfur transferase, mean blood activity in humans was 113.3 and 114.8 units, defined as μmoles of pyruvate generated per minute per 10^{10} red blood (NIH/NINDS 2016a, 2016b). In other species, activities were as follows: 15.1–18.2 units in Beagle dogs, 40.8 units in cynomolgus monkeys, 62.5 units in rabbits, 121.8 units in Swiss mice, and 532.2–639.7 in Wistar rats (NIH/NINDS 2016a, 2016b).

To identify appropriate animal models for testing the efficacy of methemoglobin-forming cyanide antidotes, Rockwood et al. (2003) compared the endogenous activities of the erythrocyte NADH-dependent enzyme methemoglobin reductase (ferricyanide reductase) in several species. Two strains of beagles had enzyme activities roughly 40–50% lower than the mean for humans and with no overlap to the range for the human data, further suggesting that dogs may not be a suitable animal model from which to extrapolate the toxicity of cyanide to humans. The enzyme activities of the other tested species had higher means than the human, but the ranges for the Rhesus and Aotus monkeys were similar to the human, indicating that these would be appropriate models. Data for the marmoset, Cynomolgus monkey, and African green monkey showed less overlap to the human data, whereas data for the ferret, chimpanzee, and baboon showed no overlap.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

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

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

From the few oral studies available, the effects of cyanide on children appear to be like those of similarly exposed adults. This is expected based on cyanide's inhibition of mitochondrial respiration in all cells (Bhattacharya and Flora 2009). Neurological (headache and coma), respiratory (tachypnea), cardiovascular (hypotension), and gastrointestinal effects (vomiting) have been reported in children who have been poisoned by eating apricot pits (Lasch and El Shawa 1981). Congenital hypothyroidism has

been observed in some children who were exposed to increased thiocyanate levels because of the maternal cassava diet during pregnancy (Ermans et al. 1980).

Developmental studies in animals (rats or hamsters) orally exposed to potassium cyanide, cassava diets, or one of the cyanogenic glycosides (amygdalin, linamarin) reported fetal toxicity (reduced fetal weight, delayed ossification) and developmental anomalies (microcephaly, limb defects, encephalocele, and rib abnormalities) in offspring (Frakes et al. 1986; Singh 1981; Tewe and Maner 1981a; Willhite 1982). These effects occurred at exposure levels that were toxic to the dam. A developmental study in pigs indicates that this species is less sensitive than rodents to gestational exposure to cyanide (Tewe and Maner 1981b). Results of a studies in lactating rats and goats indicate that cyanide and thiocyanate can be transferred through milk to nursing offspring (Soto-Blanco and Gorniak 2003, 2004).

In goats, maternal co-administration of sodium thiocyanate prevented the rise in erythrocyte cyanide levels caused by sodium nitroprusside (Curry et al. 1997). Sodium nitroprusside is infused intravenously as a vasodilator for the treatment of hypertensive emergencies (Agarwal and Kumari 2003; Curry et al. 1997; Przybylo et al. 1995; Randell and St. Louis 1996; Sipe et al. 2001). In the blood, sodium nitroprusside nonenzymatically receives one electron from oxyhemoglobin, forming the nitroprusside radical, which dissociates to nitric oxide (the vasodilator) and five cyanide ions (Przybylo et al. 1995). In practice, sodium thiosulfate is co-administered to prevent cyanide toxicity. Curry et al. (1997) infused sodium nitroprusside into gravid ewes, resulting in elevations of erythrocyte cyanide concentrations that caused the death of one ewe and one fetus from cardiac toxicity. Co-administration of sodium thiosulfate to gravid ewes prevented the elevation in erythrocyte cyanide levels in ewes and fetuses. Curry et al. (1997) concluded that sodium thiosulfate, like cyanide and sodium nitroprusside, cross the placenta in goats. The relevance of the goat study to humans is not known.

Information on exposures of cyanide to children living in the United States is mainly limited to studies on side-stream smoke. These studies show that this is an important route of exposure to cyanide for children in households with a resident smoker. Chen et al. (1990) found that serum thiocyanate concentrations of 18-month-old infants heavily exposed to environmental tobacco smoke (>20 cigarettes a day smoked in the home) were significantly higher than those of unexposed infants ($p<0.05$). Mean concentrations (±standard deviation [SD]) in these respective groups were 36.2±14.88 µmol/L (2.1±0.9 µg/mL) and 27.7±10.7 µmol/L (1.6±0.6 µg/mL). Positive correlations between fetal umbilical serum thiocyanate levels and serum thiocyanate levels of smoking mothers (Bottoms et al. 1982; Hauth et al. 1984) and mothers exposed to environmental tobacco smoke in the home (Bottoms et al. 1982) have been reported.

Hauth et al. (1984) found that the mean serum thiocyanate concentration (95 µmol/L; 5.5 µg/mL) was significantly higher ($p<0.001$) in smokers than in passive smokers (35.9 µmol/L; 2.1 µg/mL) or nonsmokers (32.3 µmol/L; 1.9 µg/mL). Similarly, the mean umbilical thiocyanate concentration in the newborn infants of smoking mothers (72 µmol/L; 4.8 µg/mL) was significantly higher than those in newborn infants of passive smokers (26 µmol/L; 1.5 µg/mL) and nonsmokers (23 µmol/L; 1.3 µg/mL). Bottoms et al. (1982) found that among newborn infants of nonsmoking mothers, fetal umbilical thiocyanate concentrations increased with passive smoking in the home ($p<0.05$).

For children without exposures to side-steam smoke, their main cyanide exposures are expected to be like those noted for the general population in Section 5.6 in air and water. Estimates of the cyanide concentration in the total diet of children in the United States were not located in the available literature. Therefore, no estimate of daily cyanide intake from food can be made. However, in the United States, exposure of children to cyanide from foods in which it occurs naturally is expected to be low, but, as noted for Section 5.6 for the general population, it is likely to exceed cyanide intake from inhalation of air and ingestion of drinking water (EPA 1981). Based on a concentration of cyanide in U.S. and Canadian drinking water of 0.001–0.011 mg/L, the daily intake of cyanide in children is estimated to be 0.001–0.011 mg, assuming a daily consumption of 1 L of water (EPA 1981; Meranger and Lo 1992). For cyanide as cyanogen chloride, the daily intake is estimated to be 0.5–0.8 µg, which is equivalent to 0.2–0.4 µg of hydrogen cyanide. This estimate is based on the quarterly median cyanogen chloride concentration in drinking water from 35 U.S. water utilities of 0.45–0.8 µg/L (0.19–0.3 µg/L cyanide) (Krasner et al. 1989) and the daily consumption of 1 L of drinking water.

Accidental cyanide poisonings in children are rare and are usually associated with exposures to combustion products in smoke (Riordan et al. 2002). Poisonings have been reported for ingestion of apricot kernels or seeds or candy made from apricot kernels. Because of their lower body weight, children tend to be more susceptible to consumption of apricot kernels than adults, with 10 or more seeds being fatal to a child (WHO 2004).

Persons with a metabolic disturbance in the conversion of cyanide to thiocyanate may be at greater risk from the toxic effect of cyanide. A defect in the rhodanese system and vitamin B_{12} deficiency have been noted in persons with tobacco amblyopia and Leber's hereditary optic atrophy exposed to tobacco smoke which contains cyanide (Wilson 1983). Iodine deficiency, along with excess chronic exposure to cyanide, may, in certain cases, be involved in the etiology of such thyroid disorders as goiter and cretinism (Delange and Ermans 1971; Ermans et al. 1972). Also, protein deficiencies and vitamin B_{12} and

riboflavin, and other deficiencies may subject people who eat foods high in cyanogenic glycosides to increased risk of neuropathies (Makene and Wilson 1972; Osuntokun 1972; Osuntokun et al. 1969). Patients with motor neuron disease (amyotrophic lateral sclerosis) possess a disorder in cyanide metabolism that may result in higher susceptibility to cyanide (Kato et al. 1985).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for cyanide from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to cyanide are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by cyanide are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Methods are available to measure levels of cyanide and its metabolite, thiocyanate, in blood and urine. High blood cyanide levels of 250–300 μg/100 mL were reported in cases of death from cyanide poisoning (Holstege and Kirk 2019; Vogel et al. 1981). The relationship between increased exposure and increased urine levels of thiocyanate was demonstrated in workers exposed occupationally to 6.4–10.3 ppm cyanide in air (El Ghawabi et al. 1975). In another study, blood cyanide concentrations varied from 0.54 to 28.36 μg/100 mL in workers exposed to ≈0.2–0.8 ppm cyanide in air and from 0.0 to 14.0 μg/100 mL in control workers (Chandra et al. 1988). Correspondingly, blood thiocyanate concentrations were 0.05–2.80 mg/100 mL in exposed workers and 0.02–0.88 mg/100 mL in control workers, respectively. Data obtained from the controls indicate that cyanide can be detected in populations exposed to low cyanide levels in the environment. Cyanide-containing food, metabolism of certain drugs, and combustion of nitrogenous polymers are among several sources of cyanide exposure. Furthermore, industrially polluted air, soil, and water may contribute to higher environmental cyanide levels. Following acute exposures, blood cyanide levels and urinary thiocyanate levels are useful for confirming cyanide exposure but may have limited value in the initial treatment of the poisoning (Holstege and Kirk 2019).

Several studies showed increased cyanide and thiocyanate levels in body fluids of smokers. Mean thiocyanate levels in smokers and nonsmokers, respectively, were found to be 7.1 and 2.0 μg/mL in plasma, 75.7 and 20.3 μg/mL in saliva, and 12.3 and 2.1 μg/mL in urine (Maliszewski and Bass 1955). Another study reported that mean thiocyanate levels were 7.1 and 2.9 μg/mL in plasma, 142 and 76 μg/mL in saliva, and 9.0 and 5.8 μg/mL in urine in smokers and nonsmokers, respectively (Jarvis 1989). The number of cigarettes smoked per day is positively correlated with the thiocyanate levels in plasma and in saliva (Yamanaka et al. 1991). Based on changes in salivary thiocyanate in six former smokers, this study estimated the half-life of salivary thiocyanate to be 9.5 days. In addition, infants living in homes with family members who smoked heavily were found to have significantly higher serum thiocyanate levels than those infants who were not exposed to cigarette smoke in the home (Chen et al. 1990). It is unclear whether passive smoking (exposure of a nonsmoker to air contaminated with tobacco smoke) is a factor in elevated fetal serum thiocyanate levels. In one study, fetal thiocyanate levels were increased in association with passive smoking in the home (Bottoms et al. 1982), while another study did not report an association (Hauth et al. 1984).

Whether it is more appropriate to use whole blood or plasma for measuring cyanide concentrations has been the subject of several reports. Cyanide plasma levels are usually about one-third to one-half of those found in whole blood, depending on the species (Ballantyne 1983a). However, plasma levels can more closely reflect the actual tissue dose. Furthermore, cyanide was found to attach more readily to plasma albumin than to hemoglobin (McMillan and Svoboda 1982). While cyanide binds to hemoglobin, hemoglobin does not play any role in the metabolism, though some authors argue that cyanide in red blood cells may be biologically active (Way 1984). Cyanide also rapidly leaves serum and plasma, especially in the first 20 minutes. Therefore, it may be appropriate to measure cyanide in both whole blood and plasma. Whole blood samples can be stored at 4°C for several weeks with little change in cyanide content.

In addition to thiocyanate, another cyanide metabolite, ATCA, has been shown to be a stable biomarker of cyanide exposure. ATCA is formed through the reaction of cyanide with l-cystine and accounts for 20% of cyanide metabolism in the human body (Logue et al. 2005). Unlike cyanide, ATCA is stable for months in biological samples stored at freezing or ambient temperatures. Logue et al (2005) report that ATCA is readily recovered from plasma or urine and analyzed by gas chromatography/mass spectrometry (GC/MS). The assay method provides for good detection limits (25 ng/mL) and recoveries (100% from plasma and 84% from urine). ATCA can also be detected in urine via liquid chromatography/mass spectrometry (LC/MS), with a detection limit of 15 ng/mL and a recovery rate ≥94% (Alwis et al. 2012). However, ATCA is produced via a minor metabolic pathway and may not be a sensitive biomarker. In rats exposed to potassium cyanide via subcutaneous injection, ATCA levels did not change in blood plasma, though elevated levels of ATCA were observed in the liver (Petrikovics et al. 2011). If human distribution of ATCA metabolism is similar, ATCA may not be a promising diagnostic biomarker in sublethal cases, but it may be useful for postmortem examinations.

In cyanide-poisoning cases, any blood levels of cyanide >0.02 mg/100 mL indicate a toxic situation (Berlin 1977). However, because cyanide binds tightly to cytochrome c oxidase, serious effects can also occur at lower levels; therefore, the clinical condition of the patient should be considered when determining proper therapy. Linden and Lovejoy (1998) presented a rough estimate of blood cyanide levels at which symptoms appear: flushing and tachycardia at 0.05–0.1 mg/100 mL, obtundation (dulled sensibility) at 0.1–0.25 mg/100 mL, coma and respiratory depression at 0.25–0.3 mg/100 mL, and death at >0.3 mg/100 mL.

While blood or urinary levels of cyanide or its metabolites are useful for confirming exposure, they may not be clinically useful in acute poisoning scenarios as results will likely not be available in time for clinical management (Graham and Traylor 2023; Holstege and Kirk 2019). In these cases, clinical presentation may be used initially for differential diagnosis in cases of suspected poisoning. Classical signs associated with cyanide exposure include an almond-like smell detected on the breath of the patient by the clinician and "cherry-red skin" (due to increased venous oxygen saturation). However, the reliability of these signs as biomarkers, on their own, has been questioned (Holstege and Kirk 2019; Parker-Cote et al. 2018). First, the ability to smell the bitter almond odor of hydrogen cyanide is genetically linked and approximately 60–70% of the population can detect it (Graham and Traylor 2023). The odor threshold for detection is estimated at concentrations of 1–5 ppm in the air. In a systematic review of 102 cases of cyanide poisoning, a detection of a bitter almond odor by the clinician was only reported in approximately 7% of cases, and a different odor was reported in about 8% of cases (Parker-Cote et al. 2018). However, it should be noted that most cases did not comment on the presence or absence of an odor. Similarly, "cherry red skin" was only present in 11% of cases reviewed by Parker-Cote et al. (2018). Arterialization of retinal veins (red presentation of retinal veins similar in color to retinal arteries) detected by funduscopic examination has also been proposed as a method to check for increased venous oxygen saturation in patients with suspected cyanide poisoning (Holstege and Kirk 2019). Other clinical abnormalities that may be observed in patients with cyanide poisoning include elevated plasma lactate, increased anion gap metabolic acidosis, increased venous oxygen saturation level, and dilated pupils (Graham and Traylor 2023; Holstege and Kirk 2019). While none of these findings alone are definitive biomarkers of exposure to cyanide, collectively, this clinical picture (particularly the known metabolic abnormalities including increased anion gap metabolic acidosis of unknown etiology), along with an altered mental status, is suggestive of potential cyanide poisoning (Holstege and Kirk 2019). Clinical signs associated with cyanide toxicity are further discussed in Section 3.3.2 (Biomarkers of Effect).

For inhalation exposures, particularly in fire victims, exhaled levels of hydrogen cyanide in the breath have been proposed as biomarker of systemic exposure (Stamyr et al. 2008). While there is concern that exhaled breath may contain unabsorbed hydrogen cyanide (from very recent exposure), Stamyr et al. (2008) determined that washin-washout kinetics from the airways would have a negligible effect on measured hydrogen cyanide levels due to the rapid half-life of hydrogen cyanide in breath (16 seconds).

Some effects of cyanide that can also be used to monitor exposure are discussed in Section 3.3.2.

3.3.2 Biomarkers of Effect

Cyanide can inhibit enzymatic activity by binding to some metallic moieties in metalloenzymes (Ardelt et al. 1989; Way 1984) and cytochrome c oxidase is especially sensitive to cyanide inhibition. Dose-related reductions in cytochrome c oxidase activity were detected in various organs of rats exposed to oral doses of potassium cyanide (Ikegaya et al. 2001); this marker was suggested as a method of diagnosis for samples taken within 2 days post-mortem. Consequent to the inhibition of cytochrome c oxidase, theoretically, oxygen cannot be used and histotoxic anoxia occurs. Lack of oxygen usage results in increased venous oxygen saturation, but this is not a useful biomarker for cyanide as several other chemical exposures (e.g., carbon monoxide) and medical conditions can cause this phenomenon (Holstege and Kirk 2019). The shift to anaerobic metabolism results in the reduction of pyruvate to lactate leading to lactate acidosis (Bhattacharya and Flora 2009). Plasma lactate concentrations have been found to be correlated to blood cyanide concentrations (Baud et al. 2002) and have been used to assess the severity of cyanide poisoning in humans (Baud et al. 1996, 2002; Haden et al. 2022). However, lactate acidosis is not specific to cyanide toxicity. Elevated anion gap metabolic acidosis is also suggestive of cyanide poisoning, but like lactate acidosis, this finding is not specific to cyanide toxicity (Holstege and Kirk 2019).

Dyspnea, palpitations, hypotension, convulsions, and vomiting are among the first effects of acute cyanide poisoning resulting in death. Death is caused by respiratory failure secondary to histotoxic hypoxia. Ingestion of amounts ≥50–100 mg sodium or potassium cyanide may be followed by almost instantaneous collapse and cessation of respiration (Hartung 1982). Data summarized by Hartung (1982) indicate that exposure to a concentration in air of 270 ppm causes immediate death; concentrations of 181 and 135 ppm are fatal after 10 and 20 minutes of exposure, respectively; concentrations between 45 and 55 ppm can be tolerated for 30–60 minutes with immediate or late effects; and 18–36 ppm may produce slight symptoms after several hours of exposure. Following chronic-duration exposure, cyanide has been associated with the development of tropical neuropathy, tobacco amblyopia, and Leber's hereditary optic atrophy (Wilson 1965). Chronic-duration exposure to cyanide arising from consumption of cyanogenic plant foods has also been connected with the occurrence of endemic goiter (Delange and Ermans 1971).

Neuropathological sequelae of acute cyanide poisoning have been detected in the brain by magnetic resonance imaging (MRI) and positron emission tomography (PET). MRI techniques identified brain lesions that developed in the weeks following a poisoning event, typically in the globus pallidus, putamen, substantia nigra, and cerebellum (Rosenberg et al. 1989; Rosenow et al. 1995). PET has been

used to localize deficiencies in dopa uptake in the striatum and reduced glucose metabolism in the cerebral cortex and other brain regions affected by cyanide (Rosenow et al. 1995). These imaging methods cannot determine that cyanide was the cause of the lesions but provide a means of monitoring the extent of brain lesions following cyanide exposure.

In the development of antidotes to cyanide, the following neurochemical biomarkers of cyanide toxicity have been considered (Isom and Borowitz 1995): inhibition of cytochrome c oxidase, activation of voltage sensitive calcium channels, activation of receptor operated calcium channels, elevation of cytosolic free Ca^{2+}, activation of intracellular calcium cascades, inhibition of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), peroxidation of membrane lipids, and generation of reactive oxygen species.

Genetic markers for cyanide-induced hypoxia have been identified in human cell lines (human intestinal epithelial T84 cells and Jurkat T cells) exposed to sodium cyanide *in vitro* (Kiang et al. 2003). Cyanide treatment upregulated the expression of inducible nitric oxide synthase (iNOs) and heat shock protein-70 (HSP-70) in both cell types, p53 in T84 cells, and the protooncogene Bcl-2 in Jurkat T cells. Cellular caspase-3 activity, indicative of apoptosis, was also significantly increased in both cell types. An inhibitor to iNOs (N^{omega}-nitro-L-arginine or LNNA) abolished the cyanide-induced increase in iNOs, HSP-70, and Bcl-2 and the increase in caspase-3 activity. In an *in vitro* study in endothelial cells, changes in mitochondrial reactive oxygen species, an increase in the mitochondria:cytosol ratio of the apoptosis regulator Bcl-2 associated X (BAX), and an increase in the expression of hypoxia inducible factor (HIF-1α) were observed (Zuhra and Szabo 2022). These studies indicate genetic responses to cyanide exposure *in vitro* and could provide a strategy for comparing tissue-specific responses to cyanide and developing therapeutic interventions following cyanide exposure *in vivo*.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Interactions in the context of this profile refer to modifications in toxic responses when an organism is exposed to another compound in addition to cyanide. A number of compounds act in synergy with cyanide to produce toxic effects. In smoke, both hydrogen cyanide and carbon monoxide would potentially increase CNS effects in exposed individuals (Birky and Clarke 1981). High blood cyanide levels were found in fire victims; however, the carboxyhemoglobin levels were also high. Thus, it is difficult to assess the relative significance of hydrogen cyanide in the toxicity from smoke inhalation.

In an investigation to examine toxicological interactions of the primary fire gases, the additive, synergistic, or antagonistic effects of combinations of hydrogen cyanide with carbon monoxide or with carbon dioxide on the 30-minute LC_{50} value for hydrogen cyanide alone were determined in rats (Levin et al. 1987). Co-exposure of rats to hydrogen cyanide (LC_{50}=110 ppm) and carbon monoxide (LC_{50}=4,600 ppm) resulted in lethal effects that were additive. In contrast, co-exposure to hydrogen cyanide and 5% carbon dioxide (not lethal by itself) resulted in an increase in lethality of hydrogen cyanide, as reflected by a decrease of the hydrogen cyanide LC_{50} value from 110 to 75 ppm. Dodds et al. (1992) also investigated the effect of simultaneous exposure to cyanide and carbon monoxide in rats, and found an additive effect on certain parameters, including lactate elevation and neurologic index. Norris et al. (1986) reported a synergistic effect on lethality in mice that were co-exposed to potassium cyanide via injection and carbon monoxide via inhalation.

Potentiation has been observed between cyanide and ascorbic acid (vitamin C). Guinea pigs exhibited increased toxic effects when treated with ascorbic acid prior to oral administration of potassium cyanide (Basu 1983). When guinea pigs were treated solely with 3.2 mg CN^-/kg as potassium cyanide, 38% exhibited slight tremors, whereas those pre-treated with 1.3 g/kg ascorbic acid for 3 consecutive days, 100% exhibited severe tremors, ataxia, muscle twitches, paralysis, and convulsions. It has been suggested that this potentiation results from the ability of ascorbic acid to compete with cyanide for cysteine, thus diminishing the detoxication of cyanide.

Antidotes for cyanide poisoning have been intensively studied and reviewed (Way 1984). Cyanide antagonists can be classified into two general groups: sulfane sulfur donors for rhodanese-catalyzed cyanide detoxification and chemicals that bind cyanide (methemoglobin inducers and cobalt compounds). Sulfur donors are used to enhance cyanide conversion to thiocyanate and include sodium thiosulfate, polythionates, and thiosulfates (Bhattacharya and Flora 2009). Sodium thiosulfate has been successfully used as an antidote against cyanide poisoning in humans for decades (Way 1984). A pharmacokinetic study in dogs demonstrated that intravenous administration of thiosulfate increased the detoxification rate of intravenously given cyanide to thiocyanate over 30 times (Sylvester et al. 1983). In this study, pretreatment with thiosulfate decreased the biological half-life of cyanide from ≈39 to ≈15 minutes and also decreased the volume of distribution of cyanide from 498 to 204 mL/kg. Thiosulfate pretreatment had prophylactic effects in guinea pigs exposed to cyanide by intravenous infusion (Mengel et al. 1989). The protection lasted for several hours depending on the dose of thiosulfate administered.

Antagonists that induce the chemical binding of cyanide to sites other than cytochrome c oxidase include methemoglobinemia inducers which provide a large pool of ferric (3+) iron to which cyanide preferentially binds when compared to cytochrome c oxidase (Bhattacharya and Flora 2009). These compounds include sodium nitrite, amyl nitrite, and 4-dimethylaminophenol (Way 1984, Bhattacharya and Flora 2009). Sodium nitrite has been effectively used in the therapy of cyanide intoxication in humans especially in combination with sodium thiosulfate (Smith 1996; Way 1984). Studies in mice demonstrated that intraperitoneal pretreatment with sodium nitrite more than doubled the LD_{50} value of intraperitoneally administered sodium cyanide from 3.18 to 7.95 mg CN^-/kg (Kruszyna et al. 1982). Peak methemoglobinemia was 35% at 40 minutes. Other methemoglobin generating agents seemed to be less effective. 4-Dimethylaminopropiophenol enhanced the LD_{50} value to 6.36 mg CN^-/kg and hydroxylamine to 4.66 mg CN^-/kg with peak methemoglobinemia being 40 and 36%, respectively, at 7 minutes. The data suggested that sodium nitrite, a slow methemoglobin former, offered prolonged protection against cyanide, while animals treated with fast methemoglobin formers died later on, probably due to the cyanide release from the cyanmethemoglobin pool. An improvement of cyanide-altered cerebral blood flow was observed in dogs treated with sodium nitrite or 4-dimethylaminophenol following intravenous injection of hydrogen cyanide (Klimmek et al. 1983). However, neither treatment prevented the progression of lactic acidosis.

Cobalt-containing compounds may also function as binders as the cobalt ion forms a stable complex with cyanide (Bhattacharya and Flora 2009). Examples include hydroxocobalamin and dicobalt ethylenediamine tetra-acetate acid (Co_2EDTA). A dramatic antagonism of the lethal effects of potassium cyanide was reported when cobaltous chloride was administered to mice along with sodium thiosulfate (Isom and Way 1973). The study authors suggested that this synergistic antidotal effect of cobaltous chloride may be associated with the physiological disposition of the cobaltous ion and its ability to chelate both thiocyanate and cyanide ions. This ability is also utilized when Co_2EDTA is used as a cyanide antidote. An improvement of cerebral aerobic metabolism and blood flow was observed in dogs treated with 10 mg/kg Co_2EDTA intravenously following intravenous application of 1.6 mg CN^-/kg as potassium cyanide (Klimmek et al. 1983). A lower molecular weight porphyrin cobalt compound than hydroxocobalamin (CoTPPS) was used as an antidote to the lethal effects of cyanide (McGuinn et al. 1994). The interaction with hydroxocobalamin (see Section 3.1.3) was also proposed as a mechanism for cyanide detoxification in cases of acute poisoning. It was demonstrated that intravenous administration of hydroxocobalamin (50–250 mg/kg) prior to or after intraperitoneal (i.p.) injection of potassium cyanide prevented lethality and decreased cyanide-induced toxic effects in mice (Mushett et al. 1952).

Several papers discuss the effects of oxygen alone or with other compounds on cyanide toxicity. Oxygen alone results in minimal antagonism in mice injected with potassium cyanide and only slightly enhances the antagonistic effects of sodium nitrite on cyanide (Sheehy and Way 1968). The antidotal effect of sodium thiosulfate alone or in combination with sodium nitrite, was enhanced by oxygen. Oxygen-treated mice did not show behavioral signs of cyanide intoxication below doses of 2.4 mg CN^-/kg as potassium cyanide, whereas air-treated animals showed effects such as gasping, irregular breathing, and convulsions at levels as low as 1.2 mg CN^-/kg as potassium cyanide (Isom et al. 1982). When mice were pretreated with sodium nitrite and sodium thiosulfate and either air or oxygen, the dose of potassium cyanide needed to cause a 59% inhibition of brain cytochrome c oxidase more than doubled in mice in an oxygen atmosphere; all points on the oxygen curve differed significantly from the air-treatment curve.

Oxygen supplementation is often a first line of supportive therapy in cyanide toxicity (Bhattacharya and Flora 2009). Enhancement of the glucose oxidation to carbon dioxide was observed when oxygen, sodium nitrite, and sodium thiosulfate were given to mice dosed with 18 mg CN^-/kg as potassium cyanide; no enhancement was observed at 4 or 6 mg CN^-/kg as potassium cyanide (Isom and Way 1974). These studies indicate that oxygen can be used in support with cyanide antagonists, but not alone as even hyperbaric oxygen alone had no effect on cyanide poisoning in mice (Way et al. 1972). The mechanism of the action of oxygen as an adjunct is not known, however, since cyanide inhibits the cellular utilization of oxygen through inhibiting cytochrome c oxidase, theoretically, the administration of oxygen should have no effect (Smith 1996).

Co administration of additional compounds with afore discussed antidotes have been examined for augmentation of the efficacy of antidotal therapy. The nucleophilic activity of cyanide to combine with carbonyl groups of ketone or aldehyde intermediary metabolites (e.g., sodium pyruvate, α-ketoglutarate) to form cyanohydrin has also been used for sequestration. Pretreatment of mice with sodium pyruvate (1 g/kg i.p.) prior to subcutaneous injection of potassium cyanide caused a statistically significant increase in the LD_{50} value from 3.1 to 5 mg CN^-/kg and prevention of convulsions (Schwartz et al. 1979). Similarly, pretreatment of mice with α-ketoglutarate (2 g/kg, i.p.) before exposure to potassium cyanide (i.p.) increased the LD_{50} value from 2.68 to 13.32 mg CN^-/kg (Moore et al. 1986). It was further demonstrated that both sodium pyruvate and α-ketoglutarate enhanced the antidotal effects of other cyanide antagonists (e.g., sodium thiosulfate, sodium nitrite) (Moore et al. 1986; Schwartz et al. 1979).

Chlorpromazine, in conjunction with a sulfur donor, significantly attenuates effects of cyanide toxicity. Adjunctive compounds include α-adrenergic blockers and calcium channel blockers (Bhattacharya and

Flora 2009). Pretreatment of rats with chlorpromazine (10 mg/kg intramuscularly) and sodium thiosulfate (1,000 mg/kg intraperitoneally) abolished or greatly diminished the increase in plasma creatine kinase observed in rats exposed to hydrogen cyanide at 200 ppm for 12.5 minutes (O'Flaherty and Thomas 1982). In an *in vitro* study, chlorpromazine and 4,4'-diisothiocyano-2,2'-stilbene disulfonic acid reduced cyanide-induced contractions in vascular smooth muscle (Robinson et al. 1985a). It was suggested that chlorpromazine prevents cyanide-induced calcium influx via reduction of lipid peroxidation of membranes (Maduh et al. 1988).

A new conceptual approach, employing carrier erythrocytes containing highly purified rhodanese (thiosulfate sulfur transferase) offers striking protection against cyanide. Several studies have shown that resealed erythrocytes containing rhodanese and sodium thiosulfate rapidly metabolize cyanide to thiocyanate (Cannon et al. 1994; Petrikovics et al. 1995). Maduh and Baskin (1994) showed that rhodanese may be regulated by protein phosphorylation and treatments that alter the phosphorylation state may affect cyanide metabolism.

An inhibitor of the enzyme cystathionine gamma-lyase, propargylglycine, significantly lowered the LD_{50} for sodium cyanide (i.p.) in rats (Porter et al. 1996). The study authors suggested that the enzyme contributes to cyanide detoxification, possibly through a pathway that provides sulfur donors.