

## CHAPTER 1

# Acute cyanide toxicity

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### At a Glance

- Cyanide intoxication can result from diet, fires, alternative and standard medical treatments, industrial exposure, and intentional exposure (e.g., suicide, homicide, terrorism).
- Cyanide blocks the oxidative respiration pathway, impeding oxygen usage within tissues; the major metabolic pathway results in the formation of less toxic thiocyanate.
- Across species, long-term effects of cyanide post-exposure include a range of behavioral and neurological dysfunction, such as Parkinsonism.
- Antidotal treatments for acute cyanide toxicity may significantly reduce adverse sequelae and provide a better quality of life post-exposure.

## 1.1 Introduction

Cyanide (CN) is a potent toxicant with rapid onset of histotoxic anoxia through inhibition of mitochondrial oxidative phosphorylation (Way, 1984), inhibition of oxidative metabolism (cytochrome C oxidase (CcOX) inhibition), and alteration of critical cellular ion homeostasis (Gunasekar *et al.*, 1996). CN exists

in a variety of forms, including gaseous hydrogen cyanide (HCN), water-soluble potassium (K) and sodium (Na) salts, poorly water-soluble mercury (Hg), copper (Cu), gold (Au), and silver (Ag) CN salts (Leybell *et al.*, 2011). Cyanogens, which are glycosides of sugar and CN-containing aglycon (Makkar *et al.*, 2007), include complex nitrile-containing compounds that can generate free CN of toxicological significance (Rao *et al.*, 2013). Within the liver, the enzyme rhodanese catalyzes the conversion of CN to thiocyanate (SCN), which is normally excreted through the kidneys. CN can bind to both the oxidized and reduced forms of CcOX, but it possesses a greater affinity for the oxidized form (Van Buuren *et al.*, 1972).

Cyanogenic compounds, such as amygdalin, can be found in certain plants, particularly in the seeds and pits of members of the genus *Prunus*, which includes apricot pits, peach pits, cherry pits, apple seeds, and almond husks (Shepherd & Velez, 2008). Other sources of CN exposure include exposure from industrial products and processes. Worldwide industrial consumption of CN is estimated to be 1.5 million tons per year, and occupational exposures account for a significant number of CN poisonings (Cummings, 2004). CN is typically used as a poison (e.g., used during World War II in concentration camps; used as a chemical for pest control). CN is an ingredient in some jewelry cleaners, photographic solutions, metal polish, and is also a by-product of the manufacture of some synthetic products such as nylon, rayon, polyurethane foam, and insulation (Hamel, 2011). In industrialized countries, the most common cause of CN poisoning is fires (Megarbane *et al.*, 2003). Toxicologic evaluation of passengers following the explosion in 1985 of a Boeing 737 during take-off in Manchester, England, revealed that 20% of the 137

victims who escaped had dangerously elevated blood levels of carbon monoxide, while 90% had dangerously elevated levels of CN (Walsh & Eckstein, 2004; Jameson, 1995). Lastly, CN exposure can also occur via acts of terrorism, murder, and suicide.

The intentional and unintentional use, or threat of use, of CN in domestic and foreign incidents has occurred in recent years. These include the 1995 Tokyo subway attack (Sauter & Keim, 2001), the 2002 recovery of stored CN in Paris, France, linked to Al-Qaeda operatives (Cloud, 2004), and the 2004 discovery by US forces of “cookbooks” on how to make HCN. Some recent threats include images of a “chemical laboratory” in a house in Fallujah, Iraq, that was allegedly used by terrorists linked to Abu Musab al-Zarqawi (Gertz, 2004), contamination of smokeless tobacco products with CN from a local merchant (Lenart *et al.*, 2010), and the 2012 London Olympic threat to distribute CN-adulterated lotions (Bromund *et al.*, 2012; Ritz, 2012). The Centers for Disease Control and Prevention (CDC) and the Occupational Safety and Health Administration (OSHA) developed regulations for CN and set permissible exposure limits at 10 ppm and 4.7 ppm, respectively ([www.cdc.gov/niosh](http://www.cdc.gov/niosh); [www.osha.gov](http://www.osha.gov)). Because of the rapidly debilitating actions of CN, it is necessary to quickly diagnose the level of exposure and provide supportive treatment to counteract the effects from CN intoxication.

Acute toxicity can be defined as the antagonistic effects resulting from a single exposure to a chemical substance or repeated exposures within a short period of time (< 24 h) (Andrew, 2009). The clinical features of acute CN poisoning are variable, and the major determinants of severity and mortality are the source of exposure (CN or CN compound), the route and magnitude of exposure (amount and duration), and the effects and the time of any treatments that may have been tried (Yen *et al.*, 1995). Acute CN toxicity can take place through ingestion, membrane absorption, and inhalation. Since there are no pathognomonic clinical signs and symptoms for its toxicity, it is pertinent to acquire a full patient or epidemiologic history and consider the diagnosis in cases of unexplained sudden collapse or acidosis (Nnoli *et al.*, 2013). In a clinical environment, CN toxicity can occur after treatment with sodium nitroprusside, which is often used in pediatric intensive care units (Baek *et al.*, 2010) for its strongly antihypertensive properties (Moffett & Price,

2008) and various pharmacokinetic advantages (Gilboa & Urizar, 1983) of rapid distribution and short half-life. Early diagnosis for acute CN toxicity is challenging because of the multitude of symptoms associated with CN intoxication (i.e., lightheadedness, nausea, pulmonary edema, restlessness, etc.). Unfortunately, instantaneous detection of CN exposure in deployed operations fields for first responders and the military is currently not available, and CN exposure often presents a narrow therapeutic window of treatment. This chapter will explore the pharmacokinetic/pharmacodynamic properties of CN, the effects of acute CN toxicity in various experimental models, and the chronic neurodegenerative implications as a result of acute CN toxicity.

## 1.2 Pharmacokinetic properties of cyanide

### 1.2.1 Absorption

The pharmacokinetic properties of CN can vary depending on the general composition of the CN (i.e., KCN, NaCN, CuCN, AgCN, and HCN) and route of exposure. CN can be rapidly lethal as a result of its fast absorption and distribution into tissues and the bloodstream, binding to metalloenzymes and rendering them inactive (Solomonson, 1981). The chemical composition of CN is one property that greatly influences the rate of absorption. The Henderson Hasselbach equation describes the ratio of ionized versus unionized at a particular pH, or vice versa. Smaller, neutral, non-ionized compounds are favored for absorption across biological membranes. Since KCN and NaCN are water soluble, they readily undergo dissolution and are absorbed in the stomach after ingestion, although the presence of food in the stomach slows the absorption of CN and potentially delays the onset of toxicity. With the pKa of > 9 for HCN, passive diffusion will be less efficient at alkaline pHs. Dermal absorption of the ionized solution is unfavorable. In a clinical and in a laboratory setting, HCN in contrast to NaCN and KCN has a faster onset of toxicity because both NaCN and KCN must first be converted to HCN in the body or skin unless equilibrium shifts to blood from stomach (Ballantyne, 1987; Curry & LoVecchio, 2001). HCN exists as a non-ionized molecule and thus can diffuse across the lipid membrane. Additionally, HCN has the lowest molecular weight in

comparison to other forms of CN, enabling it to simply diffuse readily across the membrane. Gettler and Baine (1938) studied the effects of dose and absorption rate in dogs. Three dogs were administered lethal doses of HCN via gavage, and the difference between the dose of CN given and the portion of CN remaining in the stomach and intestines was determined to represent the total amount absorbed. This difference can be attributed to enterohepatic recirculation of compounds that have phase II metabolism, where a drug is absorbed from the gastrointestinal tract (GI), goes to the liver and is passed into the bile, and then is re-secreted into the GI through the bile. Dogs were administered 20 mg, 50 mg, or 100 mg HCN, and all subsequently died within 2.5 hours. The absorbed fraction was determined to be 72%, 24%, and 17% respectively, suggesting that zero-order kinetics is independent of the CN concentration (Gettler & Baine, 1938). In another study Sousa *et al.* (2003) assessed the absorption rate of CN in rats and pigs given 1.2 mg/kg KCN via gavage. Blood CN concentrations in rats reached a peak after 15 min (0.15 mg/100 ml) whereas in pigs the blood CN concentrations reached a peak within 30 min (0.23 mg/100 ml). Irrespective of the route of exposure, species, or impeding factors such as the presence of food in the stomach, CN absorption into the bloodstream occurs within seconds to minutes after exposure (Sousa *et al.*, 2003).

### 1.2.2 Distribution

CN is rapidly distributed throughout the body after absorption (Ahmed & Farooqui, 1982; Djerad *et al.*, 2001). Subsequently, tissues with the highest oxygen demand (i.e., brain, heart, liver, kidney, and stomach) are the most drastically affected (Yamamoto *et al.*, 1982; Ballantyne, 1983a; Saito *et al.*, 2000). Furthermore, absorptive tissues in direct contact with CN, such as the lungs in the case of inhalation exposure or the stomach in the case of oral exposure, maintain high levels of measurable CN. Although several factors may influence distribution, the brain and heart are the primary targets in acute CN intoxication regardless of the route of exposure or species. Disturbances of perception and consciousness, convulsions, and impaired or lost control of respiratory and cardiovascular systems all indicate that oxygen dependent organs such as the brain and heart have been affected by exposure to CN (Ballantyne, 1987; Egekeze & Oehme, 1980; Ballantyne, 1983b). Ballantyne (1983a) conducted a series of experiments

exposing rabbits to lethal doses of HCN via different routes of exposure and then measured the concentration of CN in the brain and myocardium. CN levels were consistently high in these tissues of the exposed rabbits. In a follow-up study assessing the distribution of CN, Ballantyne (1983a) injected various species (rabbit, pig, monkey, rat, and sheep) with 8 mg/kg KCN intraperitoneally (IP) and measured the concentration of CN in the brain and myocardium. These results supported previous experiments demonstrating that species differences do not change the general pattern of CN distribution.

CN has also been shown to cross the plasma membrane and accumulate in the mitochondria and membrane elements of neuronal cells. In a study tracing radiolabeled CN ( $^{14}\text{CN}$ ) using mouse brain slices and rat pheochromocytoma (PC12) cells, Borowitz *et al.* (1994) illustrated that CN distribution with neural tissue are not uniform, but rather CN accumulates in the hypothalamus to a greater extent than in the cerebellum or hippocampus. The distribution of CN into the brain depends on the effect of respiratory acidosis/alkalosis on: (i) the binding of cyanide to plasma proteins, (ii) the ratio of non-ionized to ionized forms of cyanide, and (iii) the cerebral blood flow (Goldberg *et al.*, 1961). Conversely, another study using a nonlethal dose of CN suggests a more uniform distribution and that the accumulation differences across brain regions are the result of a 47% reduction of the permeability-area product of CN into the brain under alkaline conditions compared with acidosis in relation to the ranges of arterial pHs used (Djerad *et al.*, 2001). It is difficult to clarify the brain structure(s) in which  $^{14}\text{CN}$  activity accumulates (Djerad *et al.*, 2001). In the study of Clemedson *et al.* (1960), the central nervous system seemed to have the lowest activity of all the tissues examined (Djerad *et al.*, 2001).

### 1.2.3 Metabolism

The metabolism of CN has been well studied, and multiple metabolic pathways, both major and minor, have been identified. The major pathway for CN metabolism is the conversion of CN to SCN by either rhodanese or 3-mercaptopyruvate sulfurtransferase (MST) (Sörbo, 1975; Ballantyne, 1987; Logue *et al.*, 2010). These enzymes catalyze the transfer of a sulfane sulfur atom from sulfur donors to CN irreversibly, yielding the compound SCN which is readily excreted in the urine. Rhodanese and MST are found throughout the body

primarily in the mitochondrial membrane with high concentrations in the liver and kidney (Himwich & Saunders, 1948; Auriga & Koj, 1975; Nagahara *et al.*, 1998). SCN formation accounts for approximately 80% of CN metabolism (Wood & Cooley, 1955; Sousa *et al.*, 2003; Aminlari *et al.*, 2007). Another secondary metabolic pathway is the chemical conversion of CN to 2-amino-2-thiazoline-4-carboxylic acid (ATCA) and its tautomer 2-iminothiazolidine-4-carboxylic acid (ITCA) (Ruzo *et al.*, 1978; Salkowski & Penney, 1994; Borowitz *et al.*, 2001) by reacting with cystine. Conversion to ATCA accounts for approximately 15% of CN metabolism when assessed in rats (Wood & Cooley, 1955) and has potential as a biomarker of CN exposure (Petrikovics *et al.*, 2011).

### 1.2.4 Elimination

After CN is converted to the more polar thiocyanate, it is primarily excreted in the urine. Sousa *et al.* (2003) studied the rate of elimination in rats, pigs, and goats. All species were administered 3.0 mg/kg KCN orally (PO), and CN and SCN blood plasma concentrations in the blood were measured within 24 hours. The elimination half-life of CN was determined to be 0.64, 0.54, and 1.28 h for rats, pigs, and goats, respectively, with goats also having a higher volume of distribution (0.41 l/kg). Conversely, the CN metabolite SCN had a much slower elimination half-life of 5.8, 4.95, and 13.9 h in rats, pigs, and goats, respectively. Renal function has a significant role in modulating the elimination of CN from the body as well as rhodanese activity. A study involving eight patients with renal failure and seven healthy patients compared the rate of elimination of SCN after the administration of either oral SCN or intravenous (IV) injections of nitroprusside. Schulz *et al.* (1979) determined that the elimination half-life of SCN in patients with renal failure was on the order of nine days, three times that of healthy patients. Another less significant route of CN elimination occurs via exhaled HCN. Okoh and Pitt (1982) demonstrated that in rats exposed to a chronic intake of KCN, approximately 4% of CN was excreted in expired air after 12 hours.

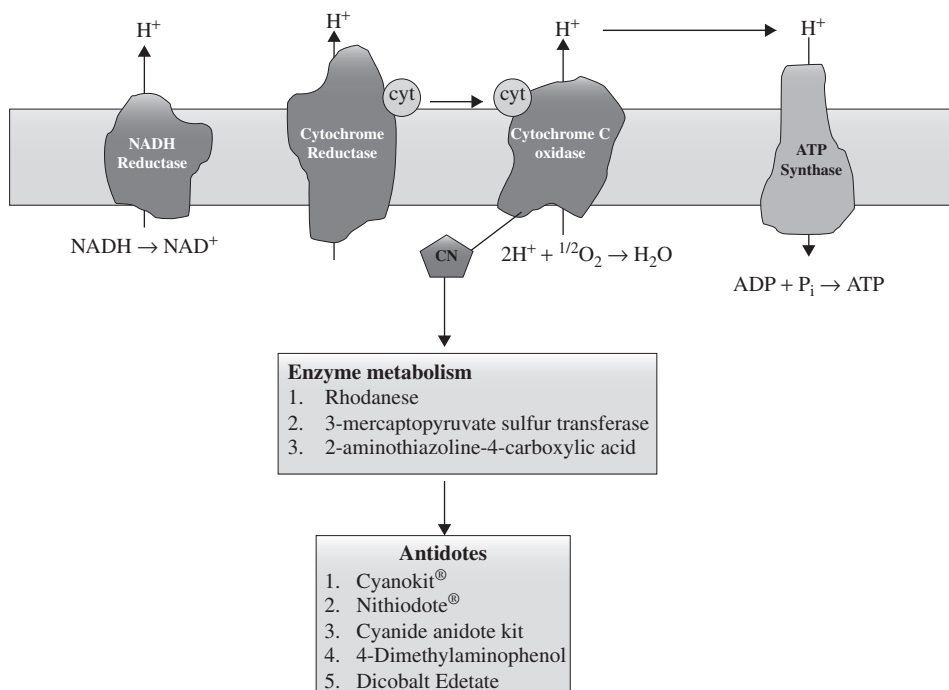
### 1.2.5 Other Determinants of Toxicity

The balance between exposure, absorption, metabolism, and elimination of CN through various mechanisms and pathways previously discussed can greatly influence the degree of toxicity and onset of symptoms. An

acute dose of sufficient CN can overwhelm the body's defense mechanisms of metabolizing and eliminating CN from the body. Other factors that influence CN's pharmacokinetic properties and toxicity are species, route of exposure, and age. Early studies by Fitzgerald (1954) illustrated that younger mice were more adversely affected by CN than adult mice. Mice were administered subcutaneous (SC) NaCN which produced an LD<sub>50</sub> value near 5 mg/kg in adult male mice and almost half the LD<sub>50</sub> (2.0–2.5 mg/kg) for neonatal mice. Neonates are more affected by CN exposure since their body mass and size is smaller in comparison to adult mice. Furthermore, it is unclear if neonates have the fully functional enzymes needed to metabolize CN (Fitzgerald, 1954). Other variables such as species and route of exposure will be discussed later in the Routes of Administration section.

## 1.3 Pharmacodynamic properties of cyanide

Cyanide's rapid and lethal effects are due to its interference with the respiratory chain within the mitochondria. CN inactivates CcOX at the ferric ion on the cytochrome *a*<sub>3</sub> enzyme (Sykes, 1981; Way, 1984; Pearce *et al.*, 2003; Cooper & Brown, 2008). CcOX, also referred to as complex IV, is the final membrane protein in the electron transport chain, primarily responsible for reducing molecular oxygen to two molecules of water. In the process, protons are pumped across the membrane creating a proton gradient that fuels the enzyme adenosine triphosphate (ATP) synthase to convert adenosine diphosphate (ADP) to ATP (Figure 1.1). CN inhibits this natural process, diverting the cell into anaerobic metabolism, which is one of the hallmarks of CN poisoning. Anaerobic metabolism induces a rise in plasma lactate concentrations (Nelson, 2006; Megarbane *et al.*, 2003). Not surprisingly, there is a positive correlation between plasma lactate and blood CN levels, both in fire victims and in victims of incidental CN poisoning (Baud, 2007; Baud *et al.*, 2002; Borron *et al.*, 2007; Anseeuw *et al.*, 2012). A plasma lactate concentration  $\geq 10$  mmol/l in fire victims without severe burns and  $\geq 8$  mmol/l in pure CN poisoned patients is a sensitive and specific indicator of CN intoxication (Megarbane *et al.*, 2003). For example, lactic acid in normal non-exposed humans ranges between 0.5 to



**Figure 1.1** Cyanide disrupts the proton gradient during cellular respiration, reducing ATP production. Cyanide (CN) binds to and inhibits cytochrome c oxidase, disrupting the proton gradient generated by the reductase and oxidase enzymes in the respiratory chain. Decreased hydrogen protons ( $H^+$ ) reduce the ability of ATP synthase to synthesize ATP. (Cyt = cytochrome c, CN = cyanide).

2.2 mmol/l; however, those exposed to CN display increased levels of lactate which has been shown to exceed 8 mmol/l (Baud *et al.*, 2002).

In addition to blocking cellular anaerobic metabolism, CN affects multiple neurotransmitter systems, including dopaminergic, GABAergic, and glutamatergic pathways, either directly or indirectly through changes in ion regulation (Persson *et al.*, 1985). For example, rats treated with NaCN (5–20 mg/kg IP) displayed decreased dopamine (DA) levels in the striatum. Other alterations included increases in glutamate levels in the cerebellum, striatum, and hippocampus of rats treated with NaCN (5–10 mg/kg IP), whereas higher doses of NaCN (10 and 20 mg/kg IP) decreased glutamate levels (Persson *et al.*, 1985).

CcOX, several neurotransmitter systems, and a large number of enzymes are inhibited by CN (Table 1.1), which may account for some of the sequelae of acute toxicity such as those listed in Table 1.2. CN interferes with several neurotransmitters including  $\gamma$ -aminobutyric acid (GABA) (Tursky & Sajter, 1962;

Cassel *et al.*, 1991), glutamate (Cassel *et al.*, 1991), acetylcholine (Owasoya & Iramain, 1980), dopamine (Cassel *et al.*, 1995), other excitatory amino acids (McCaslin & Yu, 1992; Gunasekar *et al.*, 1996) and nitric oxide (Gunasekar *et al.*, 1996). Phenotypic symptoms and signs that manifest with CN exposure are: dizziness, headache, mydriasis, weakness, tachycardia, and flushing of the skin to more pronounced symptoms such as diaphoresis, dyspnea, hyperventilation, seizures, coma, and asystole (Ballantyne *et al.*, 2007). In goats, the clinical signs of toxicity were seen four to five days after KCN dosing, and the delayed onset of clinical signs could be related to toxin distribution (Soto-Blanco *et al.*, 2008).

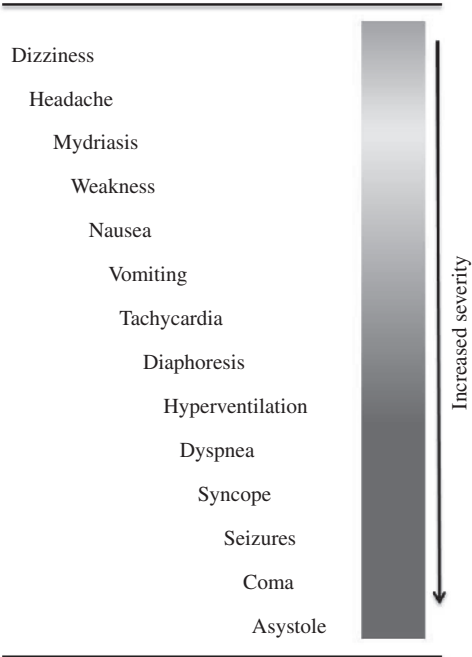
#### 1.4 Acute cyanide toxicity – routes of administration

CN exposure can occur via various routes of exposure for a number of reasons in humans. For example, CN inhalation exposure occurs in cases of cigarette

**Table 1.1** Enzymes inhibited by cyanide.

Enzyme	Reference(s)
Acetoacetate decarboxylase	Autor and Fridovich, 1970
D-amino acid oxidase	Porter <i>et al.</i> , 1972
Carbonic anhydrase	Feeney <i>et al.</i> , 1973
Copper-containing amine oxidases	Shepard <i>et al.</i> , 2004
Cytochrome c oxidase	Keilin, 1929; Sykes, 1981; Way, 1984
Formate dehydrogenase	Ohjama and Yamazaki, 1975
Glutamate decarboxylase	Tursky and Sajter, 1962
Glutathione peroxidase	Prohaska <i>et al.</i> , 1977
Guaiacol peroxidase	Ghamsari <i>et al.</i> , 2007
2-Keto-4-hydroxyglutarate aldolase	Hansen and Dekker, 1976
Lipoxygenase	Aharony <i>et al.</i> , 1982
Mercaptopyruvate sulfurtransferase	Porter and Baskin, 1996
Nitric oxide	Leavesley <i>et al.</i> , 2007; Correia and Ortiz de Montellano, 2005
Nitrite reductase	Lafferty and Garrett, 1974
Quinone oxidoreductase	Theissen and Martin, 2008
Ribulose diphosphate carboxylase	Marsho and Kung, 1976
Succinic dehydrogenase	Zanetti <i>et al.</i> , 1973
Superoxide dismutase	Feeney <i>et al.</i> , 1973; Borders and Fridovich, 1985
Tyrosine aminotransferase	Yamamoto <i>et al.</i> , 1982
Uricase	Conley and Priest, 1980
Xanthine dehydrogenase	Coughlan <i>et al.</i> , 1980
Xanthine oxidase	Massey and Edmondson, 1970

**Table 1.2** Progressive symptoms and signs of acute cyanide exposure in humans.



smokers (Centers for Disease Control and Prevention *et al.*, 2010), industrial workers during manufacturing activities (Mudder & Botz, 2004), and in fire victims (Geldner *et al.*, 2013; Grabowska *et al.*, 2012). Similarly, oral CN exposure can occur in cases of consuming certain improperly prepared foods such as cassava (Teles, 2002), although toxicity is generally developed chronically rather than acutely. Oral CN in humans is also implicated in cases of attempted suicides and homicides. Other routes of CN exposure can occur infrequently in humans – dermally in mining operations (Bismuth *et al.*, 1987; Obiri *et al.*, 2006), SC (Prieto *et al.*, 2005; Abeyasinghe *et al.*, 2011), and IP or IV via the administration of nitroprusside (Nand *et al.*, 1995; Thomas *et al.*, 2009).

Animal models of CN exposure have been developed to verify, identify, and control for a wide range of variables that otherwise confound human exposure data. For example, dermal absorption of toxic gases is largely uncharacterized in humans, as is management advice for individuals potentially exposed to CN. Designing an animal model of dermal exposure can unveil mechanistic actions of an agent as well as provide



insight on supportive care. When modeling any route of CN exposure within the laboratory, it is important to consider which routes of exposure most likely parallel human exposure, what species will best represent the model, the exposure regimen, the exposure dose, and the likelihood of other contributing factors such as age, gender, and concurrent morbidity.

### 1.4.1 Inhalation toxicity

Inhalation exposure of HCN is one of the most harmful forms of CN toxicity, where the gas evades first pass metabolism and rapidly enters the bloodstream. HCN has a distinct odor of bitter almonds with an odor threshold of 0.2–5.0 ppm (Musschoff *et al.*, 2002). In individuals presumed to be affected by CN intoxication, one method of detection is by smelling the breath of the affected individual. However, up to 40% of humans cannot detect the bitter almond odor of HCN and may therefore be at greater risk for toxicity (Corn, 2012).

The inhalation of HCN along with other chemical compounds such as carbon monoxide contributes to a number of deaths in household and building fires. The exact contribution of HCN in fire-related deaths relative to other chemical compounds is difficult to assess because of the breakdown of CN in the blood postmortem (Moriya & Hashimoto, 2001, 2003) and the lack of rapid analytical methods (Baud, 2007; Hall,

2007). Purser (2000) and Simonson *et al.* (2000) suggest that HCN is a significant factor in mortality. One reason is that CN has a strong “knock down” effect, that is, a fire victim could lose consciousness as a result of high concentrations of HCN, consequently preventing an escape, and therefore die from carbon monoxide poisoning or carbon monoxide and HCN (Purser, 2000). Furthermore the smell of HCN masked by many other components present in fire smoke poses additional problems for accurate detection (Baud, 2007). Cigarette smoke is another common source of HCN exposure. Although HCN present in cigarette smoke is not as deleterious acutely, levels in inhaled mainstream cigarettes range from 10 to 400 µg per cigarette and to a lesser extent in secondary or sidestream smoke from 0.06 to 108 µg (Fiksel *et al.*, 1981; Swauger *et al.*, 2002).

Modeling acute inhalation toxicity exposure in experimental animals can be challenging. The effect of a gas always depends on two parameters: the concentration and the duration of exposure (Anseeuw *et al.*, 2012). A lower concentration of HCN over a longer period of time can be as deleterious as a higher concentration of HCN within a short exposure period. In female rabbits, the LC<sub>50</sub> of HCN decreased from 2432 mg/m<sup>3</sup> to 208 mg/m<sup>3</sup> as the time of exposure increased from 45 seconds to 35 minutes (Table 1.3) (Ballantyne, 1984a). In primates, as the dose of HCN doubled from 100 ppm

**Table 1.3** Acute inhalation toxicity data from animals exposed to hydrogen cyanide (HCN) gas.

Species	Sex	Time of exposure	LC <sub>50</sub> (mg/kg)	95% CI	Reference
Mouse	M	30 min	176	129–260	Matijak-Schaper and Alarie, 1982
Mouse	M	30 min	177	131–266	Esposito <i>et al.</i> , 1988
Mouse	M	30 min	451	424–480	Chan <i>et al.</i> , 2010
Mouse	N/A	5 min	553	443–689	Higgins <i>et al.</i> , 1972
Rabbit	F	45 sec	2,432	2,304–2,532	Ballantyne, 1984a
Rabbit	F	5 min	409	321–458	Ballantyne, 1984a
Rabbit	F	35 min	208	154–276	Ballantyne, 1984a
Rat	F	10 sec	3,778	3,771–4,313	Ballantyne, 1984a
Rat	F	1 min	1,129	664–1,471	Ballantyne, 1984a
Rat	F	5 min	493	372–661	Ballantyne, 1984a
Rat	N/A	5 min	503	443–689	Higgins <i>et al.</i> , 1972
Rat	N/A	10 min	290	250–340	Levin <i>et al.</i> , 1987
Rat	N/A	20 min	170	160–180	Levin <i>et al.</i> , 1987
Rat	F	30 min	151	141–164	Ballantyne, 1984b
Rat	F	60 min	158	144–174	Ballantyne, 1984a
Rat	F	30 min	173	159–193	Ballantyne, 1984a

to 200 ppm, the time to incapacitation decreased from 25 minutes to 2 minutes (Purser, 2000). Similar results also occurred in rats exposed to HCN, although it may not be the case for mice (Table 1.3). The majority of animals exposed to various doses of HCN displayed typical acute toxic signs such as ataxic movements, convulsions, tachycardia, and respiratory depression.

Inhalation of HCN is unique in that death may be delayed as a result of differences in respiration rate, tidal volume, and time, which dictate the total concentration of CN inhaled during an exposure. In a study assessing the efficacy of antidotal cyanide compounds against HCN inhalation, Chan *et al.* (2010) describe an exposure model developed for rodents. Briefly, C57Bl/6 mice were placed in exposure chambers under isoflurane anesthesia and exposed to HCN via mixing KCN with NaOH. The  $LC_{50}$  was found to be 451 ppm (95% CI, 424–480 ppm). Chan *et al.* (2010) reported that the  $LC_{50}$  value in this model appeared to be slightly higher in comparison to other studies (Esposito, 1988), primarily due to the mice being anesthetized during exposure. It is known that anesthesia controls breathing rate and depth thereby reducing hyperventilation and total exposure in comparison to non-anesthetized rodents. Nevertheless, mice left untreated with antidote died immediately. Other HCN exposure models (Table 1.3) demonstrated similar exposure paradigms of untreated subjects following HCN inhalation.

Acute human HCN exposure leads to a chain of effects to include altered sense of smell, tachypnea, dyspnea, nausea, ataxia, unconsciousness, palpitations, convulsions, and asphyxiation (Chandra *et al.*, 1980; Blanc *et al.*, 1985; Penden *et al.*, 1986; Gerberding, 2006). Barcroft (1931) described an experiment where a 70 kg man and a 12 kg dog were placed inside the same exposure chamber and subjected to HCN. Muscular activities made by the dog were imitated by the man to account for potential respiratory differences. After nearly 2 minutes, the dog showed apparent signs of CN intoxication and eventually died, whereas the man felt no apparent symptoms, but had impaired memory for up to one year. Another author describes a case report wherein a fatal human poisoning occurred after cleaning the bottom of a silver plating tank. The individual was found unconscious by coworkers after being exposed to 200 ppm HCN for an unknown length of time (Singh *et al.*, 1989). In another case report described by Bonsall (1984), an industrial worker was

accidentally exposed to approximately 500 ppm HCN for 3 minutes while conducting an inspection of the tank. After being fitted with a mask and transported to the hospital, the exposure victim fully recovered over a period of 3 days with supportive therapy.

### 1.4.2 Oral cyanide toxicity

The database for acute oral toxicity of CN consists of a few case studies on human poisoning incidents and a limited number of studies in laboratory animals exposed to a single dose of CN salts (EPA, 2010). In humans who ingest 4.6–15 mg/kg as KCN, characteristic clinical signs, such as Parkinsonian-like symptoms, decreased verbal fluency, reduced information processing, coma, hyperventilation, enlarged heart, inaudible heart sounds, nausea, vomiting, albuminuria, and generalized muscular rigidity are observed in addition to pathologic analysis in several organ systems where brain lesions, and shallow pulse are exhibited (Feldman & Feldman, 1990). In rodents, single doses of 4–22 mg/kg as K-, Na- or CaCN resulted in 50–90% lethality (Ferguson, 1962; Smyth *et al.*, 1969). Studies in pigs and rats with administration of CN salts by oral gavage showed behavioral changes (reduced activity) at doses between 0.14 and 0.8 mg/kg/day, and more serious effects (tremors, convulsions, death) were observed at 7.8 mg/kg/day, a lethal dose (EPA, 2010).

Oral CN has been implicated in suicide cases and homicides. Death can occur within minutes after ingestion of CN (Holland & Kozlowski, 1986). In the southeast part of Nigeria, a 29-year-old male died from acute myocardial infarction following acute CN poisoning from ingestion of CN salts by intentional poisoning. CN concentration was detected in stomach content (260 ppm), bile fluid (272 ppm), blood (256 ppm), and mouth swab (265 ppm) (Nnoli *et al.*, 2013). One of the limitations of the case study was the inability to retrieve and analyze the sample of drink(s) and/or the glass from which the victim drank (Nnoli *et al.*, 2013).

In a different case, a 17-year-old male was admitted to a community emergency room, unresponsive, apneic, and hemodynamically unstable. Supportive care was initiated in the emergency room beginning shortly after the onset of the toxicity and continuing into the pediatric intensive care unit; unfortunately the 17-year-old patient did not receive any antidotal therapy until the CN poisoning was diagnosed approximately 4 hours after symptom onset (Peddy *et al.*, 2006).



An investigation concluded that the death was caused by KCN (1.5 g) intentionally added to a beverage (Peddy *et al.*, 2006). This case illustrates many of the difficulties associated with rapid confirmation of CN poisoning and the delay in treatment to individuals of acute CN poisoning (Borron, 2006). A fruit-flavored drink laced with KCN and painkillers was used in the mass suicide of 913 members of the People's Temple in Jonestown, Guyana, in 1978 (Thompson *et al.*, 1987), where the drink was given to children first, then to most of the adults (Moore, 2011). These incidents of oral CN exposure have reignited the concern of potential intentional or accidental usage through this route. In either circumstance, one of the greatest challenges in confirming oral CN exposure is that often the actual amount of CN administered during a murderous intent and/or suicide is unknown, and determining the initial CN dose post-exposure is often difficult.

The edible portions of dietary plant species commonly used in the United States contain relatively low levels of cyanogen glycosides (linamarin and lotausralin), although some pits and seeds of common fruits (e.g., apple, apricot, peach) contain significantly higher concentrations (EPA, 2010). In tropical countries, cassava (*Manihot esculenta* Crantz), an important tropical root crop that provides energy to about 500 million people (Padmaja, 1995; El-Sharkawy, 2004), contains high toxic content of cyanogens (Braidotti, 2011). In a study assessing a group of 73 subjects in Liberia consuming cassava, the mean daily ingestion of CN ion was calculated to be 0.61 mg/kg of body weight (Jackson, 1988). In comparative animal studies, hamsters fed a similar cassava diet were noted to exhibit adverse effects, such as stunted growth and decreased ossification (Frakes *et al.*, 1986). Tropical ataxic neuropathy (TAN) and epidemic spastic paraparesis (Konzo) are two neurological disorders associated with the consumption of cassava in several African countries (Adamolekun, 2011). It is important to note that the toxic cyanogenic glycosides can be removed by a number of processing methods. Methods to reduce the after effects of CN poisoning include sun-drying, heap fermentation (Kobawila *et al.*, 2005; Oboh & Elusiyan, 2007), and the wetting method (Cumbana *et al.*, 2007; Bradbury *et al.*, 2011). Treatment of cassava peels by sun-drying, heap fermentation or soaking reduced the CN toxicity to below 100 mg CN/kg of dry matter at 48, 72, and 96 hours respectively, but

heap fermentation or soaking gave the lowest residual CN after 120 hours (Tweyongyere & Katongole, 2002).

Various animal studies have also been conducted to establish the lethal toxicity of oral CN and to better understand the implications of acute exposure. In a study by Wiemeyer *et al.* (1986) sensitivities of six avian species, Black vultures, American kestrels, Japanese quail, domestic chickens, eastern screech-owl, and European starling species, to acute poisoning by NaCN were compared by single LD<sub>50</sub>. The LD<sub>50</sub> values across species ranged from 4 mg/kg to 21 mg/kg for an acute single oral dose (Table 1.4). The three carnivores (Black vulture, American kestrel, and eastern screech-owl; LD<sub>50</sub> 4.0–8.6 mg/kg) were more sensitive to NaCN than the other three species (Japanese quail, domestic chicken, and European starling; LD<sub>50</sub> 9.4–21 mg/kg) that feed predominantly on plant material (Wiemeyer *et al.*, 1986).

Several studies (Gerhart, 1986; Jackson, 1988; Soto-Blanco *et al.*, 2002) conducted in rats and pigs report neurological, thyroid, and gastrointestinal effects following gavage administration of acute CN doses. However, their usefulness for dose-response assessment is limited because the bolus dosing may overwhelm the endogenous detoxification process and is not characteristic of typical general population exposures to CN in drinking water. A wastewater refinery north of Mashhad, Iran, was evaluated in three stages (March 2009, June 2010, and July 2010) for CN concentration in the drinking water and irrigation water wells in the industrial plants (Mousavi *et al.*, 2013). Although the CN concentrations were within the standard range (0.07 mg/l for CN) and not deemed a health problem at the time of the study, regular estimations of the toxic chemicals was recommended because of the development of the industrial plant (Mousavi *et al.*, 2013). A study in Tabriz, Iran, found the maximum of 0.0069 mg/l CN concentration in industrial effluents (Mirmohseni & Alipour, 2002). It is important to note that some CN in water will be biotransformed into less harmful chemicals by microorganisms (Gerberding, 2006).

The management of oral CN exposure demands extra care from health-care professionals and first responders. In cases of oral CN ingestion, extreme caution should be used by health-care providers to avoid secondary contamination (e.g., bodily fluids, spilled liquid, etc.) (Hamel, 2011). Although, activated charcoal may not be highly effective in countering acute poisoning because

**Table 1.4** Compilation of LD<sub>50</sub> cyanide values in various species.

Species	Sex	LD <sub>50</sub> (mg/kg)	95% CI	Route of exposure	CN solution	Reference
Black vulture	M/ F	4.8	4.4–5.3	Oral	NaCN	Wiemeyer, 1986
American kestrel	M/F	4.0	3.0–5.3	Oral	NaCN	Wiemeyer, 1986
Japanese quail	M/F	9.4	7.7–11.4	Oral	NaCN	Wiemeyer, 1986
Domestic chicken	F	21	12–36	Oral	NaCN	Wiemeyer, 1986
Eastern screech-owl	M/F	8.6	7.2–10.2	Oral	NaCN	Wiemeyer, 1986
European starling	M/F	17	14–22	Oral	NaCN	Wiemeyer, 1986
Rabbit	F	2.49	2.26–2.81	Oral	HCN	Ballantyne, 1984a
Rat	F	3.62	3.08–3.87	Oral	HCN	Ballantyne, 1984a
Rat	F	5.72	5.23–7.08	Oral	NaCN	Ballantyne, 1984a
Mouse	M	8.5	8.1–9.0	Oral	KCN	Sheehy and Way, 1968
Rabbit	F	5.82	5.5–6.31	Oral	KCN	Ballantyne, 1984a
Rat	F	9.69	8.6–11.3	Oral	KCN	Ballantyne, 1984a
Mouse	M	9.8		s.c	KCN	Rockwood, 2012 (unpublished)
Mouse	M	12	10.8–13.3	s.c.	KCN	Isom and Way, 1973
Swine	M/F	5	2.5–6.3	i.v.	KCN	Muncy <i>et al.</i> , 2012

of the high potency of CN, the rapid onset of poisoning, and the small size of the CN molecules, it might be useful in patients who may have ingested corrosive agents (i.e., alkalis, lye, strong acids, boric acid, lithium, petroleum products, or alcohols) in addition to CN (Shepherd & Velez, 2008).

### 1.4.3 Dermal toxicity

Dermal exposure although rare, is likely to occur due to accidental exposure. Minimal occurrences of this route are described in the literature. Forty-two percent of workers exposed to 15 ppm HCN developed rashes (Blanc *et al.*, 1985). Additionally, a study conducted by Obiri *et al.* (2006) evaluated the human health risk assessment from exposure to free CN via dermal contact of surface/underground water by resident adults close to mining companies with wastewater effluent and found risks for acute exposure very high. In this community, many of the residents attributed most of the unknown causes of deaths to dermal contact with CN water and accidental ingestion (Obiri *et al.*, 2006).

CN in solution is absorbed across intact skin because of its lipid solubility (WHO, 2004). In general, when modeling the dermal route of exposure within the laboratory, it is important to consider several factors. Species differences can pose an issue and give different results depending on the CN composition (i.e., KCN, NaCN, HCN). LD<sub>50</sub> values calculated for dermal

exposure to cyanides in rabbits were 6.7 mg/kg when applied as HCN, 7.7 mg/kg as NaCN, and 8.9 mg/kg as KCN (Ballantyne, 1983a). The dermal LD<sub>50</sub> of CN as NaCN was slightly lowered by moistening the skin and substantially lowered by abrading the skin (Ballantyne, 1987). Walton and Witherspoon (1926) showed substantial evidence to indicate a similar variation in the reactions of individual dogs to dermal absorption of HCN gas as well as by inhalation exposure of HCN suggesting that skin composition (i.e., moist, dry, intact, or abraded) greatly impacts dermal absorption, and ultimately, toxicity.

Other factors also affect the rate of dermal absorption such as, follicle concentration, skin hydration, occlusion of skin, thickness of stratum corneum, lipid content of skin, adnexal structures, and physiochemical properties of CN. In amphibians, the exterior cell surface of skin epithelium, which is exposed to environmental contaminants, has a higher permeability, while the basal surface exposed to the extracellular fluid maintains a lower permeability to the contaminant (Ling, 1990). Ballantyne (1984a) demonstrated that abraded rabbit skin enhances the penetration of CN and increases toxicity (WHO, 2004). Ballantyne (1984a) applied variations of both dry and moist CN to abraded or intact skin in female rabbits. In comparison to the cyanide salts, HCN proved to be the most potent of all CN

solutions with an LD<sub>50</sub> value ranging from 2.34–6.89 (mg/kg), depending on the skin condition. Fairley *et al.* (1934) concluded that environments containing HCN readily pass through the skin surface in guinea pigs and will produce death if the exposure is prolonged. Acute dermal exposure to HCN (concentration not reported) in these guinea pigs resulted in submucous hemorrhages in the stomach (Fairley *et al.*, 1934). Despite the great volatility of HCN, the danger resulting from spilling of the liquid on bare skin was determined to be slight as long as evaporation was unimpaired.

#### 1.4.4 Subcutaneous toxicity

Acute toxicity from subcutaneous (SC) exposure to CN is unlikely to occur in terrorist acts, murders, or suicides in humans. CN poisoning by injection is rare, however, a case of SC injection was reported in Sri Lanka (Abeyasinghe *et al.*, 2011). In another case, a comatose patient was brought to a hospital after a SC self-injection of CN. Although only hemodialysis was used (to correct the severe metabolic acidosis), the patient survived (Prieto *et al.*, 2005). As an injection, CN may not result in the displaying of traditional autopsy findings such as bright pink or red discoloration of mucosal tissues, indicators that typically revealed from oral exposure poisoning (Abeyasinghe *et al.*, 2011). SC administration is commonly used by researchers in experimental animal models because of its ease of administration and moderate rate of absorption into the bloodstream when compared to other routes of administration such as the intravenous (IV) route and also because it bypasses stratum corneum as major impediment to absorption in realistic exposure.

#### 1.4.5 Intravenous toxicity

Intravenous administration permits direct infusion of CN into the blood stream resulting in a rapid onset of clinical signs. Larger animals such as pigs and rabbits are often used for this method of exposure in a laboratory setting because of the ease of intubation, less variability as with intraperitoneal (IP) injection and instrumentation (e.g., arterial and venous catheters as well as cardiac output monitors). Ballantyne (1984a) showed that the IV LD<sub>50</sub> values in female rabbits for HCN, NaCN, and KCN were 0.59 mg/kg, 1.23 mg/kg, and 1.89 mg/kg respectively. When expressed on a molar basis there was no significant difference in acute lethal toxicity of HCN and NaCN, however, KCN appears to be slightly less toxic.

Other IV models of CN exposure have also been developed (Bebarta *et al.*, 2010; Muncy *et al.*, 2012). Briefly, Yorkshire pigs of both sexes were mechanically ventilated under isoflurane to allow for monitoring of arterial and cardiac output throughout the experiment. KCN was then infused at a rate of 0.16 mg/kg/min until severe hypotension occurred, which produced 100% lethality when untreated. All animals reached severe hypotension within 40 minutes, with the mean CN dose near 5 mg/kg (range 2.5–6.3 mg/kg) (Muncy *et al.*, 2012).

#### 1.4.6 Intraperitoneal toxicity

Exposure to CN using intraperitoneal (IP) administration is frequently practiced in rodent models to ensure accurate delivery, and in the majority of cases, to evaluate the efficacy of established or potential antidotes. Ballantyne (1984a) characterized the acute toxicity of IP injected NaCN and KCN in mice, rats, rabbits, and guinea pigs. LD<sub>50</sub> values ranged between 4.55–6.70 mg/kg for mice, 4.72–5.55 mg/kg for rats, 2.79–3.99 mg/kg for rabbits, and 5.51–6.49 mg/kg for guinea pigs. No human data are available implicating cases of IP CN exposure.

#### 1.4.7 Antidotes for acute cyanide poisoning

The onset of CN poisoning can vary depending on the route of exposure (i.e., inhalation, oral), duration of exposure, dose of CN, and form of CN (i.e., NaCN, KCN, HCN). In general, symptoms can range from a mild headache to more drastic symptoms such as seizure, bradypnea, coma, and death. Therefore, it is extremely important to rapidly detect and manage treatment with specific CN antidotes and supportive therapy (oxygen). Hall *et al.* (2009) articulated that the ideal CN antidote should possess the following properties:

- 1 rapid onset of action;
- 2 neutralize CN without interfering with cellular oxygen use or oxygen transport;
- 3 have safety and tolerability profiles for use outside of the hospital;
- 4 safe for use with smoke-inhalation victims;
- 5 innocuous in non-poisoned patients;
- 6 easily administered.

Antidotes for CN poisoning have been intensively studied and reviewed (Dumestre & Nickerson, 2014; Way, 1984). CN antagonists can be classified into two

general groups: those that act as sulfane sulfur donors (e.g., polythionates and thiosulfates) and those that induce direct chemical binding of CN (EPA, 2010). In the first group, sodium thiosulfate acts as a sulfur donor to rhodanese, which catalyzes the conversion of CN to SCN, which is then readily excreted in the urine. Sodium thiosulfate has been successfully used as an antidote against CN poisoning in humans for decades (Way, 1984; Chen *et al.*, 1933). Within the second group, nitrites induce the formation of methemoglobin, which is able to bind CN, forming cyanmethemoglobin and freeing the mitochondria to produce more ATP. It is theorized that methemoglobin sequesters CN away from cytochrome c oxidase, which leads to CN detoxification (Flora *et al.*, 2004), although it is also emerging that nitrites may exert their primary antidotal effects via nitric oxide-centered mechanisms (Pearce *et al.*, 2003).

CN can interfere with multiple enzyme systems. Multidrug therapy, as opposed to a single-drug therapy, may be the most practical solution to provide efficacy in cases of CN poisoning. The combination of a sulfur donor (i.e., sodium thiosulfate) and a methemoglobin former (i.e., sodium and/or amyl nitrite) has a long history of successfully countering CN-induced poisoning (Chen *et al.*, 1933; Hug, 1934). Although sulfur donors are beneficial, a few limitations exist such as solubility and sustainability of substrate supply for detoxification (Brenner *et al.*, 2010). In 2011, the Food and Drug Administration (FDA) approved Nithiodote®, which consists of co-packaged sodium thiosulfate and sodium nitrite for the treatment of acute CN poisoning. The following year the FDA approved separate packaging for injections of sodium nitrite and sodium thiosulfate to be used sequentially to prevent incompatibility issues with the combination therapy. Limitations such as the requirement for IV administration (sodium thiosulfate and sodium nitrite), the slow time to action associated with sodium thiosulfate, and the potentially dangerous hypotension associated with sodium nitrite have led to the need for more effective and safer CN antidotes.

Hydroxocobalamin binds with CN to form cyanobalamin which is subsequently renally excreted. The cobalt compounds in hydroxocobalamin have the ability to bind and sequester CN (Mushett *et al.*, 1952). Additionally, hydroxocobalamin does not produce methemoglobin intermediates, which would otherwise impede the oxygen-carrying capacity of hemoglobin. The efficacy of hydroxocobalamin was first used in a

mouse model (Mushett *et al.*, 1952). Hydroxocobalamin is an antidote that displays many of the characteristics of the ideal CN antidote to include the following: rapid onset of action, neutralization of CN without interference with cellular oxygen use, tolerability and safety profiles conducive to pre-hospital use, safe for use with smoke-inhalation victims, safe when administered to non-poisoned patients, and ease of administration (Hall *et al.*, 2009). Hydroxocobalamin was approved (as Cyanokit®) as a CN antidote by the FDA in 2006. Noted limitations of Cyanokit include large IV administration volume, the need for reconstitution, and cost. Further discussion of CN antidotes appears elsewhere in this book.

## 1.5 Neurological and behavioral effects following acute cyanide exposure

Although many organ and biological systems are affected by CN exposure, adverse effects on the central nervous system (CNS) are of particular concern and may be most important to the organism because of the high metabolic demand for oxygen in neurons, and CNS control of respiratory function (EPA, 2010). A crucial component involved in movement control that is impacted by CN is the basal ganglia. The basal ganglia play a crucial role in modulating the activity of dopaminergic neurons (Lee & Tepper, 2009). A majority of dopaminergic (DA)-containing cells develop from a single embryological cell group that originates at the Mesencephalic-diencephalic junction and projects to various forebrain targets (Hynes & Rosenthal, 1999). The DA neurons in the brain account for less than 1% of the total neuronal population, yet they have a profound effect on brain function (Björklund & Lindvall, 1984; Björklund & Dunnett, 2007). The loss of DA neurons, which can occur following CN poisoning, disrupts normal DA tone (i.e., which is associated with brain stimulation and reward (Hernandez *et al.*, 2012) and basal ganglia function. Brain regions abundant with DA neurons have several functions in the brain, including important roles in behavior, cognition, motivation, motor activity, reward, inhibition of prolactin production, sleep, attention, mood, and learning (Wang & Lupica, 2014; Happel *et al.*, 2014; Ben-Jonathan & Hnasko, 2001; Simon *et al.*, 1980).

### 1.5.1 Neurodegenerative effects and implications

Dopaminergic systems appear to be highly susceptible to the action of CN and the mitochondrial respiratory rates of sensitive brain areas that are affected result in predisposition to neuronal injury (Kanthasamy *et al.*, 1993). A major neurodegenerative disorder associated with dopaminergic cell loss is Parkinson's disease (PD). CN (about 500–1500 mg/kg orally) in humans has been shown to result in Parkinsonian or dystonic Parkinsonian syndrome, and it also generates lesions in the basal ganglia (i.e., caudate-putamen, substantia nigra, etc.) (Finelli, 1981; Uitti *et al.*, 1985; Carella *et al.*, 1988; Messing & Storch, 1988). The Parkinsonian brain has over 50% fewer dopaminergic neurons within the midbrain than age-matched human normal brains, and the cell loss occurs within the combined substantia nigra (SN) (dopaminergic nucleus A9) and retrorubral (dopaminergic nucleus A8) areas (> 61%) and the ventral tegmental area (dopaminergic nucleus A10) (> 42%) (German *et al.*, 1989). Progressive loss of neuromelanin-containing dopaminergic neurons in the SN of the ventral midbrain is characteristic of PD in humans (Arias-Carrión & Pöppel, 2007). In a feline model, animals infused with NaCN through a femoral vein catheter (Funata *et al.*, 1984) displayed severe brain damage in the deep cerebral white matter, corpus callosum, pallidum, and SN, but not in the cerebral cortex or hippocampus (Yen *et al.*, 1995), a pattern similar to PD cases in humans. An additional reported CNS effect following CN intoxication is memory impairment in animals and humans (Chin & Calderon, 2000; Kimani *et al.*, 2013).

MRI investigations have revealed effects in the basal ganglia including multiple areas of reduced signal intensity in the globus pallidus and posterior putamen (Borghain *et al.*, 1995; Grandas *et al.*, 1989; Messing, 1991). High metabolic demands in CNS structures such as the basal ganglia, cerebral cortex, and sensorimotor cortex (Rachinger *et al.*, 2002; Uitti *et al.*, 1985; Zaknun *et al.*, 2005) are attributed to direct toxicity and secondary CN intoxication as a consequence of cerebral hypoxia from apnea (Rosenberg *et al.*, 1989). A slow recovery from severe dystonia syndromes arising from CN intoxication has been noted in some cases and has involved treatment with Parkinsonism therapies such as levodopa (Rachinger *et al.*, 2002; Zaknun *et al.*, 2005; Borghain *et al.*, 1995; Valenzuela *et al.*, 1992).

### 1.5.2 Behavioral abnormality assessments in animal models

In light of the typical dose-dependent signs observed with CN toxicity, several experimental models have been developed to assess behavioral toxicity in laboratory animals exposed to acute sublethal doses of CN. The use of animal models presents the opportunity to better assess the behavioral characterization of motor impairments and deficits to gain insight regarding the behavioral correlates of acute CN intoxication and the impact on performance of the warfighter and return to duty. CN poisoning can cause permanent neurological disabilities, ranging from various extrapyramidal syndromes to post-anoxic vegetative states (Rachinger *et al.*, 2002), which are also classical characteristics of neurological disorders such as PD and dystonia syndrome (Finelli, 1981; Uitti *et al.*, 1985; Rosenberg *et al.*, 1989; Messing, 1991).

The cardinal symptoms of PD include tremor, rigidity, postural instability, and bradykinesia, which form the basis of most behavioral testing in mouse models of PD (Taylor *et al.*, 2010). Motor behavioral tests in PD mouse models have been used to identify the most suitable predictor of dopaminergic cell loss to assess the relationship of cell loss to behavioral alterations (Iancu *et al.*, 2005). One familiar test is the righting reflex or the inverted screen test, which is an innate response of the body to compensate itself when orientation is compromised. The test is often conducted by placing the test subject (e.g., mouse) on a mesh screen, then inverting it. Mice will typically revert or right themselves immediately; however, mice exposed to sublethal doses of CN will take much longer. In a recent study by Chan *et al.* (2010), mice injected with NaCN (1.764 mg/kg; IP) were able to right themselves within 70 min as opposed to untreated mice which righted themselves instantaneously (Coughenour *et al.*, 1977; Crankshaw *et al.*, 2007).

In a test designed to assess spatial navigation through the utilization of the Morris water maze (MWM) swim test, rats were placed in a round water tank and trained to use spatial cues to locate an escape platform submerged slightly below the waterline (Baskin & Rockwood, 2002). Blokland *et al.* (1993) demonstrated that microgram concentrations of NaCN (5.0 µg) administered intracerebroventricularly (ICV) significantly increased the total time to find the escape platform when administered within 5 min of testing. The effects



displayed were transient and showed no effects when NaCN was administered 10 or 15 min ICV prior to testing (Prickaerts *et al.*, 1998).

## 1.6 Summary

Intentional (i.e., suicide, homicide, terrorism) and unintentional (i.e., accidental poisoning, industrial exposure, and food sources) acute CN exposure occurrences have increased the awareness about the toxic effects of CN and, as a result, have broadened the scope of research of chemical agents and related toxic industrial chemicals. CN rapidly binds to CcOX preventing aerobic respiration, and in turn, diverts the cell into anaerobic respiration. The progression of events results in the depletion of ATP and a reduction of ATP formation. Animal model research and case studies from human exposures give us a broader perspective on the pharmacokinetic and pharmacodynamic properties of acute CN toxicity. CN is rapidly absorbed and distributed ubiquitously throughout the body, affecting highly perfused organs particularly the brain, liver, and lungs. No model alone is flawless; however, each model gives valuable insight to specific issues that can be integrated into a clinical or field setting. For example, a dermal pig exposure to NaCN may simulate contact corrosive injury indicative of CN exposure in the human, whereas an inhalation model adapted for other species may more closely approximate human CN exposure from building fires. Ultimately, the onset and severity of toxicity depend on the dose, chemical composition (i.e., NaCN, KCN, and HCN), duration of exposure to CN, availability and timing of antidotal treatment regimens, and supportive therapy. The pharmacodynamic parameters of how the body absorbs, distributes, metabolizes, and excretes CN to reduce morbidity and mortality are keys to treating toxicity.

With the numerous experimental models that have been established, more research is needed to fully understand the actions of acute CN toxicity in living organisms. In low level acute exposure environments/situations of first responders and military personnel in combat when behavioral aberrations occur, this information will provide additional clarity for the “trigger-to-treat.” Current and emerging technologies such as global gene expression profiling and metabolomic/proteomic profiles will provide better

insight to elucidate the mechanisms of acute CN exposure and will contribute to a deeper understanding of the full range of effects (e.g., cellular, molecular, behavioral, toxicological and pharmacological).

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