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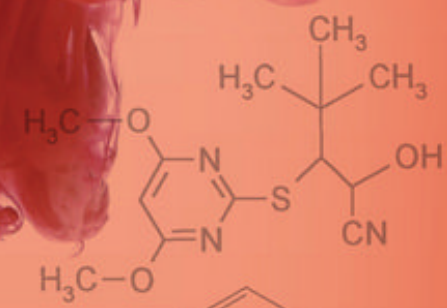
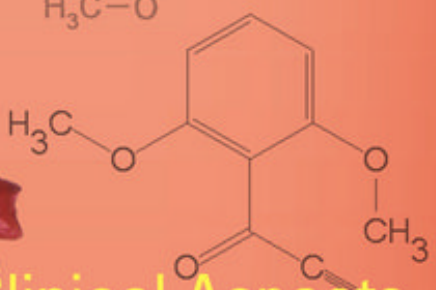
Alan Hall • Gary Isom • Gary Rockwood

Toxicology of Cyanides and Cyanogens

Experimental, Applied and Clinical Aspects





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EDITED BY

Alan H. Hall, BA, MD

Toxicology Consulting and Medical Translating Services
Springtown and Azle, TX;
Colorado School of Public Health
University of Colorado-Denver
Denver, CO

Gary E. Isom, BPharm, PHD

Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University
West Lafayette, IN

Gary A. Rockwood, BA, MS, PHD

U.S. Army Medical Research Institute of Chemical Defense
Analytical Toxicology Division
Aberdeen Proving Ground, MD

WILEY Blackwell

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111 River Street, Hoboken, NJ 07030-5774, USA

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This book is dedicated to the memory of the late Steven I. Baskin, PharmD, PhD, FCP, FACC, DABT, FATS

Dr. Steven I. Baskin passed away on September 29, 2014. He had planned to be a co-editor and chapter author for this book, but was prevented from doing so by serious illnesses. Dr. Baskin was a major contributor to research on cyanide and new countermeasures to treat cyanide poisoning. He was for many years affiliated with the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) where he conducted numerous studies on cyanide poisoning and its treatment. Dr. Baskin was known worldwide for his significant contributions to knowledge in the area of cyanide poisoning, lectured widely, authored/co-authored a very large number of publications, and was a major contributor to numerous workshops, symposia, and scientific programs nationally and internationally. His intelligence and ability to see new approaches were exemplary. He was also a good friend and colleague. We dedicate this book to his memory.

Contents

List of Contributors

Prasad Abraham, PharmD, BCPS, FCCM
Department of Pharmacy and Drug Information
Grady Health System, Atlanta, GA

Andrea R. Allen, Ph.D.
U.S. Army Medical Research Institute of Chemical Defense
Analytical Toxicology Division
Aberdeen Proving Ground, MD

Kelly A. Basi, Ph.D.
U.S. Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD

Y. Bentur, MD
Israel Poison Information Center
Rambam Health Care Campus, The Rappaport Faculty of Medicine
Technion-Israel Institute of Technology, Haifa, Israel

R. Bhattacharya, M.Sc., Ph.D.
Division of Pharmacology and Toxicology
Defence Research and Development Establishment, Gwalior, India

Lamont Booker, Ph.D.
Food and Drug Administration
Center for Devices and Radiological Health, Silver Spring, MD

Joseph L. Borowitz, Ph.D.
Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University, West Lafayette, IN

Stephen W. Borron, MD, MS
Division of Medical Toxicology
Department of Emergency Medicine
Paul L. Foster School of Medicine
Texas Tech. University Health Sciences Center
El Paso, TX

Gerry R. Boss, MD
Department of Medicine
University of California, San Diego, CA

Matthew Brenner, MD
Beckman Laser Institute and Medical Clinic
University of California, Irvine

G. Capellier, MD, Ph.D.
Emergency Department
University Hospital Jean Minjoz, Besançon, France

Katleen Chester, PharmD, BCPS
Department of Pharmacy and Drug Information
Grady Health System, Atlanta, GA

Julie Cliff, MBBS, FRCP, MSc, DTM&H
Department of Community Health, Faculty of Medicine,
Universidade Eduardo Mondlane, Maputo,
Mozambique

Margaret R. DeFreytas, BS, MS
U.S. Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD

T. Desmettre, MD, Ph.D.
Emergency Department
University Hospital Jean Minjoz, Besançon, France

Jason D. Downey, Ph.D.
U.S. Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD

A. Eisenkraft, MD, MHA
Israel Poison Information Center
Rambam Health Care Campus, Haifa, Israel

A. Falk, Ph.D.
Israel Poison Information Center
Rambam Health Care Campus, Haifa, Israel

J.-L. Fortin, MD
Army Medical Centre, Quartier Joffre, Besançon
Emergency Department, University Hospital Jean Minjoz,
Besançon Cedex, France

Richard J. Geller, MD, MPH
Children's Hospital Central California and the
California Poison Control System, Madera, CA

Robert J. Geller, MD
Georgia Poison Center, Atlanta, GA

Tee L. Guidotti, MD, MPH
Vice President for HSE/Sustainability
Medical Advisory Services, Rockville, MD

Alan H. Hall, MD
Toxicology Consulting and Medical Translating Services
Springtown and Azle, Texas
Clinical Professor
Colorado School of Public Health
University of Colorado-Denver, Denver, CO

Gary E. Isom, Ph.D.
Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University, West Lafayette, IN

Wendy Klein-Schwartz, PharmD, MPH
Associate Professor at the University of Maryland College of Pharmacy and
Coordinator of Research and Education at the Maryland Poison Center, Baltimore, MD

Thomas L. Kurt, MD, MPH
Clinical Professor, Department of Internal Medicine, University of Texas
Southwestern Medical Center and Consultant, North Texas Poison Center
Parkland Memorial Hospital, Dallas, TX

Alissa Lockwood, PharmD
Department of Pharmacy
Parkland Health System, Dallas, TX

Brian A. Logue, Ph.D.
Associate Professor, Department of Chemistry and Biochemistry
Associate Director, Center for Security Printing and Anti-counterfeiting Technology
South Dakota State University

Daniel Lugassy, MD
Assistant Professor of Emergency Medicine
New York University School of Medicine
Attending Physician
Bellevue Hospital Center and New York University Langone Medical Center, New York

P. Luporsi, MD
Emergency Department
University Hospital Jean Minjoz, Besançon, France

Sari Mahon-Brenner, Ph.D.
Beckman Laser Institute and Medical Clinic
University of California, Irvine

Samantha L. Malone, MPH, CPH
University of Pittsburgh, Graduate School of Public Health
Environmental and Occupational Health Department, Pittsburgh, PA.

Dana B. Mirkin, MD
Saint David's Occupational Health Service
Austin, TX

Brendan L. Mitchell, Ph.D.
Associate Professor, Department of Chemistry and Biochemistry
Associate Director, Center for Security Printing and Anti-counterfeiting Technology
South Dakota State University

Ashraf Mozayani, PharmD., Ph.D., FBFT
Department of Administration of Justice
Barbara Jordan-Mickey Leland School of Public Affairs
Texas Southern University, Houston, TX

Humberto Muquingue, MD, MSc, Ph.D.
Department of Biochemistry, Faculty of Medicine
Universidade Eduardo Mondlane, Maputo, Mozambique

Lewis Nelson, MD
Professor of Emergency Medicine
Department of Emergency Medicine, New York University School of Medicine
Attending Physician
Bellevue Hospital Center and New York University Langone Medical Center
Director, Fellowship in Medical Toxicology
New York City Poison Control Center and New York University School of Medicine

Hipolito Nzwalo, MD, MSc, FEBN
Faro Central Hospital, Faro, Portugal

John Patka, PharmD, BCPS
Department of Pharmacy and Drug Information
Grady Health System, Atlanta, GA

Steven E. Patterson, Ph.D.
Center for Drug Design, University of Minnesota

Linda L. Pearce, Ph.D.
University of Pittsburgh, Graduate School of Public Health,
Environmental and Occupational Health Department,
Pittsburgh, PA.

Jim Peterson, Ph.D.
University of Pittsburgh, Graduate School of Public Health
Environmental and Occupational Health Department,
Pittsburgh, PA.

Ilona Petrikovics, Ph.D.
Sam Houston State University
Huntsville, TX

Steven H. Lamm, MD, DTPH
Georgetown University, Washington, DC

René Pita, Ph.D., Lt.Col.
Chemical Defense Department
NBC Defense School, Madrid, Spain

Marina Rabinovich, PharmD, BCPS
Department of Pharmacy and Drug Information
Grady Health System, Atlanta, GA

Gary A. Rockwood, Ph.D.
U.S. Army Medical Research Institute of Chemical Defense
Analytical Toxicology Division
Aberdeen Proving Ground, MD

Jennifer Sutherland, PharmD
Department of Pharmacy and Drug Information
Grady Health System, Atlanta, GA

David E. Thompson, Ph.D.
Sam Houston State University
Huntsville, TX

Jorn Chi-Chung Yu, Ph.D.
Department of Forensic Science, College of Criminal Justice
Sam Houston State University, Huntsville, TX

Foreword

The classical acute poisons such as cyanide and arsenic have long fascinated the general public and toxicologists. The former is the subject of this book, which arises from a book proposal originally put together by the late Dr. Ballantyne in 2009, which was envisaged as a successor to *Clinical and Experimental Toxicology of Cyanides* edited by Dr. Ballantyne (and me), published by Wright of Bristol, UK, in 1987. Since that time there have been numerous developments in our knowledge of the toxicology of cyanide, which makes the book very welcome because it is some years since there has been a book entirely devoted to cyanide toxicology.

Inorganic and organic cyanides, the latter usually termed nitriles, are ubiquitous. There are natural compounds containing cyanide moieties and there is the opportunity for exposure from the industrial use of cyanides and from their use as fumigants. Further cyanides have been used for murder and suicide and were used in the past as chemical warfare agents. Cyanides may also be produced during fires (see below).

The oldest cyanides are the natural ones: many plants contain cyanogenic glycosides where cyanide is combined with sugars as a defence against predators. Such plants include almonds (*Prunus dulcis*), peaches (*Prunus persica*), apricots (*Prunus armeniaca*), and black cherries (*Prunus serotina*) where the glycosides are found in the kernel; and apples (*Malus domestica*) where the glycoside is in the pips. Here the noxious material is amygdalin, which was isolated and investigated in the early 19th century. Acute cyanide poisoning has been reported with *inter alia* apricot kernels. A case of lethal poisoning from drinking the first glassful from a new bottle of "noyeau liqueur" (Crème de Noyaux, which is made from apricot kernels) was recorded in *Taylor's Principle and Practice of Medical Jurisprudence*. The author stated, "there had accumulated in the bottle and floated to the top a sufficient quantity of prussic acid to kill" (Smith, 1957). A semi-synthetic form of amygdalin, laetrile, has been promoted as an alternative treatment for cancer, but there is no scientific evidence to support claims that laetrile is effective when used for this purpose (National Cancer Institute, 2010); indeed the material has serious side effects. The use of laetrile as an anti-cancer treatment was the subject of a Cochrane review (Cochrane Gynaecological Cancer Group *et al*., 2011); the story of laetrile is weird even by the standards of alternative medicine. Another plant which contains cyanogenic glycosides is cassava (*Manihot esculenta*), which is a shrub of the spurge family (*Euphorbiaceae*) from South America. Cassava has an edible starchy tuber and is widely cultivated in tropical and subtropical regions. To avoid getting ill from cassava some method of processing such as soaking, cooking, or fermentation is necessary to remove the cyanide. There have been reports of poisoning from these sources and cassava is associated with a number of chronic conditions of ill-health, a subject of chapter 7 of this book. Cyanide is also found in the leaves of cherry laurel (*Prunus laurocerasus*) - laurel leaves have long been used by lepidopterists in their "killing bottles".

Inorganic cyanides followed later and hydrogen cyanide was first isolated from the blue pigment Prussian blue, IUPAC name iron (II,III)-hexacyanoferrate(II,III), and hydrogen cyanide was long known as prussic acid. Hydrogen cyanide was used as a fumigant in the late 19th century and as chemical warfare agent in World War 1 (see chapter 14). Zyklon B, which contained hydrogen cyanide, together with an irritant and an adsorbent, was used to kill people in German concentration camps, during the holocaust. The potassium or sodium salt was typically used in cyanide poison capsules, such as those used by Eva Braun to kill herself and were used to kill the Goebbels children in Hitler's bunker in 1945. A notable murder using cyanide (combined with attempts using other methods), was that of Gregory Rasputin in the dying days of the Russian empire.

Hydrogen cyanide may be important in the toxicology of combustion products, where nitrogen-containing polymers have burnt and this is discussed in chapter 10.

Studies of smoke inhalation victims have to be treated with some caution as there is usually exposure to both cyanide and carbon monoxide and such cases may be complicated by the presence of hypoxia. Indeed it has been opined that the human data are currently unclear about whether, or to what degree, cyanide contributes to morbidity and mortality in victims of smoke inhalation (Erdman, 2008).

As well as suicide, murder and fires, there is ample opportunity for exposure to cyanide in industry. Cyanide is used in gold and silver mining and in electroplating in the jewelry trade.

The present book includes a chapter on cyanide in chemical warfare and terrorism. This is very welcome, although the importance of this aspect of cyanide toxicity is a sad reflection of modern life. In the 1st World War hydrogen cyanide does not seem to have been particularly effective (it is a very volatile liquid and less dense than air), but hydrogen cyanide is listed under the annex on chemicals, schedule 3 of the Chemical Weapons Convention (Organisation for the Prohibition of Chemical Weapons, 1993). Two problems with this convention are that (i) a few countries are still not states parties to the convention and (ii) the convention does not really deal with non-state organizations such as terrorist groups, still less rogue lone wolves.

It is often not appreciated by the general public that, as well as being an acutely-acting poison, cyanides are responsible for appreciable ill-health as chronic poisons, often from the natural cyanide glycosides discussed above. In developed countries tobacco amblyopia (nutritional optic neuropathy) and subacute combined degeneration of the spinal cord are important and disabling conditions. In some countries the long-continued consumption of cyanide-containing foods, often accompanied by malnutrition can give rise to a variety of syndromes, including neuropathies and thyroid dysfunction.

Since the 1987 book was published there have been numerous developments in the toxicology of cyanides notably in antidote development, where hydroxocobalamin has found favour, further studies on 4-dimethylaminophenol have taken place and there has been much work on experimental antidotes. In the present book there are a number of chapters on antidotes (22–27). It is apt that there is a chapter on α-ketoglutarate as well as chapters on the current antidotes. The reaction of cyanide with ketones or aldehydes to form cyanhydrins is well known and the use of such compounds looks, on the face of it, an attractive method of detoxifying cyanides. Apart from glucose, which is co-administered with dicobalt edetate, these substances have not been used clinically but a number have been studied experimentally including pyruvic acid and dihydroxyacetone, in addition to α-ketoglutarate. It should be noted that the glucose that is co-administered with dicobalt edetate is intended to reduce cobalt toxicity rather than act as an antidote to cyanide in its own right. It has often been remarked that the number of current clinically-used cyanide antidotes and also the number that have been studied experimentally in animals is a reflection of the fact that none is entirely satisfactory clinically. However, the fundamental problem is the very rapid onset of cyanide poisoning, especially with hydrogen cyanide, and the difficulty in getting access to an antidote in a comparable time. It may be that this difficulty cannot be surmounted.

TC Marrs

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CHAPTER 1
Acute cyanide toxicity

Andrea R. Allen, Lamont Booker, and Gary A. Rockwood

Disclaimer: the views expressed in this chapter are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or the U.S. Government.

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

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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; 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}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}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_{50} value near 5 mg/kg in adult male mice and almost half the LD_{50} (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_3 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

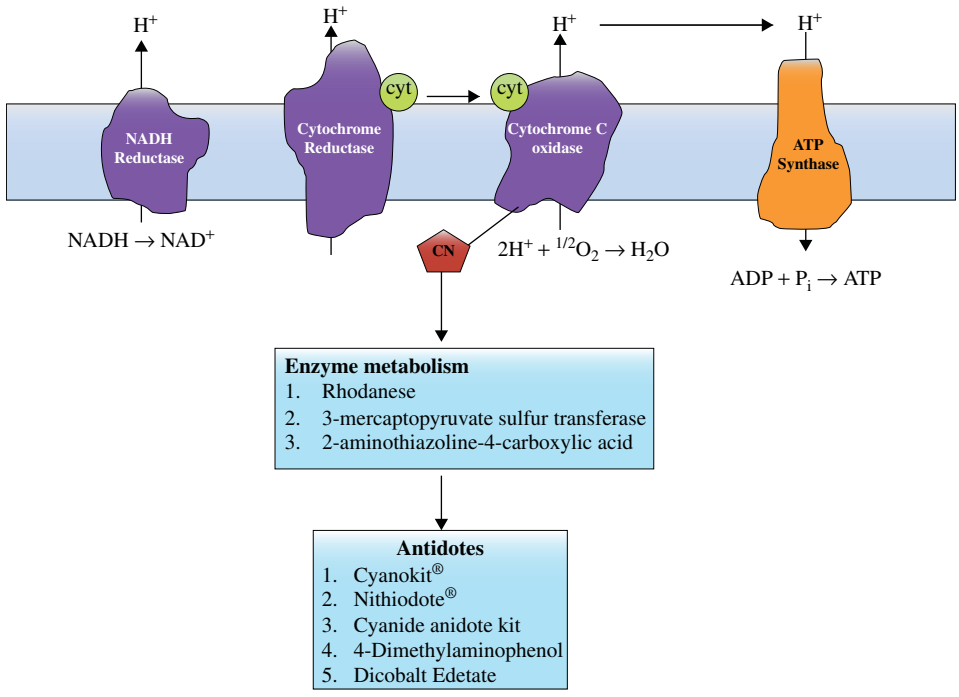


Figure 1.1 Cyanide disrupts the proton gradient during cellular respiration, reducing ATP production. Cyanide (CN) binds to and inhibits cytochrome c oxidase, disrupting the proton gradient generated by the reductase and oxidase enzymes in the respiratory chain. Decreased hydrogen protons (H^+) reduce 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 γ-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).

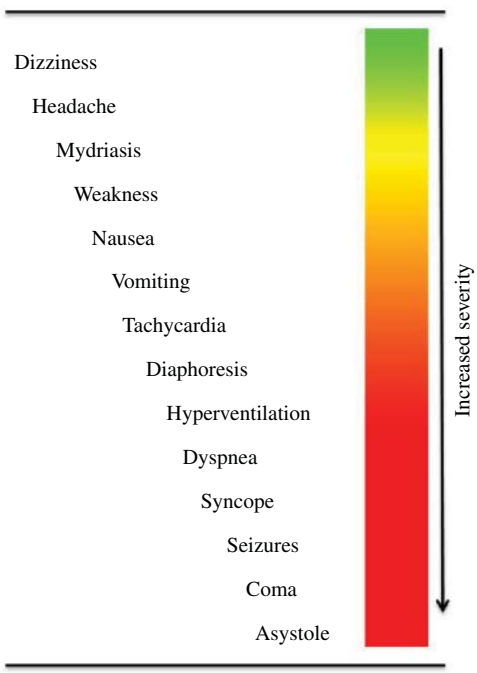
1.4 Acute cyanide toxicity – routes of administration

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 *et al.*, 1972
Carbonic anhydrase	Feeney *et al.*, 1973
Copper-containing amine oxidases	Shepard *et al.,* 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 *et al.,* 1977
Guaiacol peroxidase	Ghamsari *et al.,* 2007
2-Keto-4-hydroxyglutarate aldolase	Hansen and Dekker, 1976
Lipoxygenase	Aharony *et al.*, 1982
Mercaptopyruvate sulfurtransferase	Porter and Baskin, 1996
Nitric oxide	Leavesley *et al.*, 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 *et al.,* 1973
Superoxide dismutase	Feeney *et al.*, 1973; Borders and Fridovich, 1985
Tyrosine aminotransferase	Yamamoto *et al.*, 1982
Uricase	Conley and Priest, 1980
Xanthine dehydrogenase	Coughlan *et al.*, 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 (Musshoff *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_{50} of HCN decreased from 2432 mg/m^3 to 208 mg/m^3 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_{50} (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 *et al*., 1988
Mouse	M	30 min	451	424–480	Chan *et al*., 2010
Mouse	N/A	5 min	553	443–689	Higgins *et al*., 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 *et al*., 1972
Rat	N/A	10 min	290	250–340	Levin *et al*., 1987
Rat	N/A	20 min	170	160–180	Levin *et al*., 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 accidently 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_{50}. The LD_{50} 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_{50} 4.0–8.6 mg/kg) were more sensitive to NaCN than the other three species (Japanese quail, domestic chicken, and European starling; LD_{50} 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 was 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_{50} cyanide values in various species.

Species	Sex	LD_{50} (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 *et al*., 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_{50} 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_{50} 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_{50} 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 by passes stratum corneum as major impedance 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_{50} 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_{50} 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 (Borgohain *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; Borgohain *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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CHAPTER 2

Chronic cyanide exposure

Case studies and animal models

Jason D. Downey, Kelly A. Basi, Margaret R. DeFreytas, and Gary A. Rockwood

Disclaimer: The views expressed in this book chapter are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

At a Glance

- The most common sources of long-term cyanide exposure are fires, some industrial processes, improperly processed foods, and smoking.
- Long-term consumption of improperly processed cassava damages the descending motor pathways and leads to konzo and tropical ataxic neuropathy.
- Exposure to cyanide in the workplace or through smoking affects thyroid function and normal fetal development and sometimes causes Parkinsonian syndromes.
- Several animal models have been developed to study the toxicity of chronic cyanide exposure in adults fed cyanide or a cyanogenic diet and the developing fetus.

2.1 Introduction

Chronic human cyanide poisoning may result from a broad range of exposures (e.g., fire, industrial, medical, dietary, and cigarette smoke) and may present clinical manifestations in cases of repeated low-dose cyanide exposure (Finkel *et al*., 1983). Animal and human studies link cyanide to several disease states, specifically neuropathies. Cyanide disrupts aerobic cellular respiration by binding to cytochrome c oxidase. Cyanide toxicity is characterized by histotoxic hypoxia where cellular oxidative phosphorylation is blocked. Since the central nervous system is highly dependent on aerobic metabolism, chronic cyanide exposure can produce neuronal and/or axonal degeneration that manifests as a Parkinson-like condition (Ballantyne, 1987; Rachinger *et al*., 2002).

2.2 Sources of chronic cyanide exposure

Humans are regularly exposed to cyanide from a variety of different sources, and healthy humans were found to have a blood cyanide concentration between 0.4 and 1.8 μM (Tian *et al*., 2013). Cyanide can be liberated during the combustion of products containing both carbon and nitrogen. These products include wool, silk, polyurethane (insulation/upholstery), polyacrylonitriles (plastics), melamine resins (household goods), and synthetic rubber (Vogel *et al*., 1981). It is therefore not surprising that in industrialized countries, the most common cause of acute cyanide poisoning is domestic fires (Mégarbane & Baud, 2003). Furthermore, for firefighters, the risk of chronic cyanide exposure may be increased (Guidotti & Clough, 1992). Common sources of cyanide exposure include manufacturing, alternative and standard medical treatments, certain foods, and tobacco cigarettes.

Cyanide is widely used in industry, with the worldwide industrial consumption of cyanide estimated to be 1.5 million tons per year (Cummings, 2004). Cyanide poisonings may derive from metal extraction in mining, electroplating in jewelry production, manufacturing of plastics and rubber, the unhairing process of tanning

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hides, and production of rodent pesticides and fumigants. Cyanide poisonings often result from the reaction of ammonia with methane and air forming hydrocyanic acid. Industrial hygiene standards to limit occupational exposure to cyanide have been developed (NIOSH, 2010), yet exposure to cyanide in the workplace still occurs from accidents or other shortcomings.

Cyanide exposures can result from alternative and standard medical treatments. Laetrile was developed in the 1950s as a synthetic derivative of amygdalin, a cyanogenic glycoside found naturally in bitter almonds and peach pits and used as an antineoplastic agent since the 1800s. Early formulations of purified amygdalin were poorly tolerated, and laetrile was reportedly a nontoxic alternative. Laetrile and amygdalin are marketed as vitamin supplements and used as an alternative therapeutic for cancer, and they can still be obtained today through the Internet. However, amygdalin and laetrile are now known to cause severe cyanide toxicity (Bromley *et al*., 2005; Hall *et al*., 1986; O'Brien *et al*., 2005). Commonly, preparations claiming to be laetrile are either heavily contaminated with amygdalin or preparations of amygdalin mislabeled as laetrile. Cyanogenic glycosides release hydrogen cyanide (HCN) when hydrolyzed in the gastrointestinal tract.

Another therapeutic, sodium nitroprusside (SNP), often used in the treatment of hypertensive emergencies, contains five cyanide ligands per molecule, which can result in cyanide poisoning (Vesey *et al*., 1976). One use for SNP is to induce hypotension during surgery. SNP infusion produces a dose-dependent increase in red blood cell cyanide concentration, as high as 50 μM in some instances (Pasch *et al*., 1983). Simultaneous infusion of thiosulfate with nitroprusside provides the necessary sulfur donor to prevent cyanide accumulation by forming the less toxic intermediate, sodium thiocyanate (Davies *et al*., 1975; Hall & Guest, 1992; Humphrey & Nash, 1978).

A frequent source of chronic cyanide ingestion is through the diet. Certain foods, such as cassava root and lima beans, are staples in some parts of the world and contain varying levels of cyanide. Free cyanide in food and drink is usually derived from the hydrolysis of cyanogenic glycosides and includes linamarin in cassava, as well as amygdalin and prunasin in almonds and apricot pits (Figure 2.1). The amount of cyanide in these foods is usually below acutely lethal levels for humans, although in uncooked cassava, the levels can be dangerously high. If consumed in sufficient quantities over time, the toxic insult can be cumulative. Cassava root is a high-yield crop popular in tropical Africa, Asia, and Latin America. Cassava can survive extreme drought, making it an attractive food source in more arid regions as well (Baskin & Rockwood, 2002; Howlett *et al*., 1990). Over 276 million metric tons were produced worldwide in 2013 (Faostat, 2015). If cassava is not processed properly (e.g., soaked in water for 3 to 5 days) cyanide removal is not complete and consumption may be dangerous.

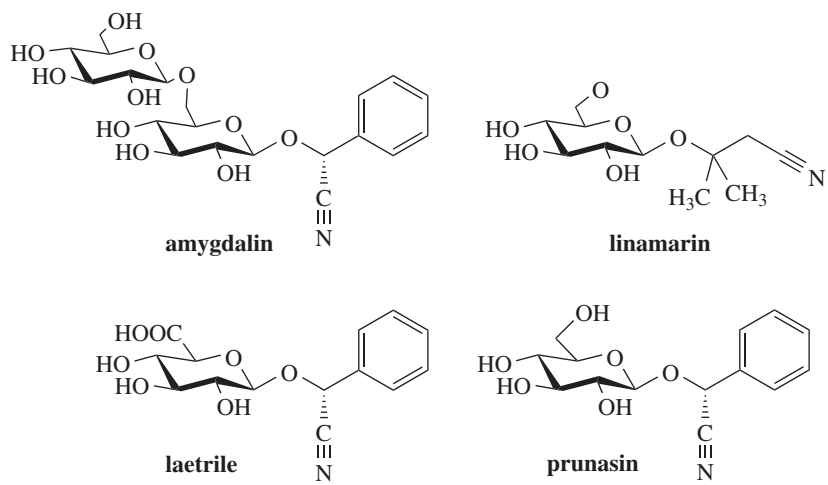


Figure 2.1 Common cyanogenic glucosides.

Lastly, a small amount of cyanide intake occurs during the process of smoking tobacco (Chandra *et al.*, 1980; Harris, 1996; Logue *et al.*, 2009; Lundquist *et al.*, 1987; Tsuge *et al.*, 2000). Consequently, cyanide and thiocyanate concentrations in the plasma, whole blood, saliva, and tissues of such smokers are elevated relative to non-smokers. Tobacco smokers have 115–415% of the circulating cyanide concentrations of non-smokers (Youso *et al.*, 2010). In certain settings, chronic cyanide exposure through inhalation of tobacco cigarette smoke has been associated with disease, for example, *in utero* cyanide toxicities (Doherty *et al.*, 1982; Singh, 1981; Umebese & Okeke, 2004) and exacerbation of vitamin B_{12} deficiencies (Chisholm *et al.*, 1967; Smith, 1961; Wokes & Moore, 1958).

2.3 Chronic cyanide exposure in human disease

Epidemiological, therapeutic, and experimental studies suggest a role of chronic cyanide exposure in human disease. Humans have developed robust, endogenous mechanisms of cyanide metabolism to detoxify cyanide from food and the environment (Figure 2.2). Chronic cyanide exposure may overwhelm these endogenous detoxification mechanisms and produce adverse biological effects (Banerjee *et al.*, 1997; El Ghawabi *et al.*, 1975; Kalyanaraman *et al.*, 1983; Vesey *et al.*, 1976). Furthermore, nutritional inadequacies, such as low sulfur or iodine intake (Adamolekun, 2010; Cliff *et al.*, 1985; Osuntokun *et al.*, 1968) and inborn metabolic deficiency may exacerbate the toxicity of chronic cyanide exposure (Wilson *et al.*, 1971) by exceeding the capacity of the body to detoxify the cyanide, systems for which substrates of dietary origin may be rate limiting. Cyanide is detoxified largely through the rhodanese mechanism (Figure 2.2). In the presence of a sulfur donor, rhodanese converts cyanide to thiocyanate, which can then be excreted in urine. If a diet lacks foods with sulfur-containing amino acids, cyanide detoxification may be slowed or even halted (Cliff *et al.*, 1985; Swenne *et al.*, 1996; Tor-Agbidye *et al.*, 1999).

2.3.1 Neurological syndrome related to ingestion of cassava

Cassava, known also as tapioca, manioc, and yuca, originated in South America, but is the major nutritional

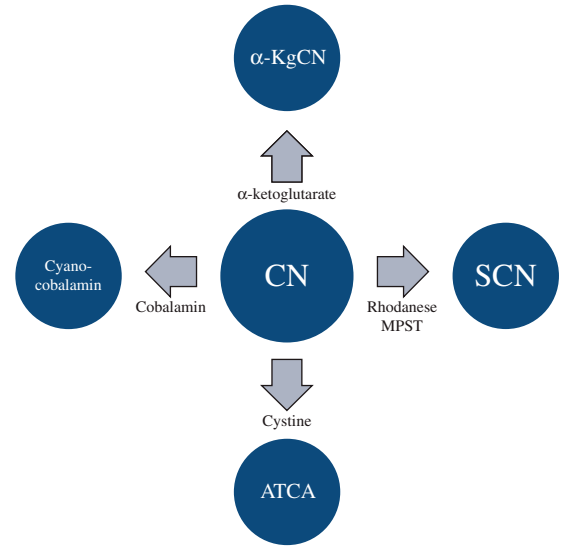
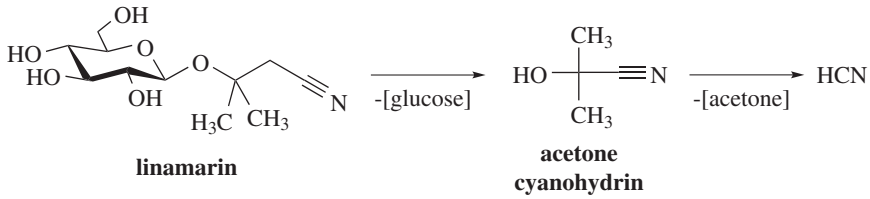


Figure 2.2 Endogenous cyanide detoxification mechanisms. A-KgCN – a-ketoglutarate cyanohydrin; MPST – mercaptopyruvate sulfurtransferase; ATCA – 2-amino-2-thioazoline-4-carboxylic acid.

supply for 20–40% of the population in sub-Saharan Africa. Cassava can withstand drought and pests, grow in poor soil without fertilizer, and requires less labor for cultivation than other crops (Nzwalo & Cliff, 2011). The disadvantages of using cassava as a food source include the potential cyanide toxicity of both the roots and leaves and the low protein content of the roots. Bitter cassava has a higher amount of glucosides (Cliff, 1984) and must be processed to remove the toxic compounds as compared to the sweeter version, which contains low levels of glucosides and can be eaten fresh. Drought and poor soil increase the level of cyanogenic glucosides (Vandegeer *et al.*, 2012). Linamarin is the main cyanogenic glucoside in cassava (Figure 2.3). When plant tissue is mechanically disintegrated or fermented, the glucoside is broken down into cyanohydrins by β-glucosidase (linamarase) in the plant cell (Cooke *et al.*, 1978; Wood, 1966), and cyanohydrins are metabolized to, or spontaneously decompose into, HCN (Cooke & De la Cruz, 1982). Careful preparation of the cassava root, such as fermentation or soaking in water three to five days, reduces this cyanogenic potential to levels safe for consumption (Tylleskär *et al.*, 1992).

Tropical ataxic neuropathy (TAN) is a syndrome common to a particular area in Nigeria where large amounts



linamarin **acetone cyanohydrin**

Figure 2.3 Generation of HCN from the breakdown of linamarin.

of cassava are consumed without the addition of protein-rich supplementary foods. TAN is characterized by visual impairment and weakness and spasticity of the lower limbs (Osuntokun *et al.*, 1968). The most common symptoms are tingling of the extremities and blurring of vision, where unsteadiness and weakness occur as the condition advances, often leading to an uncoordinated gait (i.e., ataxia). Although associated deficiencies of certain B vitamins may also play a role, chronic cyanide exposure is likely the dominant etiological factor in this neuropathy. Osuntokun and colleagues found the disease confined to areas of Nigeria where cassava was most cultivated, and disease prevalence correlated with cyanide exposure as determined by serum thiocyanate levels (Osuntokun, 1981). All cases were reported from areas where cassava is consumed in large quantities and not in nearby areas where other food staples predominate (Osuntokun, 1981). The inadequate supply of sulfur-containing amino acids in cassava further limits the capacity of the cyanide detoxification pathways (Diasolua Ngudi *et al.*, 2002; Nassar *et al.*, 2009).

A study involving nine patients with ataxic neuropathy compared plasma amino acid concentrations to healthy controls. Investigators found an absence or diminution of the sulfur-containing amino acids cysteine and methionine and a variable concentration of most other essential amino acids (Osuntokun *et al.*, 1968). The authors attribute the low concentrations of sulfur-containing amino acids in the plasma to an inadequate intake of sulfur-containing proteins and/or a conditioned deficiency resulting from excessive cyanide detoxification. Reduction in the dietary intake of cyanide and introduction of a more balanced diet to incorporate the needed sulfur for cyanide conversion to thiocyanate may help decrease the incidence of TAN (Osuntokun & Monekosso, 1969). However, agricultural crises precipitated by drought, war, or surges in cassava trade can lead to food shortages and necessitate that families again rely on insufficiently processed cassava, thereby increasing the incidence of TAN (Banea *et al.*, 1997; Cliff *et al.*, 1997; Howlett *et al.*, 1990).

Spastic paraparesis, another chronic neurological syndrome, has been reported in Tanzania and Mozambique. Studies have shown that over a period of several weeks, ingestion of inadequately prepared cassava with high cyanide content can result in this neurological dysfunction (Carton *et al.*, 1986; Cliff, 1984; Howlett *et al.*, 1990; Tylleskär *et al.*, 1991). Spastic paraparesis has been reported among people whose regular diets contain cassava and other related cyanogenic-containing substances. This disease is referred to as "konzo" and is a specific neurological disorder that is similar to paralysis. It is characterized by an abrupt onset of varying degrees of spastic paraparesis due to isolated bilateral upper motor neuron damage (Tshala-Katumbay, Eeg-Olofsson, *et al.*, 2002; Tylleskär *et al.*, 1991). Only the pyramidal tracts are affected, although vision abnormalities and dysarthria may occur in severe cases. Mild cases may recover, but symptoms are irreversible in more severe cases.

Konzo was first reported in 1938 in the southern part of the Bandundu region in Zaire (Trolli, 1938). Thirty-nine uniform clinical cases from Tanzania were reported describing abrupt symmetric isolated and permanent damage to the upper motor neurons (Howlett *et al.*, 1990). The diet consisted almost exclusively of bitter cassava roots. Drought conditions and a shortage of other foods led to an increase in the natural occurrence of cyanogenic glucosides in the cassava roots, and the preparation time to remove the cyanide from the roots prior to consumption was shortened. Patient symptoms, cassava dependence, and blood chemistries resembled those reported from outbreaks of spastic paraparesis.

Cyanide exposure in regions affected by konzo has been studied in the Democratic Republic of the Congo (Tylleskär *et al.*, 1992). The cyanogenic glucoside, cyanohydrin, and HCN content of various cassava products in the region were analyzed. Proper treatment

of the cassava root (recommended 3- to 5-day soak in water; here, a 3-day soak) reduced the cyanogenic glucoside content to clinically insignificant levels. However, shortcuts in the processing of the cassava root (single-day soak) were self-reported to be extremely common, which allows the majority of cyanohydrins to remain in the flour. On average, flour consumed by affected households contained 32 mg/kg cyanide, half from cyanohydrins and half from glucosides. Using urinary thiocyanate concentration as a marker for cyanide exposure, children fed the short-soaked product had a 15-fold higher urinary thiocyanate concentration than children fed a properly processed product.

To identify the causative lesions in konzo, symptomatic patients of varying severity were studied by MRI and electrophysiology. Two konzo-afflicted subjects showed no apparent abnormalities on MRI (Tylleskär *et al.*, 1993). However, using transcranial magnetic stimulation, impulse transmission to the lower limbs was impaired or absent. To better study the nerve conduction deficiency in the descending motor pathways of konzo subjects, two larger studies were conducted utilizing transcranial magnetic stimulation and transcranial electrical stimulation in konzo subjects. Again, motor-evoked potentials were either delayed or not observed (Tshala-Katumbay, Edebol Eeg-Olofsson, *et al.*, 2002). The pattern of deficiencies in these patients indicates that the defect lies in the upper motor neurons of the descending motor pathways (Banea-Mayambu *et al.*, 1997; Howlett *et al.*, 1990; Tylleskär *et al.*, 1993). Additionally, evidence of defects in the ascending somatosensory pathways has also been reported (Tshala-Katumbay, Edebol Eeg-Olofsson, *et al.*, 2002).

Recently, Adamolekun (2010, 2011) suggested that the symptoms of konzo are inconsistent with cyanide toxicity and instead more closely resemble those of thiamine deficiency. Konzo patients are known to consume large amounts of linamarin-containing cassava while also consuming inadequate amounts of sulfur-containing amino acids necessary for cyanide detoxification. The author suggests that with sulfur-containing amino acids depleted, thiamine is consumed to provide sufficient sulfur donor to rhodanese and that this in turn can generate a thiamine deficiency (Adamolekun, 2010). To our knowledge, no published literature is available regarding thiamine levels in konzo patients or the therapeutic application of thiamine in the konzo patient population. Also, konzo patients lack typical manifestations of thiamine deficiency (e.g., loss of vision, peripheral sensory impairment, peripheral edema, hypertension), such as those seen in beriberi and Wernicke-Korsakoff syndrome (Nzwalo & Cliff, 2011; So & Simon, 2012). Alternatively, thiocyanate is thought to be a harmless intermediate of cyanide detoxification, but some authors have proposed that it may play a role in konzo (Tor-Agbidye *et al.*, 1999). The precise neurotoxic mechanism remains to be elucidated.

Other effects correlated with cassava consumption include pancreatic diabetes, vitamin B_{12} deficiency, and thyroid dysfunction (Jantz & Uluwaduge, 1997). Epidemiological studies describe a unique type of pancreatic diabetes that develops in the tropics. This so-called tropical malnutrition diabetes or malnutrition-associated diabetes mellitus is found in regions that coincide with populations known for heavy nutritional dependence on cassava. Patients typically are malnourished, especially lacking significant protein intake, indicating a probable deficiency in sulfur-containing amino acids (McMillan & Geevarghese, 1979). Cyanide exposure, through either diet or tobacco smoking, has been suggested as the cause of alcoholic and tropical pancreatitis, possibly leading to diabetes mellitus (Pitchumoni *et al.*, 1988). In a laboratory setting, rats, rabbits, pigs, and goats administered cyanide in their feed for several months showed no changes in fasting blood glucose or histology of the pancreas, possibly due to the absence of a sulfur-containing amino acid deficiency (Okolie & Osagie, 2000; Soto-Blanco, Sousa, *et al.*, 2001).

2.3.2 Thyroid dysfunction and chronic cyanide exposure

Iodine is required for the normal function of the thyroid gland, which transports iodide into the thyroid through the sodium-iodide symporter and incorporates iodide in the thyroid hormone molecules. Insufficient delivery of iodide to the thyroid gland triggers a compensatory hyperplasia of the gland referred to as a goiter. Goiter as a result of iodine deficiency affects more than 180 million people worldwide, 2.72% of the global population (Vos *et al.*, 2012). Additionally, since the thiocyanate ion is similar to the iodide ion in size and charge, thiocyanate competitively inhibits iodine uptake by the thyroid gland, and the iodine is excreted in the urine (Barker, 1936; Bourdoux *et al.*, 1978; Carrasco, 1993; Dohán *et al.*, 2003). Therefore, under conditions of high ingestion of inefficiently prepared cassava, the

potential for chronic cyanide overload exists, which in turns creates a high level of serum thiocyanate. This results in a decreased absorption of iodide by the thyroid and a subsequent increase in iodide excretion into the urine. Endemic goiter develops with concomitant inadequate dietary iodine intake (< 100 mg/day). Neurologic abnormalities and severe mental retardation occur in cases of severe iodine deficiency and may be exacerbated at high enough thiocyanate concentrations. In studies of populations from the Democratic Republic of the Congo, the prevalence of goiter and cretinism was significantly correlated with serum thiocyanate concentration and urinary iodide excretion (Ermans *et al*., 1980).

Bourdoux and colleagues described a population in the Democratic Republic of the Congo that consumed large amounts of cassava with concurrent severe iodine deficiency (Bourdoux *et al*., 1978). This population had elevated serum and urinary thiocyanate concentrations, and the urinary thiocyanate concentrations correlated with degree of thyroid hyperplasia. In a subset of patients that did not initially have elevated thiocyanate levels, three days of a cassava-rich diet induced thiocyanate elevations comparable to the rest of this patient population, which resolved when the cassava-rich diet was withdrawn.

When iodine supplements are given, the prevalence of goiter is reduced in spite of the continued consumption of cassava. Ataxic neuropathy and endemic goiter have not been reported in tribes in the Amazon jungle, even though they consume large volumes of cooked fresh cassava. One possible reason this population is resistant to the neuropathy is that they eat a considerable amount of fish and animal protein, thus having higher levels of sulfur-containing amino acids and iodine in their diet than do the konzo- and TAN-afflicted populations for whom alternative food sources are scarce.

In addition to dietary sources, endocrine disorders can also arise from long-term industrial exposure to cyanide. Several industrial processes used in mining, jewelry production, rubber and plastic manufacturing, and other industries use chemicals that present a risk of chronic exposure to cyanide. Electroplating workers in three Egyptian factories were studied (El Ghawabi *et al*., 1975). Cyanide concentrations in the air were measured and found to be between 4.2 and 12.4 ppm. The National Institute for Occupational Safety and Health has recommended a short-term exposure limit of 4.7 ppm and an 8-hour time-weighted average permissible exposure limit of 10 ppm (NIOSH, 2010). All exposed workers had increased hemoglobin and lymphocyte counts. More than half of the exposed workers had thyroid enlargements. Thiocyanate urinary excretions also correlated with the duration of exposure to HCN in the work environment. The symptoms in the exposed workers included headache (81%), weakness (78%), and altered sense of taste or smell (78%). Exposed workers had significantly impaired ^{131}I uptake and elevated urinary thiocyanate concentrations compared to unexposed controls.

Similarly, cyanide exposure and thyroid function were estimated in employees of a cable manufacturing plant. Researchers considered the "exposed" population to be a group of non-smoking males who worked in the electroplating process area for more than five consecutive years. The "nonexposed" population was a group of workers who worked outside of the manufacturing building. Exposed workers had more than three times the plasma thiocyanate concentration than that of nonexposed workers, strongly suggesting that this population had been exposed to cyanide. Exposed workers had significantly reduced serum T_3 and T_4 and significantly increased thyroid-stimulating hormone, indicating a failure of T_3/T_4 synthesis (Banerjee *et al*., 1997).

Another study examined workers at a silver-reclaiming plant in Illinois that used a cyanide-extracting process to recover silver from used photographic and X-ray film (Blanc *et al*., 1985). At peak operation, the plant consumed an estimated 5 tons of sodium cyanide (NaCN) each month. Twenty-four hours after regulators closed the plant, the air HCN concentration was measured at 15 ppm. Workers experienced symptoms consistent with cyanide exposure, and symptom severity correlated with estimated cyanide exposure. Serum analysis indicated that workers had an average increase in thyroid-stimulating hormone, suggesting thyroid dysfunction, and decreased vitamin B_{12} and folate.

2.3.3 Chronic cyanide intoxication from laetrile

Amygdalin is a cyanogenic glycoside composed of two glucose molecules and a mandelonitrile aglycone group found in many of the species of the genus *Prunus* (e.g., apples, pears, bitter almonds, cherries, etc.). In the past, laetrile, a synthetic derivative of the amygdalin that was

originally patented for use as a meat preservative (Krebs & Krebs, 1961), has been promoted to remedy cancer (Milazzo *et al.*, 2007) and relieve chronic pain (Euler, 2012; Wilson, 2012). This can still be purchased over the Internet as a vitamin supplement of "Vitamin B17" or apricot kernel extract. Therapies marketed as laetrile are oftentimes heavily contaminated with amygdalin or are entirely amygdalin preparations (Milazzo *et al.*, 2007). The safety and efficacy of laetrile have been debated in the literature. An extensive review of the laetrile literature reported that all animal and human cancer studies reported to date have failed to show prolongation in survival or a substantial reduction in tumor size (Lewis, 1977; Milazzo *et al.*, 2007). However, the possibility of chronic cyanide poisoning from daily ingestion of laetrile is overwhelming when one compares the approximately 8.25 mg of cyanide ingested daily by those who subsist on cassava diets with the 30 mg of cyanide ingested with every 500 mg tablet of laetrile (Newton *et al.*, 1981). Amygdalin has demonstrated human toxicity, and there have been several cases of lethality in humans (Braico *et al.*, 1979; Hall *et al.*, 1986; Humbert *et al.*, 1977; Sadoff *et al.*, 1978), as well as a number of other cases in which a subacute or chronic syndrome has followed its intensive use (Bromley *et al.*, 2005; Kalyanaraman *et al.*, 1983; O'Brien *et al.*, 2005; Ortega & Creek, 1978; Smith *et al.*, 1978). Intestinal β-D-glucosidases break down amygdalin into glucose and mandelonitrile, which is further broken down into benzaldehyde and HCN (Figure 2.4). This enzymatic reaction explains why only gastrointestinal exposure, in contrast to intravenous administration, results in toxicity (Hall *et al.*, 1986). Smith and colleagues reported that a 46-year-old man took 500 mg laetrile daily for six months and developed generalized muscle weakness, paralysis of both eyelids, and paresis of the upper and lower and upper extremities. These neurologic symptoms were consistent with chronic cyanide poisoning. After discontinuing use of the drug, his neurologic symptoms improved over the next six days.

Another case involved a 67-year-old woman with lymphoma who developed a neuromyopathy following ingestion of three tablets of laetrile daily for an unknown period (Kalyanaraman *et al.*, 1983). Each tablet contained 25–75 mg cyanide. Nerve biopsies showed a combination of demyelination and axonal degeneration. Muscle biopsies showed denervation and myopathy/atrophy. These findings were found to be unrelated to her chemotherapy and closely resembled the ataxic neuropathy seen in the Nigerian populations. Furthermore, she had significant elevation of blood and urinary thiocyanate and cyanide levels. It was concluded that cyanide toxicity secondary to laetrile therapy and nutritional deficiency caused the neuromyopathy, and after discontinuation of laetrile, the patient improved.

Studies in rats displayed neurologic damage 80 minutes after oral administration of amygdalin. Animals were initially ataxic and progressed to complete loss of

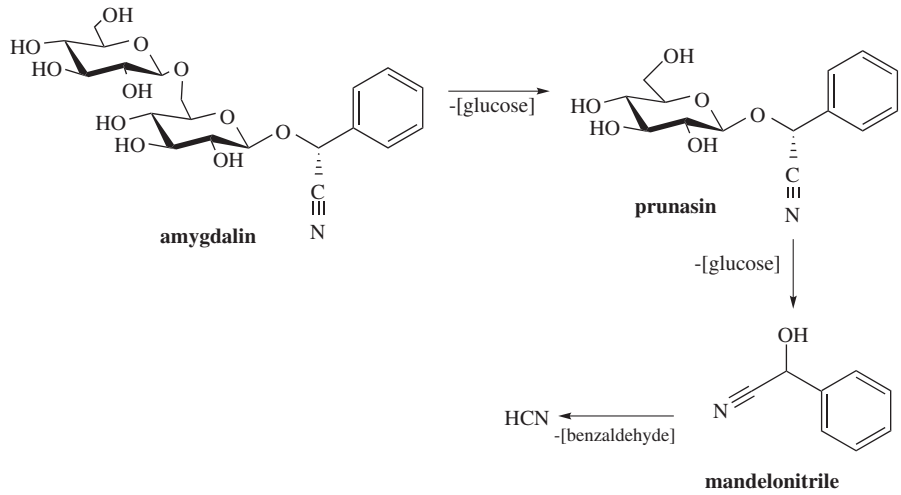


Figure 2.4 Generation of HCN from the breakdown of amygdalin.

righting reflex with hind limb paralysis prior to death. The cyanogenic potential of amygdalin was quantified. After oral administration to rats, 13% of available cyanide was released in the gastrointestinal tract. Exposure of amygdalin to human gut flora estimated 53% of available cyanide being released in the human gastrointestinal tract, suggesting that orally administered daily doses will expose persons to chronic levels of cyanide (Newton *et al.*, 1981). This study and the above case reports suggest that long-term consumption of cyanogenic glycoside-based therapeutics can produce neurologic damage in humans consistent with tropical ataxic neuropathy.

2.3.4 Toxic amblyopia

Chronic cyanide exposure may directly cause dietary deficiencies. Tobacco amblyopia is a vitamin B_{12} (cobalamin) deficiency and is characterized by vision loss, which is often restored after smoking cessation. Varying by brand, tobacco cigarettes can contain as much as 150 μg HCN in each cigarette (Gori & Lynch, 1978). Studies suggest that chronic exposure to cyanide in cigarette smoke produces derangements of cobalamin concentrations in humans. In a study of vitamin B_{12} handling in smokers, urinary thiocyanate excretion and urinary vitamin B_{12} excretion were increased in smokers compared to non-smokers. Serum B_{12} levels were lower in smokers versus non-smokers, and urinary thiocyanate excretion was significantly and negatively correlated with serum vitamin B_{12} concentrations (Linnell *et al.*, 1968). Cyanocobalamin, a form of vitamin B_{12} inactivated by chelation of cyanide, was detected in the plasma of heavy cigarette and pipe tobacco smokers (Lindstrand *et al.*, 1966). Defective absorption of vitamin B_{12} along with reduced serum vitamin B_{12} concentrations has also been shown in almost half of the patients with tobacco amblyopia. The magnitudes of these effects correlated with the amount of tobacco consumed (Foulds *et al.*, 1969). This vitamin B_{12} depletion is thought to underlie tobacco amblyopia.

Wokes and Moore suggested that cyanide from tobacco smoke was a contributory factor in tobacco amblyopia, and Smith and colleagues produced more evidence to show that this was due to reduced concentration of vitamin B_{12} since hydroxocobalamin was overwhelmed by large amounts of cyanide to form metabolically inert cyanocobalamin (Smith, 1961; Wokes & Moore, 1958). Moreover, traces of cyanocobalamin were later shown to be present in the plasma of 25% of normal non-smokers, and just fewer than 50% in normal smokers. The amount of plasma cyanocobalamin in patients with tobacco amblyopia was higher than the plasma cyanocobalamin in smokers in general (Wilson *et al.*, 1971). In ten patients with tobacco amblyopia, but without vitamin B_{12} deficiency, eight had approximately 10% of plasma cobalamin as cyanocobalamin. Furthermore, depletion of hydroxocobalamin by tobacco-derived cyanide may also be involved in optical neuritis in the setting of pernicious anemia (Chisholm *et al.*, 1967; Smith, 1961).

Some investigators argue that cyanide in tobacco smoke is the etiologic or aggravating factor in the development of the optic neuropathies of pernicious anemia and Leber's hereditary optic neuropathy (LHON) (Dreyfus, 1976; Grant, 1974; Wokes & Moore, 1958). However, Victor and colleagues (Victor, 1963; Victor & Dreyfus, 1965) suggest that this condition is a nutritional deficiency, rather than a set of toxic reactions, and that it should be called "nutritional retrobulbar neuropathy." Plasma and urinary thiocyanate concentrations in normal non-smokers were lower than in cigarette and pipe tobacco smokers, while corresponding levels in patients with LHON were similar to that of non-smokers. It was concluded that in normal non-smokers, thiocyanate in body fluids was likely from dietary sources and did not represent detoxified cyanide, whereas in smokers, this was detoxified cyanide. The lack of increased thiocyanate in LHON is consistent with the hypothesis of defective cyanide metabolism. Furthermore, there is a massive increase in cyanocobalamin concentration in plasma of patients with LHON, regardless of smoking status, which further supports this hypothesis (Wilson *et al.*, 1971). A defect in rhodanese activity in LHON patients was hypothesized and thoroughly debated (Cagianut *et al.*, 1981; Grant, 1974; Nikoskelainen *et al.*, 1984; Poole & Kind, 1986; Whitehouse *et al.*, 1989). However, it is now known that mutations associated with LHON are all localized to mitochondrial DNA, mostly within genes encoding subunits of electron transport chain complex I (Riordan-Eva & Harding, 1995).

2.3.5 Cyanide toxicity in pregnancy

Long-term exposure to cyanide through the diet has been studied epidemiologically and in animal models for effects on pregnancy, fetal development, delivery,

and fertility. One study describes babies born with severe congenital limb malformations to mothers that subsist primarily on garri, a preparation of cassava root (Umebese & Okeke, 2004). Mothers of healthy babies in the region typically acquired their garri from the area markets, for whom it is customary to age the garri at least five days before sale. The garri consumed by the mothers with the affected babies had aged less than 48 hours. The improperly aged garri was found to have 81 mg/kg cyanide content, compared to the properly processed garri with < 31 mg/kg cyanide. The authors offer the chronic cyanide consumption from the improperly processed garri as a possible explanation or contributor to the congenital limb defects seen in this study. Bolstering this conclusion, studies of the offspring of rats fed large amounts of cassava starting 15 days post-fertilization have shown an increased frequency of growth retardation and limb defects (Singh, 1981).

Plasma thiocyanate concentrations are increased in mothers who smoke and in their fetuses (Andrews, 1973; Pettigrew *et al*., 1977). Birth weight for babies born to mothers who smoke is also known to be lower than for babies of non-smokers (Chanoine *et al*., 1991; McGarry & Andrews, 1972; Meberg & Marstein, 1986; Pettigrew *et al*., 1977). Furthermore, reduction or cessation of smoking during pregnancy significantly improves infant birth weight (Li *et al*., 1993; Sexton & Hebel, 1984). Pettigrew and Logan hypothesize that since detoxification of cyanide from inhaled tobacco smoke consumes vitamin B_{12} and sulfur-containing amino acids, this may divert nutrients from fetal development. Serum vitamin B_{12} levels were significantly lower in pregnant women who smoke compared to non-smokers (McGarry & Andrews, 1972). Cassava-fed, pregnant hamsters had offspring that showed evidence of fetotoxicity, and these hamsters gained significantly less weight while pregnant than did control animals (Frakes *et al*., 1986). The fetotoxicity included reduced fetal body weight and a low incidence of gross congenital malformations which was significantly higher than in litters from dams fed either low protein or laboratory stock diets.

The Investigational New Drug application filed with the Food and Drug Administration seeking approval for marketing laetrile included data demonstrating fetal toxicity in rats when dams were fed laetrile (Lewis, 1977). Pregnant rats were fed 5 or 25 mg/kg per day of laetrile orally. In rats receiving the lower dose of laetrile, one fetus was found to have a kidney defect and one had hydrocephalus. In the higher dose cohort, two fetuses had abnormal kidneys. Additionally, the teratogenic effects of laetrile on hamsters manifest with oral laetrile administration, but not intravenous, and were prevented with thiosulfate treatment. The results indicate these developmental defects were a result of cyanide freed from laetrile by the gut flora (Willhite, 1982).

In regions prone to iodine deficiencies, the potential association between prenatal cyanide exposure from mothers who smoke during pregnancy and neonatal thyroid volume was studied (Chanoine *et al*., 1991). Cord serum thiocyanate elevation was confirmed, and the thiocyanate concentration was found to be correlated with maternal smoking frequency, indicating that thiocyanate crosses the placenta and is likely derived from cyanide detoxification. The ratio of thyroid volume to birth weight increased significantly as a function of cord serum thiocyanate concentration, and this increase was due to a concomitant increase in thyroid volume and a decrease in birth weight.

Aside from interfering with fetal development, cyanide exposure may also affect fertility and delivery. Smoking has long been associated with dysfunctions in fertility (Centers for Disease Control and Prevention, 2010). Cyanide concentrations well below those found in cigarette smoke were sufficient to inhibit the function of oviductal cilia in hamsters. The authors hypothesize that this mechanism contributes to the increase in ectopic pregnancies and infertility seen in smokers (Talbot *et al*., 1998). Additionally, cyanide exposure may affect the normal functioning of the myometrium and contribute to dysfunctional labor. Calcium handling and contractility of human myometrium samples were studied ex vivo. It was determined that cyanide rapidly inactivated spontaneous and agonist-invoked calcium transients and attenuated the contractile force generated by depolarizing the myometrium. This study suggests that cyanide exposure interferes with the contractility and oxytocin-induced contractions of the human myometrium, possibly contributing to dysfunctional labor and unpredictability of oxytocin efficacy in labor and delivery (Monir-Bishty *et al*., 2003; Phoenix & Wray, 1993). Similar experiments in rat myometrial strips indicate that metabolic inhibition by cyanide increases potassium efflux from the myometrium, hyperpolarizing the cells and decreasing excitation of the muscle (Heaton *et al*., 1993).

Animal studies of prenatal chronic cyanide exposure consistently demonstrate the teratogenicity of chronic cyanide exposure. Chronic exposure to high doses of potassium cyanide (KCN; 3 mg/kg by gavage) in pregnant goats produced congenital defects and spontaneous abortions. Abnormalities in the thyroid, liver, and cerebellum were seen in fetuses exposed to cyanide (Soto-Blanco & Gorniak, 2004; Soto-Blanco *et al.*, 2009). In rats, prenatal exposure to cyanide increased frequency of visceral developmental defects. Histopathological examination revealed damage to hepatocytes and brain and abnormal biliary duct proliferation (De Sousa *et al.*, 2007). Prenatal cyanide exposure in hamsters also induced developmental defects, mostly neural tube defects and a low incidence of cardiac morphogenesis defects (Doherty *et al.*, 1982).

2.4 Experimental models of chronic cyanide exposure

The effects of chronic cyanide intoxication have been studied and discussed in humans (see previous sections), and cyanide is associated with diseases such as tropical ataxic neuropathy, konzo, and possibly toxic amblyopia. The exact mechanisms responsible for the pathologies associated with chronic exposure to cyanide remain to be elucidated. Studies indicate that at least some of the diseases thought to be caused by chronic cyanide intoxication may also require, or be exacerbated by, concomitant dietary deficiencies of sulfur-containing amino acids, cobalamin, iodine, or other nutrients. A variety of animal models in several species covering different routes of administration and cyanide sources have been developed to understand the consequences of chronic cyanide exposure. A selection of these models are described below and summarized in Table 2.1.

2.4.1 Chronic cyanide exposure in pregnancy and development

In addition to the concern in humans, the teratogenic and low birth-weight potential of prenatal cyanide exposure is of concern in livestock production. The effects on prenatal and postnatal chronic cyanide exposure have been studied in several species, including rats, hamsters, and goats. In a study for prenatal cyanide toxicity in goats, cyanide was delivered by gavage to pregnant goats starting at 24 days post-fertilization (dpf) and extending to delivery (Soto-Blanco & Gorniak, 2004). While cyanide exposure produced clear signs of cyanide toxicity in the dams, length of gestation of live births and the number of live kids were indistinguishable from controls. At high doses of cyanide exposure from gestational days 24 through 150, two kids had congenital birth defects and a third was spontaneously aborted. Histological lesions were only seen in the dam and fetuses euthanized a few weeks before term. These lesions showed characteristic defects of the thyroid and spongiosis of the white matter. A follow-up study looked at pregnant goats given KCN by gavage starting on 24 dpf, and in this study all animals were euthanized a few weeks before term at 120 dpf (Soto-Blanco *et al.*, 2009). Defects of the thyroid, liver, and cerebellum were seen in both the dams and the fetuses. The dams also had spongiosis of the cerebrum. Cyanide and thiocyanate concentrations were higher in maternal blood than in fetal blood, showing some, but incomplete, transfer of cyanide to the fetus.

Using rats, general pathology of prenatal oral cyanide exposure was studied by supplementing the drinking water with KCN from 6 to 20 dpf (De Sousa *et al.*, 2007). The doses of cyanide used were low enough to not induce any overt signs of toxicity. Serum thiocyanate levels were increased in each of the experimental groups consuming KCN. The overall number of fetuses found to have defects in visceral development was increased in the 30 mg/kg per day KCN group. Also at this dose, maternal serum glucose concentrations were significantly elevated. Histological analysis of the dams and pups revealed KCN dose-dependent lesions. High-dose KCN induced significant vacuolization of hepatocytes in the dams and milder hepatocyte damage in the pups. In dams, brain histopathological examination showed regions of focal necrosis and gliosis, as well as congestion and vacuolization of the cerebellar white matter, with similar and milder lesions seen in pups. Lesions to the pancreatic islets were seen in the high-dose KCN dams. Pups in this group had indications of biliary duct proliferation. Cyanide exposure did not affect the number of implantations or live births. Histopathological analysis of the lung and spleen was unremarkable across groups.

Introducing cyanide to animals through food or drinking water often lacks dosing precision because food and water consumption is difficult to standardize in group-housed animals, alterations in taste may affect

Table 2.1 Summary of studies of long-term cyanide exposure reports in animals.

Dose	Duration	Outcome	Reference
Inhalation			
200 ppm HCN inhaled 230 ppm HCN inhaled 430 ppm HCN inhaled	12.5 minutes once per day every 5th day for 5 total exposures	Injury to myocardium and increased number of stimulated ectopic heartbeats.	O'Flaherty & Thomas, 1982
Orally			
0.05% KCN feed	*Ad libitum* for gestation + 1–50 days postpartum	Prenatal KCN had no effect. Postnatal KCN delayed maturation of the cerebellum.	Malomo *et al.*, 2004
0.15% KCN feed	*Ad libitum* for 11.5 months	Attenuated weight gain, decreased thyroid activity, myelin degeneration of spinal cord white matter.	Philbrick *et al.*, 1979
~ 60μg/kg KCN per day orally	40 weeks	Injury to liver, kidney, and lung, but not heart and pancreas.	Okolie & Osagie, 1999, 2000
0.15, 0.3, or 0.6 mg/kg KCN per day orally	3 months	Injury to spinal motor pathway, hippocampus, and cerebellum, absence of pancreatic or thyroid lesions.	Soto-Blanco *et al.*, 2002a, 2002b
0.3, 0.9, 3, or 9 mg/kg KCN per day orally	15 days	Attenuated weight gain, injury to liver and kidney, thyroid dysfunction.	De Sousa *et al.*, 2002
0.3, 0.6, 1.2, or 3 mg/kg KCN per day orally	5 months	Absence of fasting blood glucose aberrations and pancreatic lesions, attenuated weight gain, thyroid dysfunction, injury to brain stem, cerebellum, and spinal motor pathway.	Soto-Blanco, Sousa, *et al.*, 2001; Soto-Blanco, Gorniak, *et al.*, 2001; Soto-Blanco *et al.*, 2002a
1, 2, or 3 mg/kg KCN per day orally	Gestational days 24–150	Spontaneous abortion, thyroid dysfunction and cerebellum injury in dam and its fetuses.	Soto-Blanco & Gorniak, 2004; Soto-Blanco *et al.*, 2009
1, 3, or 30 mg/kg KCN per day orally	Gestational days 6–20	Injury to pancreas and bile duct malformation.	De Sousa *et al.*, 2007
1.4 mg/kg KCN per day orally	90 days	Decreased motor coordination, depleted glutathione, lipid peroxidation in brain and liver.	Mathangi *et al.*, 2011
2, 4,or 6 mg/kg KCN per day	10 weeks	Thyroid dysfunction, injury to cerebellum, kidney, and liver.	Manzano *et al.*, 2007
2, 4, or 6 mg/kg KCN per day orally	74 days	Absence of fasting blood glucose aberrations and pancreatic lesions.	Soto-Blanco, Sousa, *et al.*, 2001
2.5 mg/kg HCN per day	30 days	Thyroid dysfunction and injury to mesencephalon.	Soto-Blanco *et al.*, 2008
3.8 mg/kg KCN per day orally	30 days	Thyroid dysfunction and injury to mesencephalon.	Soto-Blanco *et al.*, 2008
4.5 mg/kg HCN per day orally	30 days	Thyroid dysfunction, injury to liver and mesencephalon.	Soto-Blanco & Gorniak, 2010
9 or 12 mg/kg KCN per day orally	*Ad libitum* for 15 days	Absence of fasting blood glucose aberrations and pancreatic lesions.	Soto-Blanco, Gorniak, *et al.*, 2001; B. Soto-Blanco, Sousa, *et al.*, 2001
10, 20, or 30 g unprocessed cassava per day orally	6 weeks	Attenuated weight gain, injury to liver.	Oyeyemi *et al.*, 2012

(*continued*)

Table 2.1 (*continued*)

Dose	Duration	Outcome	Reference
Subcutaneous or Intraperitoneal			
0.126, 0.127, 0.1275, or 0.1295 mmol/kg/h NaCN subcutaneously	2–5 days	Neural tube defects, attenuated weight gain.	Doherty *et al*., 1982
0.5 mg KCN once per week subcutaneously	22 weeks	Neuronal degeneration and loss in cortex, hippocampus, and cerebellum.	Smith *et al*., 1963
12 mg/kg KCN per day subcutaneously	7 days	Akinesia and reduced locomotor activity, increased lipid peroxidation in striatum and hippocampus, reduced TH^+ cells in substantia nigra.	Kanthasamy *et al*., 1994
12 mg/kg KCN per day intraperitoneally	12 days	Apoptotic lesions of the motor cortex, necrotic lesions of the substantia nigra.	Mills *et al*., 1999

consumption rate, and animals sleep for several hours a day making exposure discontinuous. One alternative, demonstrated in the study of developmental defects due to chronic cyanide exposure in hamsters, is the steady delivery of cyanide solutions using subcutaneously implanted osmotic pumps (Doherty *et al*., 1982). In this study, NaCN was administered subcutaneously via osmotic pumps to pregnant hamsters only between 6 and 9 dpf. Elevations of whole blood cyanide and thiocyanate concentrations were confirmed in animals with implanted NaCN pumps; with the lowest dose rate produced a mean whole blood cyanide concentration of 25 μM. Overt cyanide toxicity of the dams (e.g., weight loss, ataxia, and dyspnea) was observed only in the groups with the highest cyanide exposure. Cyanide exposure induced a number of fetal abnormalities, mainly neural tube defects, but also defects in cardiac morphogenesis.

The effect of chronic prenatal and postnatal cyanide exposure on the developing cerebellum was studied in rats. Soon after conception, pregnant rats were fed standard rat chow or chow supplemented with 500 ppm KCN (Malomo *et al*., 2004). After birth, rats continued to receive the same diet they consumed prenatally. Morphometric comparisons of the developing cerebellum were made between the two treatment groups, starting at postnatal day one out to postnatal day 50. No significant difference was measured in the thickness of the external granular layers of control and cyanide-exposed rats at postnatal days one and nine. By day 28 through the end of the experiment at day 50, the molecular layer of the cerebellum of cyanide-exposed rats was significantly thinner than that of control rats. The Purkinje layer of the cerebellum did not differ at any time between control and cyanide-exposed rats. These data would suggest postnatal and not prenatal chronic exposure to cyanide in rats affects some aspects of cerebellar development, at least as determined by histopathological exam.

2.4.2 Damage to the central nervous system by chronic cyanide exposure

Cyanide intoxication has been well documented to induce central nervous system dysfunction. Cyanide has been associated with syndromes affecting the central nervous system in animals. Ataxia has been observed in sheep, cattle, and horses grazing sorghum (Adams *et al*., 1969; Bradley *et al*., 1995; McKenzie & McMicking, 1977). Sorghum contains toxic levels of the cyanogenic glucoside dhurrin, a compound similar to prunasin and amygdalin (Dunstan & Henry, 1902). Several animal studies in multiple species have been conducted to characterize the lesions to the central nervous system in chronic cyanide exposure.

Similar to the motor defects associated with chronic cyanide exposure in humans, motor activity abnormalities in rats chronically exposed to cyanide have been reported. KCN was delivered to rats by gavage for 90 days (Mathangi *et al*., 2011). Motor coordination was determined by the rotarod test prior to cyanide exposure and again every 15 days throughout the 90-day KCN exposure. Motor coordination, reported as latency for a

rat to fall from the rotarod, was significantly impaired in cyanide-exposed animals starting at the first time point measured, 15 days after starting KCN exposure, and remained attenuated throughout the study. Biochemical studies showed increases in lipid peroxidation and a relative depletion of antioxidant molecules in the blood, brains, and livers of rats exposed to cyanide, indicating ongoing oxidative damage in these animals. This study demonstrated liver histopathology after cyanide exposure (hepatocyte necrosis), but no changes to brain histology.

The role of cyanide exposure leading to demyelination was initially prescribed to anoxic injury secondary to acute, high-dose cyanide exposure. In an effort to demonstrate a direct role of cyanide in demyelinating lesions, chronic, low-dose cyanide exposure in rats was studied. Low-dose KCN was injected subcutaneously once weekly for 22 weeks. In cyanide-exposed rats, neuronal degeneration and neuron loss of pyramidal cells of the cortex and Purkinje cells of the cerebellum were observed. Staining for myelin in the corpus callosum of cyanide-exposed rats was weak, but not conclusive enough to declare a demyelinating lesion; myelin of the optic nerves was unaffected (Smith *et al.*, 1963).

Konzo, TAN, and tobacco amblyopia may require dietary deficiencies to produce cyanide toxicity. This hypothesis has been tested in a rat model of chronic cyanide exposure. Weanling rats were fed either a complete diet or a diet deplete of methionine, vitamin B_{12}, and iodine. Rats on each diet were further separated into groups in which the diet was supplemented with KCN or lacked KCN (Philbrick *et al.*, 1979). Exposure to cyanide by this route was carried out for 11.5 months. Cyanide exposure significantly stunted the growth of the rats compared to non-exposed controls. As seen in several human and in other animal studies, thyroid function was affected in cyanide-exposed rats. Younger cyanide-exposed rats had significantly reduced plasma T_4 concentrations, and this reduction was more severe in rats on the nutrient-deficient diet. Plasma T_4 concentrations normalized in mature rats, but T_4 secretion rates were still impaired. Histological studies of these animals demonstrated the typical gliosis and damage to the ventral column of the spinal cord. Electron microscopy revealed myelin degeneration in the spinal cord white matter. These lesions were less severe in animals fed the nutrient-complete, cyanide-containing diet.

Degeneration and loss of dopaminergic neurons are pathologies associated with Parkinsonian movement disorders like those seen in patients with konzo and TAN. To test the hypothesis that chronic cyanide exposure induces similar lesions, these dopaminergic pathways were characterized in chronic cyanide exposure mouse model. Mice given twice-daily subcutaneous KCN injections for 7 days had reduced locomotor activity and increased duration of akinesia, both of which were reversed by treatment with L-DOPA (Kanthasamy *et al.*, 1994). Neurochemical analysis of the mice after seven days of cyanide exposure indicated a reduction in dopamine content in the hippocampus and striatum. Tyrosine hydroxylase-positive staining was reduced in the substantia nigra by half as compared to saline-injected controls. This neurodegeneration was further characterized in an effort to determine whether the neuron loss was apoptotic or necrotic in nature. Mice were treated with twice-daily KCN IP injections for up to 12 days, and brain histology was determined (Mills *et al.*, 1999). The results showed areas of apoptotic neurons in motor cortices of mice after three days of KCN. At the same time, progressive, bilateral necrotic lesions appeared in the substantia nigra of the same mice with a ring of glial fibrillary acidic protein (GFAP) immunoreactivity surrounding the lesions, a marker of reactive gliosis. No indications of necrosis or increases in GFAP immunoreactivity were seen in the cortices of these mice. Virtually all other brain regions evaluated were unchanged.

Two separate studies of oral administration of varying doses of KCN to goats for five months (Soto-Blanco *et al.*, 2002a) and to rats for 3 months (Soto-Blanco *et al.*, 2002b) revealed neuropathic lesions similar to those seen in other animal studies. Histopathological analysis of brains from goats in the higher KCN experimental group revealed axonal degeneration in the pons, medulla oblongata, and ventral horn of the spinal cord, gliosis and spongiosis in the medulla oblongata, gliosis in the pons, and damaged Purkinje cells. At a moderate KCN dose, congestion and hemorrhage in the cerebellum and axonal degeneration in the spinal cord were observed. Immunohistochemistry for markers of apoptosis suggested that apoptosis was not involved in the lesions. In rats, similar axonal degeneration in the ventral horn of the spinal cord was observed, as well as neuronal loss in the hippocampus, damaged Purkinje cells, and loss of cerebellar white matter.

To confirm these pathologies are consistent with those of chronic cyanide ingestion, a similar study was conducted in a goat model of chronic dietary cyanide exposure. The leaves and seeds of chokecherry trees, like most of the genus *Prunus*, contain toxic levels of amygdalin and prunasin. Ground chokecherry leaves and flowers have been used in a laboratory settings as a dietary source of cyanide. Goats were fed this mixture for four weeks at a target dose of 2.5 mg/kg HCN per day (Soto-Blanco *et al.*, 2008). For the duration of the experiment, plasma thiocyanate concentrations were more than five times higher in chokecherry-fed goats compared to hay-fed goats. Thyroid dysfunction was evident by increased total vacuoles in the thyroid follicles of chokecherry-fed goats. In contrast to previously described animal experiments, investigators here found significantly elevated fasting blood glucose in chokecherry-fed goats compared to control goats. Histological lesions were not detected in any tissues outside of the brain and thyroid. Again, spongiosis and axonal dystrophy in the midbrain were seen in chokecherry-fed goats.

2.4.3 Other pathologies in animal models of chronic cyanide exposure

Studies of the general toxicity of long-term cyanide exposure have been conducted in several species. The effects of a 15-day cyanide exposure have been studied in rats using KCN dissolved in drinking water (De Sousa *et al.*, 2002). Elevated thiocyanate concentrations were confirmed in each of the cyanide-exposed cohorts. Kidney, liver, and thyroid tissues were compared by histopathological analysis. Degeneration of renal tubular epithelium and hepatocytes was evident in the high-dose cyanide cohorts. Histopathological examination of thyroid tissue revealed a cyanide dose-dependent increase in vacuoles within the thyroid follicular colloid, consistent with the thyroid changes seen in other cyanide-exposed animals.

Susceptibility of liver and kidney to damage from extended cyanide exposure has been studied in a rabbit model of chronic cyanide ingestion. KCN was administered to rabbits in feed for 40 weeks. Activity of liver enzymes within hepatocytes was reduced, and serum liver enzyme activity was increased in cyanide-exposed rabbits, indicating hepatotoxicity of long-term cyanide exposure. Blood urea nitrogen (BUN) and serum creatinine, measures of kidney function, both increased significantly with cyanide exposure, suggesting renal insufficiency in chronically exposed animals. Significant degenerative histopathologic lesions were evident in the livers and kidneys of cyanide-exposed rabbits (Okolie & Osagie, 1999).

Based on initial reports of cyanide involvement in cardiac dysfunction, pulmonary edema (Graham *et al.*, 1977), and possible involvement in the pathogenesis of diabetes (Pitchumoni *et al.*, 1988), subsequent studies have established cardiac, pulmonary, and pancreatic damage in rabbits after long-term cyanide exposure (Okolie & Osagie, 2000). KCN in grower mash was delivered to rabbits for 10 months. Fasting blood glucose was not significantly different between control and KCN-fed rabbits. Cardiac and pancreatic tissues were indistinguishable between groups on histopathological examination. While control rabbit lung tissue was unremarkable, the lungs of cyanide-fed rabbits displayed foci of edema and necrosis from long-term dietary cyanide ingestion.

A goat model of chronic cyanide ingestion was developed to study the association of goiter and diabetes mellitus with long-term cyanide exposure (Soto-Blanco, Gorniak, *et al.*, 2001). Goats were orally dosed with KCN for five months. Thyroid and pancreatic dysfunction were monitored by blood chemistry and histopathology. Cyanide-exposed goats had significantly suppressed plasma T_3 concentrations and increased vacuoles in the thyroid follicular colloid. No alterations in blood glucose or pancreatic histology were observed in goats exposed to four different KCN concentrations.

Adding to previous studies in which exogenous cyanide salts are chronically administered to animals, natural sources of cyanide have also been used experimentally to study chronic exposure (Soto-Blanco & Gorniak, 2010). Goats fed cassava leaves at a target HCN dose of 4.5 mg/kg per day had no differences in their weekly blood chemistry panels compared to hay-fed animals. Goats fed cassava had evidence of thyroid hormone synthesis defects, damage to periportal hepatocytes, and spongiosis of the midbrain. The lesions described are consistent with those found in a similar study in which goats were chronically administered KCN (Soto-Blanco, Gorniak, *et al.*, 2001; Soto-Blanco, *et al.*, 2002a, 2008, 2009). Similarly, rats fed diets in which increasing proportions are made up of unprocessed cassava showed a dose-dependent increase in

serum concentration of liver enzymes and degeneration of hepatocytes (Oyeyemi *et al.*, 2012).

The whole-animal toxicology of chronic KCN exposure in pigs has been studied. Pigs were fed different doses of KCN twice a day for 70 days (Manzano *et al.*, 2007). Cyanide exposure was confirmed by significant elevations in serum thiocyanate in the cyanide-exposed cohort. Similar to human and other animal studies, chronic cyanide exposure increased the weight of the thyroid gland in pigs. Histopathological examination showed several dose-dependent lesions including the following: increased vacuoles in thyroid follicular colloid, degeneration of cerebellar white matter and Purkinje cells, degeneration of renal tubular epithelium, and hepatic necrosis. No pancreatic lesions were evident in any group.

Epidemiological studies of malnutrition-related diabetes mellitus have suggested a role of cyanide in the development of diabetes mellitus (Pitchumoni *et al.*, 1988). In contrast, comprehensive studies in rats, pigs, goats, and rabbits have failed to demonstrate aberrations in fasting blood glucose or the histology of the endocrine pancreas (Manzano *et al.*, 2007; Okolie & Osagie, 2000; Soto-Blanco, Gorniak, *et al.*, 2001; Soto-Blanco, Sousa, *et al.*, 2001). One possible explanation is the lack of concomitant malnutrition in these animal studies.

Lastly, inhalation of pyrolysis products during structural fires is a route of acute cyanide exposure. Inhalation of cyanide from fires is also chronic, occupational cyanide exposure risk for first responders. HCN is one of the toxic compounds formed during fires and contributes to the mortality of smoke-inhalation victims. To characterize the toxicity of cyanide exposure from repeated inhalation of pyrolysis products, O'Flaherty and Thomas demonstrated the toxicity of HCN in smoke in a rat model of smoke inhalation (O'Flaherty & Thomas, 1982). Rats were repeatedly exposed to combustion products of polyurethane foam, which generates carbon monoxide and HCN by pyrolysis, or hemlock, which generates carbon monoxide but not HCN by pyrolysis. Rats were exposed five times each, one exposure per day with a four-day interval between exposures. Combustion of polyurethane on average produced 230 ppm HCN, whereas combustion of hemlock generated no detectable HCN; each produced comparable amounts of carbon monoxide. Exposure of rats to polyurethane foam smoke produced blood cyanide concentrations in excess of 1 μg/ml. Polyurethane smoke caused cyanide-dependent cardiotoxicity, as these mice had significantly increased markers of myocardial damage that could be prevented by pretreatment with thiosulfate. Rats exposed to polyurethane smoke had significantly more ectopic heartbeats than those exposed to hemlock smoke. Polyurethane smoke inhalation caused focal lesions in the myocardium that were not seen in rats exposed to hemlock smoke (O'Flaherty & Thomas, 1982). These findings parallel those seen in human victims of smoke inhalation (Fortin *et al.*, 2010).

2.5 Conclusion

Chronic cyanide exposure can occur through several routes. Poorly regulated industry and insufficiently processed crops high in cyanogenic glycosides are particular risks for long-term exposure to cyanide. Robust detoxification mechanisms have evolved to metabolize dietary and environmental cyanide, however these endogenous detoxification pathways are saturable (e.g., due to cyanide dose or nutritional status). Inadequate detoxification can result in serious morbidity and mortality, to include motor control deficits and paralysis as seen in konzo and tropical ataxic neuropathy, loss of vision, endocrine dysfunction, and death. To better understand the pathophysiology of chronic cyanide exposure and to aid in development of medical countermeasures, animal models have been developed to simulate the different routes of exposure and subsequent insults that result from chronic cyanide exposure.

Several animal models have been developed to characterize chronic cyanide exposure. Models delivering cyanide by injection or infusion, by ingestion in food or drink, by gavage, or by repeated inhalation of pyrolysis products have been developed to recapitulate the modes through which chronic cyanide exposure is known to take place. Lesions to the central nervous system and thyroid are seen consistently, which damage to the liver and heart is frequently present. Studies indicate cyanide exposure is detrimental to fertility and fetal development. These chemical insults are similar across the different modes of administration. The literature on the diabetes risk associated with chronic cyanide exposure is less clear.

Several strategies are available to mitigate the risks of cyanide exposure. New lines of cassava are being

generated with lower cyanogen profiles and increased sulfur-containing amino acid content. Properly processing cassava has been shown to reduce cyanogenic content of the roots to safe levels, even in cultivars known to be rich in cyanogenic glycosides. Personal protective equipment has been developed to help prevent occupational exposure to cyanide. Point-of-care detection of cyanide exposure and next-generation cyanide medical countermeasures are needed to rapidly identify and treat cyanide exposure victims, especially in cases of acute cyanide exposure.

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CHAPTER 3

Physicochemical properties, synthesis, applications, and transport

David E. Thompson and Ilona Petrikovics

At a Glance

- HCN can be produced by natural abiotic synthesis and is hypothesized to have been a prebiotic precursor for the formation of certain amino acids and nitrogen compounds important for life.
- Cyanide is produced biotically and used naturally by various plant and animal species.
- Cyanogenic glycosides are present in a variety of foodstuffs, particularly cassava which is a dietary staple for millions of persons.
- There are a number of chemical processes for producing cyanide and its salts, both for large-scale industrial uses and small-scale laboratory uses.
- One major use is in mining for the separation of gold and silver from ores (~10% of total commercial cyanide production).
- Another major industrial use (~90% of total commercial cyanide production) is in organic synthesis.
- Soil microorganisms have a role in cyanide bioremediation.

3.1 Introduction

The term cyanide commonly refers to the cyanide anion, CN^-; to compounds that release CN^- into aqueous solution, including most prominently hydrocyanic acid (HCN), salts of CN^- such as sodium cyanide (NaCN) and potassium cyanide (KCN); and to complexes that form when CN^- ligands bind a transition metal ion. Abiotically produced cyanide is a naturally occurring component of the universe that is thought to have played a key role in the formation of the first terrestrial amino acids. Cyanide is also produced by many plants for purposes of nitrogen storage and self-defense. It was via plants such as peaches and cassava tubers that humans first encountered cyanide. Procedures were developed to isolate foods from cyanide so that foods could be safely eaten, and to concentrate the poison via extraction and distillation for purposes of execution or assassination. The scientific discovery of cyanide and its structure arose, not from studies on bio generated cyanide, but from basic chemistry investigations of a synthetic blue pigment. As chemical investigation progressed, it became clear that cyanide was a ligand that could bind and solubilize gold. This made cyanide a valuable commodity in the mining of noble metals. To meet this industrial demand, methods were devised to synthesize cyanide in industrial quantities.

Cyanide was subsequently found to serve as an effective building block in organic chemistry with a wide variety of applications. Inevitably some of this industrially produced cyanide finds its way back into the environment. This chapter closes with a brief discussion of the environmental transport properties of cyanide.

3.2 Natural sources of cyanide

3.2.1 Natural abiotic synthesis of cyanide

Radio astronomical observations have shown that HCN is abundant in the cosmos. HCN was first detected in

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the interstellar medium (Huebner *et al*., 1974). It can be observed from ground-based telescopes through a number of atmospheric windows (Treffers *et al*., 1978). Other abiotic sources of environmental cyanide include volcanic eruptions, and lightning induced reactions. Through reactions with itself and other small organic molecules, HCN is hypothesized to have been a prebiotic precursor for the formation of adenine, guanine, and other simple nitrogen compounds that are important to life (Oro and Lazcano-Araujo, 1981; Marrs and Ballantyne, 1987).

3.2.2 Biosynthesis of cyanide (Plant, fungi, bacteria, and animal synthesis of cyanogenic glycosides and lipids, and cyanide)

Cyanide compounds are produced and used naturally by various plant- and animal- species including bacteria, fungi, and algae as an important defense mechanism against herbivores (Møller, 2010). More than 200,000 different bio-active molecules (also called secondary metabolites) are known and identified in plants (Zagrobelny *et al*., 2008). Cyanogenic glycosides are bio-active molecules that liberate HCN as a defensive mechanism in response to the damaging of plant tissue by herbivores and pathogens (Lieberei *et al*., 1989). They have been reported to be present in more than 2500 different plant species, including ferns, gymnosperms and angiosperms. The conserved structure and widespread distribution of cyanogenic glycosides suggests shared and ancient genetic roots (Zagrobelny *et al*., 2004).

Cyanogenic glycoside plant defenses provide sophisticated mechanisms of protection against consumption of various parts of the living plant (fruit, leaves, etc.), and against consumption of the seeds carrying the genetic material for the next generation. Cyanogenic glycosides (alpha-hydroxynitrile glycosides) are derived from the amino acids Val, Ile, Leu, Phe, Tyr and the amino acid derivate cyclopentenyl glycine (Møller, 2010). The approximately 75 documented cyanogenic glycosides are all O-beta-glycosidic derivatives of alpha-hydroxyl-nitriles (however, some plants have beta-and gamma-hydroxynitrile glycosides). Key enzyme classes participating in the biogenesis of cyanogenic glycosides are Cytochrome P450s and glycosyltransferases (Andersen *et al*., 2000; Kahn *et al*., 1997; Sibbesen *et al*., 1994). The cyanogenic glycosides are stored harmlessly in a compartment within the cells. Following tissue disruption, the cyanogenic glycoside comes into contact with beta-glycosidases and a-hydroxynitrile lyases which liberate HCN (Zagrobelny *et al*., 2007).

In the cyanogen monosaccharide the cyanohydrin moiety is connected to a single sugar residue by a glycosidic bond. In the cyanogen disaccharides (e.g., Amygdalin, Vicianin, linustatin) or trisaccharides (e.g., xeranthin), two or three sugar moieties are connected to the cyanohydrin moiety. Sulfated, malonylated, and acylated derivatives of cyanogen glycosides are also known. Cyanogenic lipids contain instead of sugars, long chain fatty acids. Cyanolipids occur in the seed oils of many sapindaceous plants, and structurally they originate from leucine (Mikolajczak, 1977). Higher plants are adversely affected by cyanide through cytochrome oxidase inhibition. Non-acclimatized soil bacteria are also adversely affected at 0.3 mg HCN/kg; acclimatized populations can degrade waste containing up to 60 mg total cyanide per kg (Eisler, 2000). Certain soil bacteria and fungi can also produce cyanide as part of their defense mechanisms (Towill *et al*., 1978).

Although these chemical defenses work well against occasional predators, certain herbivores have developed methods to overcome these defenses. Some have even evolved means to use these chemicals to their advantage (Gleadow and Woodrow, 2002). Among Chilopods (centipedes), 7 out of the 3,000 species are able to produce cyanogenic glycosides or sequester them from their food. Forty-six out of the 7,500 species of Diplopods (millipedes) and 68 out of the 750,000 species of Insecta (insects) are able to do the same (Dzombak, 2005). These species are able to metabolize cyanogenic glycosides or sequester them for use in their own defense. This also allows these species to ingest cyanogenic plants as a food source (Møller, 2010). Among the millipedes, little is known about their process of metabolism, but it is thought that their process mirrors that of plants in that a precursor is processed to produce a cyanogenic glycoside. Insecta, however, are able to manufacture cyanogenic glycosides from certain amino acids such as Val and Leu. They can also convert consumed cyanogenic glycosides into other cyanogenic glycosides (Dzombak, 2005). It is estimated that cyanogenesis has been used as a plant defense mechanism for about 430 million years (Zagrobelny *et al*., 2008). That is enough time for certain invaders, such as fungal pathogens (Fry and Evans, 1977) to develop tolerance against HCN. They have the ability to form formamide from HCN when catalyzed by formamide hydrolase. This formamide can then be hydrolyzed to produce ammonia, a source of

reduced nitrogen (Møller, 2010). The same organism (*Chromobacterium Violaceum*) can also produce (Nazly *et al*., 1981) and degrade (Rodgers, 1981) cyanide. Cyanide production was reported from glycine by snow mold fungus (Bunch and Knowles, 1981) and by algae (Vennesland *et al*., 1981). There are also at least 45 known species of Zygaenidae (burnet moths) that are able to produce the cyanogenic glycosides linamarin and lotaustralin. They also sequester cyanogenic glycosides from their food (Zagrobelny *et al*., 2007). Their larvae use this combination of consumption and production to keep a constant ratio between the cyanogenic glycosides despite variations in the cyanogenic content of their diet. Before mating, the females secrete HCN to attract males and are in turn attracted to males with high cyanogenic glycoside concentrations. The males then give the female a nuptial gift including cyanogenic glycosides during mating (Møller, 2010). Bacteria are also able to neutralize cyanogenic glycosides. They can react HCN with cysteine to form β-cyanoalanine when catalyzed by β-cyanoalanine synthase. They then convert this toxic β-cyanoalanine into aspartic acid, via the NIT4-type nitrilase enzyme, which can be used as a nitrogen source (Møller, 2010).

In contrast to the taxonomically widespread distribution of cyanogenesis in the plant and moderate distribution in the arthropod kingdom, the production of HCN by animals is highly restricted (Duffey, 1981). One animal with the known ability to neutralize cyanogenic glycosides is the golden bamboo lemur. It consumes about 12 times the dose of HCN that is lethal to other animals per day yet suffers no ill effects. It is not known, however, how the golden bamboo lemur is able to survive these large quantities (Møller, 2010), but this ability may have allowed the golden bamboo lemur to achieve a greater geographic distribution than similar bamboo lemur species that lack this capacity to eat cyanogenic plants (Gleadow and Woodrow, 2002). Grazing animals including cows, sheep, donkeys, horses, and chickens are sensitive to HCN, but the degree of sensitivity differs between species (Towill *et al*., 1978).

3.3 Isolation and characterization of cyanide

3.3.1 Early human encounters with cyanide from plant sources

Low levels of cyanogenic glycosides are present in many human foods (e.g., apricots, bean sprouts, cashews, cherries, chestnuts, corn, kidney beans, lentils, nectarines, etc.). A few dietary plants such as the bitter almond and the cassava root (or manioc) have sufficiently high HCN content to be rendered inedible without processing (Vetter, 2000; Jones, 1998). Standard cooking preparation for food containing cyanogenic glycosides includes HCN removal by heat or by hydrolysis before consumption. HCN has been reported causing deaths from diet.

Before the molecular nature of the cyanide poisoning was understood humans identified these cyanogenic plants as poisonous and with time developed strategies to avoid and to concentrate the cyanide. For example, bitter almond extract was employed medicinally, but had severe side effects when used in small doses and was deadly when used in larger doses (Ahmad, 2010; Sykes, 1981).

3.3.2 Scientific discovery and structure determination of cyanide

It was not through studies of cyanogenic food stuffs, medicines or poisons that cyanide came to be chemically identified. The modern scientific discovery of cyanide arose serendipitously from chemical study of the pigment "Prussian Blue," $Fe(III)_4[Fe(II)(CN)_6]_3$. Prussian blue was first accidentally produced and isolated by the paint maker Diesbach in Berlin in 1706, in a failed attempt to produce the red pigment carmine from cochineal insects (Coleby, 1939). Its excellent properties as a pigment enabled Prussian Blue to come into wider use. In 1752, the French chemist Pierre Macquer demonstrated that Prussian Blue could be oxidized to yield a gas and iron oxide, and that the reverse reaction yielded Prussian Blue (Macquer, 1752). In 1782, the Swedish chemist Carl Wilhelm Scheele generated the same volatile gas by heating Prussian blue in the presence of dilute sulfuric acid, realized that the gas was combustible, trapped it in aqueous solution, and found that the dissolved gas rendered the solution acidic (Clenell, 1910). In 1811, Claude Louis Berthollet demonstrated that the same gas was distinct from other known acids because it did not contain oxygen (Clenell, 1910). Joseph Louis Gay-Lussac condensed the acidic gas into a light blue liquid in 1811, and determined its chemical formula to be HCN in 1815 (Crosland, 1978). In recognition of these seminal experiments and the blue color of the condensate, the Greek word cyan, meaning blue, forms the root of the names for HCN (hydrocyanic acid), CN^- (cyanide anion), and NCCN (cyanogen). The blue color was later found to be due,

Table 3.1 Physicochemical properties of cyanide and compounds (Homan, 1987; ChemSpider, 2014; HSDB, 2014).

Properties	Hydrogen cyanide	Sodium cyanide	Potassium cyanide	Calcium cyanide	Cyanogen	Cyanogen chloride
Molecular weight	27.03	49.01	65.11	92.112	52.04	61.47
Color	Colorless	White, colorless	White, colorless	White	Colorless	Colorless
State (~ 25°C)	Liquid/Gas	Solid	Solid	Solid	Gas	Gas
Melting point, °C	−13.4	563.7	634.5	Decomposes at > 350°C	−27.9	−6
Boiling point, °C	25.60	1496	No data	–	−21.1	12.8
Density, g / cm^3	0.687 (Liquid)	1.60 (Solid)	1.55 (Solid)	1.853 (Solid)	–	–
Odor	Faint bitter almond odor	Faint bitter almond odor in damp air	Faint bitter almond odor in damp air	Faint bitter almond odor in damp air	Almond like odor	Highly irritating
Water Solubility	Miscible	Soluble	Soluble	Soluble	Soluble	Soluble
Organic Solvents Solubility	Soluble in ethanol, ether	Slightly soluble in ethanol and ether	Slightly soluble in ethanol; Insoluble in ether	soluble in ethanol; Insoluble in ether	Soluble in ethanol and ether	Soluble in ethanol and ether

Sources: Data were compiled from the Royal Society of Chemistry's ChemSpider website, available at www.chemspider.com; the Hazardous Substances Database of the National Library of Medicine (US), available at http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm; and Homan 1987.

not to cyanide itself, but rather to small amounts of iron impurity that had been complexed by cyanide.

3.3.3 Physicochemical properties of cyanide and cyanogenic compounds

HCN is a linear molecule, with molecular weight of 27.03 g/mol that consists of one hydrogen, carbon and nitrogen covalently bonded together with the carbon in the middle. Pure HCN is colorless in condensed form, melts at −13.24°C and boils at 25.7°C under atmospheric pressure. The vapor pressure of HCN is 107.6 kPa at 27.2°C. Although HCN gas is colorless, most humans are able to recognize its bitter, almond-like odor (Maxwell, 2007). HCN is soluble in water, alcohol, and slightly in ether. Additional physicochemical properties of HCN, common CN salts, cyanogen, and cyanogen chloride are provided in Table 3.1.

In pure water, HCN behaves as a weak acid, slightly dissociating to yield a proton and the cyanide anion. The extent of dissociation can be controlled by buffering the solution. At a buffered physiological pH of 7.4 the ratio of HCN to CN^- is about 63:1. The two forms of cyanide (HCN and CN^-) rapidly interconvert in aqueous environments at physiological pH. Due to its small size and nonionic character, HCN readily crosses cell membranes, including the blood brain barrier, and readily diffuses out of aqueous solution into the air. The negative charge on the cyanide anion, CN^-, greatly reduces its ability to diffuse across biological membranes, or out of solution as a vapor. Its negative charge, compactness, and ability to engage in π-bonding enable the cyanide anion to form stable complexes with many transition metals. Important classes of transition metal cyanide complexes include: hexacyanides $[M(CN)_6]^{3-}$ (M = Ti, V, Cr, Mn, Fe, Co) which exhibit an octahedral geometry; tetracyanides, $[M(CN)_4]^{2-}$ (M = Ni, Pd, Pt) which exhibit a square planar geometry and dicyanides $[M(CN)_2]^-$ (M = Cu, Ag, Au), which are linear in geometry (Sharpe, 1976). The ability of the cyanide anion to bind transition metals in biological enzymes leads to its toxicity.

3.4 Industrial production of cyanide

3.4.1 Hydrocyanic acid synthesis

Industrial cyanide production generally begins with the synthesis of hydrocyanic acid. The HCN synthesized is used in subsequent reactions to produce cyanide salts. These serve as reactants in the formation of many industrial products. The most important industrial syntheses of hydrocyanic acid depend upon the following raw materials: natural gas and water as sources of hydrogen; natural gas and petroleum as sources of carbon; and air

as a source of nitrogen. The first step in the generation of cyanide involves reacting methane, from natural gas, with water to generate hydrogen gas in the hydrogen shift or steam reforming reaction:

$$CH_4(g) + H_2O(g) \rightleftharpoons CO(g) + 3H_2(g) \tag{3.1}$$

The hydrogen gas is then reacted with molecular nitrogen from the air, via the Haber-Bosch process, to yield ammonia:

$$3H_2(g) + N_2(g) \rightleftharpoons 2NH_3(g) \tag{3.2}$$

This ammonia is then reacted with a hydrocarbon in the presence of oxygen or in a high temperature reactor to yield the target hydrocyanic acid and either water or hydrogen according to the following unbalanced generic reactions:

$$NH_3(g) + RCH_3 + O_2 \rightleftharpoons HCN(g) + H_2O$$
$$NH_3(g) + RCH_3 + Heat \rightleftharpoons HCN(g) + H_2 \tag{3.3}$$

Due to the demand for cyanides for mining operations in the 1890s various industrial methods were investigated for large-scale production of HCN. In earlier years it was produced by the reaction of powdered coal with ammonia at 1250°C with low HCN yield. Detailed studies indicated that HCN was produced via a reaction between ammonia and the hydrocarbons in the coal (Johnson *et al.*, 1968). Coals with higher volatile matter contents gave higher yields of HCN. This theory was confirmed by the observation that yields of HCN from reaction of ammonia with gas mixtures containing methane are directly related to the methane content of the gas mixture. An important step forward involved a process in which reacting sodium amide ($NaNH_2$) formed from the reaction of ammonia and sodium was reacted with charcoal, at 850°C. This former method gave high yields of sodium cyanide that could be subsequently converted to HCN by further reaction with sulfuric acid (Wittcoff *et al.*, 2013).

The two largest modern industrial sources of cyanide are the Andrussow process (AP), and the cyanogenic ammoxidation of propene (CAP) process. The CAP process is an unintended side reaction in the production of acrylonitrile. Other industrially significant cyanide synthesis methods include the Degussa hydrocyanic methane ammonia process (DHMA), and the Shawinigan process (SP). As shown below, all of these reactions follow the generic ammonia–hydrocarbon reaction scheme.

$$\begin{aligned}
AP: \quad & NH_3(g) + \underbrace{CH_4(g)}_{\substack{methane\ from\\ natural\ gas}} + 1.5O_2(g) \\
\rightleftharpoons \quad & HCN(g) + 3H_2O(g) \\
CAP: \quad & 3NH_3(g) + \underbrace{H_2C=CHCH_3}_{\substack{propene\ from\ thermally\\ cracked\ petroleum}} + 3O_2(g) \\
\rightleftharpoons \quad & 3HCN(g) + 6H_2O(g) \\
DHMA: \quad & NH_3(g) + \underbrace{CH_4(g)}_{\substack{methane\ from\\ natural\ gas}} + heat \\
\rightleftharpoons \quad & HCN(g) + 3H_2(g) \\
SP: \quad & NH_3(g) + \underbrace{C_3H_8(g)}_{\substack{petroleum\ derived\\ propane}} + heat \\
\rightleftharpoons \quad & HCN(g) + 3H_2(g)
\end{aligned} \tag{3.4}$$

In the Andrussow process methane, ammonia, and oxygen react to form HCN in the presence of a platinum catalyst (commonly alloyed with 10–29% rhodium) at 1000 – 1200°C (Andrussow, 1935). The partial oxidation of methane and ammonia in side reactions provides the energy for HCN formation via the Andrussow process.

The second largest source of industrial cyanide is the manufacture of acrylonitrile (Fugate, 1962). Charles Moureu first synthesized acrylonitrile in 1893. Acrylonitrile began to be widely used in the polymerization of acrylic fibers for textiles and synthetic rubber in the 1930s. In the late 1950s, Jim Idol designed a catalytic process that was successfully implemented by Evelyn Jonak to produce acrylonitrile in about 50% yield from acrolein, ammonia, and air. Acetonitrile and hydrogen cyanide were by-products of this ammoxidation reaction. This research (Branan, 1959) into selective catalytic oxidation revolutionized acrylonitrile manufacturing (Weissermel and Arpe, 1993; Veatch *et al.*, 1960; Idol, 1959).

The Degussa and Shawinigan processes are less widely used. In the Degussa (Endter, 1962) process no oxygen is added, and although the reaction is entropically favored, it is endothermic with a positive reaction enthalpy of 61 kcal mol^{-1}. The reaction mixture of methane and ammonia then flows through a heated

Pt-coated tube reactor, gaining energy to react from the heated walls. This Pt-mediated coupling reaction of methane and ammonia was extensively studied experimentally and computationally (Diefenbach *et al.*, 1999), and served as a model for the industrial production of HCN. In the Shawinigan process, small hydrocarbons react with ammonia.

Other methods used for small scale preparation of HCN include acidifying solutions of cyanide salts, dehydration of amides, and reacting ammonia and carbon monoxide (Wittcoff & Reuben, 1996).

$$\begin{aligned} &\textit{acidification of cyanide salts} : H^+ + NaCN \rightarrow HCN + Na^+ \\ &\textit{amide dehydration} : CH(O)NH_2 \rightarrow HCN + H_2O \\ &\textit{ammonia / carbon monoxide} : CO + NH_3 \rightarrow HCN + H_2O \end{aligned} \tag{3.5}$$

3.4.2 Cyanide salt synthesis

Sodium and potassium cyanide are the most widely used salts of cyanide. These alkali cyanide salts are white, hygroscopic crystalline powders. They are prepared, predominantly, via aqueous reaction of hydrocyanic acid with either sodium or potassium hydroxide. They may also be prepared by heating the appropriate alkali amide with carbon or by melting the appropriate alkali chloride and calcium cyanamide in an electric furnace.

3.5 Applications and uses of cyanide

Cyanide usage in industry can be divided into two broad classes: "inorganic" uses in which cyanide serves as a ligand that forms complexes with transition metals, and "organic" uses in which cyanide serves as a reagent for industrial organic synthesis. The most important inorganic use of cyanide is the extraction of gold and silver from mining ores (Holloway, 1982). Other inorganic uses include metallurgy and pest control. Illicit uses include the stunning of fish in coral reef habitats for subsequent sale to aquarium owners. Historically, inorganic cyanide has also been employed as a chemical weapon and agent of genocide. Although gold extraction created the first large market for cyanide, present inorganic uses consume only about 10% of the total industrially produced cyanide. The remaining 90% serves as a building block in organic synthetic processes including, but not limited to, the manufacture of nylon, acrylic windows (e.g., plexiglass), phosphate free detergents, orthopedic cement, herbicides, fiber reactive dyes, chicken feed supplements, and high performance carbon composite materials.

3.5.1 Applications and uses of cyanide as a ligand of transition metals

Most gold is extracted today from open pit mines (Fleming & Cromberg, 1984). Earth is removed until veins rich in gold are exposed. These ores are then crushed, mixed with cement as an erosion preventative, piled onto plastic sheets at the top of the mine, and sprinkled with a dilute solution of potassium or sodium cyanide (Gonen *et al.*, 2004). In the well-aerated piles the cyanide anion complexes oxidized gold to form soluble anions such as $[Au(CN)_2]^-$ and $[Ag(CN)_2]^-$ as shown.

$$2Au + 4CN^- + {}^1\!/_2O_2 + H_2O \rightarrow 2[Au(CN)_2]^- + 2OH^- \tag{3.6}$$

The gold bearing water flows under gravitational pull toward a "pregnant" pool at a lower elevation in the mine. From here it is pulled into a tank where zinc is introduced and reduces the gold, displacing the cyanide. The gold and other metals precipitate and they are trapped as the solution is pumped through a filter. The metal laden filter is removed, placed in a crucible, and fired in a furnace. All the metals melt, and gold, being the densest, sediments to the crucible bottom. The crucible is allowed to cool, the block of metals removed, flipped upside down, and the gold is retrieved from the top of the block. Silver is mined in a similar manner (Xie and Dreisinger, 2007). However, when silver is found already oxidized as a sulfide, the overall complexation reaction does not involve oxygen:

$$Ag_2S + 4CN^- \rightarrow 2Ag(CN)_2{}^- + S^{2-} \tag{3.7}$$

Cyanide salts are also used for illicit capture of aquarium fish (Dee *et al.*, 2014). The salts are placed in the water where they kill and stun fish. Stunned fish can be easily captured, and those that survive exported for sale as aquarium fish. Cyanide salts are also used as hardening agents that work by adding carbon to technical steel (Gail *et al.*, 2012). Potassium ferrocyanide is used as an anti-caking agent in salts (Nguyen *et al.*2012). Cyanide fumigation has been used for insect and rodent control in grain silos, ships, and on humans at border crossings (Christianson, 2010).

Cyanide pellets have been employed for population management of mammals (Blackie *et al*., 2013).

Sodium nitroprusside $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ is used in emergency medicine and in vascular research to rapidly decrease blood pressure (Tinker and Michenfelder, 1976). The nitroprusside anion releases nitric oxide, causing vasodilation. While it is an effective blood pressure lowering drug, care must be taken due to the associated liberation of cyanide (Lockwood *et al*., 2010). Sodium nitroprusside dissolves in water to yield sodium ion, nitroprusside anion, and water:

$$Na_2[Fe(CN)_5NO] \cdot 2H_2O \rightarrow 2Na^+ + Fe(CN)_5NO^{2-} + 2H_2O \quad (3.8)$$

Cyanide is used as a complexing ligand in the artificial production of vitamin B12. Cyanocuprol (a copper cyanide compound) was briefly used in Japan, during World War I, as a tuberculosis and leprosy treatment (Takano, 1916).

The ability to complex transition metal centers in enzymes confers upon cyanide its poisonous nature. In World War II the Nazis made extensive use of cyanide gas chambers to perpetrate the mass murders of the holocaust (Baskin, 2001). In the 1980s, Saddam Hussein used various chemical weapons against the Kurds, Syrians, and Iranians some of which caused intoxication symptoms that matched those expected from cyanide exposure (Baskin, 1997). Cyanide-based gas chambers have been used for capital punishment in the United States and elsewhere (Christianson, 2010). Cyanide has been used as an agent of mass suicide, for example, by the followers of Jim Jones in Jonestown, Guyana (Thompson *et al*., 1987), and as an agent of individual suicide, for example, the Liberation Tigers of Tamil Eelam in Sri Lanka carried cyanide pellets that would enable escape via suicide from interrogation and other abuses upon capture (Roberts, 2014).

3.5.2 Applications and uses of cyanide in the synthesis of organic molecules

CN is a strong nucleophile that is readily incorporated into organic molecules as a functional group. Important classes of organic cyanide containing molecules include cyanogen, the nitriles (RCN), the cyanates (ROCN), the isocyanates (RNCO), and the thiocyanates (RSCN).

Cyanogen [$(CN)_2$] is the colorless, flammable dimer of cyanide. The toxicity of cyanogen and its halide derivatives is comparable to HCN (Eisler, 2000). In water, it forms HCN slowly. Cyanogen can be hydrolyzed to yield oxalic acid and ammonia. Common synonyms used for cyanogen include: carbon nitrile, dicyanogen, ethane dinitrile, and oxalic acid dinitrile.

Nitriles [RCN] are formed when the carbon atom in CN forms a covalent bond with a carbon in an organic molecule R. Such compounds may alternately be given the prefix "cyano." Nitrile gloves and superglue are two examples of widespread nitrile based products. Nitriles can be prepared by nucleophilic substitution of CN for a halide group.

$$RX + CN^- \rightarrow RCN + X^- \quad (3.9)$$

Nitriles can also be formed by catalytically mediated reaction of HCN with an alkene. Because the formation of a nitrile adds one additional carbon to an organic molecule, cyanide is called a C-1 synthon. Nitriles can be hydrolyzed to form carboxylic acids by refluxing in aqueous solution in the presence of a mineral acid catalyst.

$$RCN + 2H_2O \rightarrow RCOOH + NH_3 \quad (3.10)$$

Nitriles can be first reduced (e.g., with lithium aluminum hydride under reflux in dry ether), and then reacted with water to form amines.

$$RCN + 0.5LiAlH_4 + (second\ step)2H_2O \rightarrow RCH_2NH_2 + 0.5LiAl(OH)_4 \quad (3.11)$$

Aside from cyanogenic glycosides, nitriles are comparatively innocuous in the environment, low in chemical reactivity and biodegradable (Eisler, 2000). The three smallest nitriles are: ethanenitrile (acetonitrile), propane nitrile, and butane nitrile. These small nitriles are liquids at room temperature, with boiling points of 82°C, 97°C, and 117°C respectively, and water solubilities between 0.1 and 0.03 g/ml.

Cyanogenic nitriles, not to be confused with cyanogen, are nitrile-containing compounds that can liberate free hydrocyanic acid or cyanide anion. For simple mononitriles there is a clear progression, with more cyanide being released as chain length increases. A similar pattern exists in dinitriles, but corresponding compounds require a longer carbon chain than mononitriles before free cyanide is produced. The toxicity of saturated aliphatic nitriles is correlated

with metabolic cyanide liberation. According to Ahmed and Farooqui (1982), the toxicity (in correlation with brain cyanide concentration) decreases in the order of KCN > butironitrile > propionitrile > acetonitrile. However, the degree of cyanide release is not a major factor in the toxicity of unsaturated aliphatic nitriles. Cyanohydrins [$R_2C(OH)CN$] and cyanogenic glycosides [$R_1R_2C(OR_3)CN$] are special classes of nitriles that decompose to HCN and cyanide ions under appropriate conditions. Acrylonitrile, propionitrile, and succinonitrile are examples of synthetic cyanogenic molecules. Acrylonitrile is a clear colorless or slightly yellowish liquid and has a characteristic pungent odor. Acrylonitrile's boiling point is 77.3°C, freezing point is −83.5°C, density is 0.806, and vapor pressure is 11.5kPa at 20°C. Acrylonitrile is highly flammable and gets automatically ignited at temperature of around 480°C. It is photosensitive and extremely unstable and reacts vigorously with acids, bases, oxidizing agents, amines, and bromine (Bradzil, 2004).

Cyanates [ROCN] are formed industrially by the reacting a phenolic hydroxide group with a cyanide containing compound (e.g., cyanogen chloride) in the presence of a base. Alkyl cyanates are insoluble in water and form cyanurates.

Isocyanates [RNCO] are formed from inorganic cyanates, such as NaOCN and KOCN. They are also employed in the manufacture of herbicides, fungicides, insecticides, various dye-intermediates, and different types of isocyanates. Isocyanates are also used as catalysts in acrylic polymer production for keeping up fiber whiteness at certain desired levels.

Thiocyanates [RSCN] are formed from cyanides and sulfur-containing materials and are relatively stable. With Fe^{3+} the SCN^- ion gives a colored complex, $Fe(SCN)_3$, that is used for *in vitro* sulfur donor efficacy determination by a colorimetric method (Westley, 1981). Preparations of thiocyanates are reported (Guy, 2010) by the reaction of isothiocyanic acid or its salts with organic compounds; by the reaction of thiocyanogen or related reagents with organic compounds and by the cyanation of organo-sulfur compounds. Pseudohalide reactions of thiocyanates and sulfenyl cyanide reactions of thiocyanates are also reported (Guy, 2010). When ammonium thiocyanate reacts with acids of moderate strength (e.g., 40% hydrochloric acid), isoperthiocyanic acid ($C_2S_3(NH)_2$) (3-amino-5-thione-1,2,4,-dithiazole) is formed (Johar *et al*., 1972).

3.6 Environmental transport of cyanide

The widespread use of cyanide in industry, and the presence of cyanide precursors in fires, and combustion engines leads to the release of some cyanide into the environment. The majority of the atmospheric cyanide is released unintentionally from automobile exhaust and from industrial sources including mining operations, chemical processing, petroleum refineries, steel mills, and solid waste incinerators (Sorokin, 1993). The burning of nitrogen containing organic materials such as wool, silk, polyacrylonitrile, nylon, polyurethane, and paper in wildfires also releases HCN into the atmosphere. Amounts liberated vary considerably according to conditions of combustion (Pauluhn, 1992), and can constitute an important route of cyanide poisoning (Baud *et al*., 1991).

Cyanide discharged via these processes enters the atmosphere mainly as gaseous HCN, and in smaller amounts as fine dust particles, that can settle over land and water, especially with the help of rain and snow. The half-life of HCN in the atmosphere generally varies from around 30 days to 1 year (Way, 1981), however, in certain conditions it can persist for up to 11 years (Marrs and Ballantyne, 1987). Gas phase remediation strategies include biological oxidation, catalytic oxidation, or thermal treatment (Baker and Chou, 1984).

The major sources of cyanide contamination in water are mining, metal finishing, iron and steel making, organic chemical manufacturing, and water treatment (which may be downstream from cyanide releasing industries). Cyanide can also enter waterways from agricultural runoff, runoff from roads which are salted in winter, and atmospheric fallout and washout (ATSDR, 1997; Fiksel *et al*., 1981; EPA, 1980). Cyanide discharges into water have severe consequences. For example, on January 30, 2000, an embankment of the sedimentation reservoir broke open along a 25–30m section in the Romanian-Australian goldmine refuse reprocessing plant of the AURUL Company. As a consequence, about 100,000–120,000 cubic meters of cyanide and heavy metal containing slop water was discharged into open waterways. This contamination traveled through the Zazar, Lapus, and eventually the Somes (Szamos) rivers until it reached the Hungarian Tisza River. It is believed, that the pollution was the result of human negligence. There were no initial attempts to localize the disaster.

As the result, about 1240 tons of fish were killed, and many other aquatic animals perished in the river Tisza in Hungary. The contamination reached as far as Belgrade and sections of the River Danube in Bulgaria (Soldán *et al.*, 2001; Cunningham, 2005; Batha, 2000).

In water, a fraction of cyanide will naturally dissociate to yield the cyanide anion. The cyanide anion is very effective at complexing and solubilizing heavy metals such as mercury and gold. Once solubilized, hazardous heavy metals such mercury can quickly move up the food chain.

Cyanide may enter potable water systems via their source water or through leaks in the distribution system. Cyanide compounds are generally found in natural water in relatively small amounts. The average daily intake of HCN from drinking water has been estimated to be between 0.4 to 0.7 mg of HCN. This estimate is based on cyanide concentrations in potable water measured by 35 U.S. water utilities (ATSDR, 1997) in 1988. The factors affecting the rate of cyanide volatilization from a body of water include water temperature, pH, wind speed, and cyanide concentration (ATSDR, 1997; EPA, 1992, 1996). The half-life of cyanide in water is a complex function of factors such as buffering capacity, transition metal content, pH, temperature, and so on. In water, cyanide can be biochemically converted into less harmful chemicals by microorganisms, or can form stable complexes with most transition metals which may be present in water. Some cyanide complexes, such as $Zn(CN)_2$, dissociate relatively easily, while others, such as iron, cobalt, nickel, and copper cyanides (Towill *et al.*, 1978) are very stable. Some transition metal complexes, such as ferri- and ferro-cyanides, bind cyanide sufficiently strongly that, even when dissolved in water, they release cyanide only in the presence of UV light (Marrs and Ballantyne, 1987).

Cyanide ions mimic halide ions in their reactivity patterns and are sometimes referred to as "pseudo halide" ions. Both the silver halides and cyanides are almost insoluble in water. The alkali metal cyanide salts (e.g., KCN, NaCN), and halogen salts (e.g., KCl and NaCl) are highly soluble in water. Bringing such salts into contact with water or moist air results in the release of HCN (HSDB, 2005a, 2005b). The dissolving reaction itself is exothermic and the heat released upon dissolving accelerates the partitioning of HCN fumes into the air. Inhalation of cyanide salt dust brings the salts into contact with water in the nasal and lung mucosal fluids where they are dissolved to yield CN^-. The salts may also react with water in humid air and be inhaled as HCN. In both cases, there will be systemic absorption of cyanide. Cyanide salts generate HCN in water according to the following equations:

$$MCN + H_2O \rightarrow HCN + M^+ + OH^-(M = Na, K)M(CN)_2$$
$$+2H_2O \rightarrow 2HCN + M^{2+} + 2\,OH^-(M = Ca) \qquad (3.12)$$

The cyanide complexes (Table 3.2) exert different chemical and toxicological properties vs. the simple cyanides.

The stability of cyanide complexes in solution depends on the type of cations and the complex anions. Some cyanide complexes undergo reactions with weak acids to yield HCN and a salt, while others require stronger acids for HCN liberation. The most stable cyanide complexes include $Fe(CN)_6^{4-}$ and $Co(CN)_6^{4-}$; moderately stable complexes include $Cu(CN)_2^-$, $Cu(CN)_3^{2-}$, $Ag(CN)_2^-$, and $Ni(CN)_4^{2-}$; and least stable complexes include

Table 3.2 Known and characterized cyano complexes (Sharpe, 1976).

Metal	Selected spectroscopically characterized cyano complexes of the transition metals
Chromium	$K_6[Cr(CN)_6]$, $K_3[Cr(CN)_6]$
Molybdenum	$K_4[Mo(CN)_6]$, $K_2[Mo(CN)_5]$, $K_4[Mo(CN)_7]$, $K_4[Mo(CN)_8]$, $K_3[Mo(CN)_8]$
Manganese	$K_5[Mn(CN)_6]$, $K_4[Mn(CN)_6]$, $K_3[Mn(CN)_6]$, $K_2[Mn(CN)_6]$
Iron	$K_4[Fe(CN)_6]$, $K_2Fe^{II}[Fe^{II}(CN)_6]$, $KFe^{III}[Fe^{II}(CN)_6]$, $Fe^{III}[Fe^{III}(CN)_6]$, $K_3[Fe(CN)_6]$
Cobalt	$K_8[Co_2(CN)_8]$, $K_3[Co(CN)_4]$, $K_3[Co(CN)_5]$, $K_3[Co(CN)_6]$
Copper	CuCN, $K[Cu(CN)_2]$, $K[Cu_2(CN)_3]$, $K_3[Cu(CN)_4]$
Silver	AgCN, $K[Ag(CN)_2]$, $K_3[Ag(CN)_4]$
Gold	AuCN, $K[Au(CN)]_2$, $K[Au(CN)_4]$

$Zn(CN)_4^{2-}$, $Cd(CN)_3^-$, and $Cd(CN)_4^{2-}$. The release of cyanide from transition metal cyanide complexes can be accelerated by decreasing pH, increasing temperature, irradiating with UV light, or by lowering the oxidation state of the metal (Beck, 1987). However, as mentioned above, the alkali metal cyanide salts NaCN and KCN dissociate virtually completely when dissolved in water.

Alkaline chlorination, in which cyanogen chloride is hydrolyzed to cyanate ion (CNO^-), is the most important method for removing cyanide from wastewaters (Eisler, 2000). If free chlorine is present, CNO^- is further oxidized (Marrs and Ballantyne, 1987; Way, 1981). Other cyanide removal methods are ozone treatment, activated carbon absorption, ion exchange, and bioremediation with cyanide-metabolizing bacteria (Towill *et al*., 1978; Way, 1981; Marrs and Ballantyne, 1987).

Soil is a natural bio membrane, which hosts complex and diverse microbial populations and acts as a source or sink for most gases including HCN. Many natural and anthropogenic activities add cyanide to soil (Salkowski and Penney, 1994). Cyanides in contaminated soils can escape by evaporation, be degraded by microorganisms, or trapped in stable transition metal complexes. Diffusion of HCN gas is the primary mechanism of cyanide transport through soil.

The rate of cyanide migration in soils is influenced by a wide variety of factors including soil water content, soil pH, soil porosity, organic matter content, density, and clay content as well as atmospheric conditions such as barometric pressure, humidity, and temperature (Chatwin and Trepanowski, 1987).

Fortunately, a number of catabolic pathways for cyanide can be found in many eukaryotes and prokaryotes (Baxter and Cummings, 2006). Bioremediation of cyanide by soil microorganisms under aerobic conditions yields products such as ammonia, carbon dioxide, and nitrate. Bioremediation under anaerobic conditions commonly yields products such as ammonium ion, nitrogen, thiocyanate, and carbon dioxide. Ammonia generated by cyanide bioremediation can in turn be anaerobically converted to nitrogen by many species of ammonia oxidizers and nitrate-reducing or denitrifying organisms. Cyanide can also be tied up in stable complexes with transition metals (Rouse and Pyrih, 1990; Towill *et al*., 1978; Marrs and Ballantyne, 1987). Thus, anaerobic cyanide degradation is a promising approach for bioremediating cyanide spill sites (Ubalua, 2010). Some notable soil microorganisms capable of cyanide degradation include fungi species such as *Fusarium solani*, *Stemphylium loti*, and a *Pholiota* sp. as well as bacteria species such as *Corynebacterium*, *Arthrobacter, Bacillus*, *Thiobacillus*, *Pseudomonas*, *Klebsiella*, and *Escherichia* (Towill *et al*., 1978; Knowles, 1988; Silva-Avalos *et al*., 1990). At high enough concentrations cyanide becomes toxic to many bioremediating microorganisms. In such cases, remediation can be accelerated by blending cyanide contaminated soils with uncontaminated soils.

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CHAPTER 4

Cyanide metabolism and physiological disposition

Gary E. Isom, Joseph L. Borowitz, and Alan H. Hall

At a Glance

- The primary route of cyanide elimination is sulfuration to thiocyanate mediated enzymatically by rhodanese and MST.
- The availability of endogenous tissue stores of sulfur sulfane donors is rate limiting.
- Administration of sulfur sulfane antidotes can enhance the rate of cyanide elimination.
- Small amounts of cyanide can be converted by minor pathways to non-sulfurated products.
- Cyanide elimination follows first-order kinetics with an elimination half-life dependent on dose and presence of antidotes.
- Several diseases are associated with altered metabolism in which the cyanide load exceeds the availability of sulfur substrate or detoxification capacity.

4.1 Introduction

Cyanide is eliminated from the body by multiple pathways, including pulmonary exhalation, urinary excretion, and biotransformation. The primary route for detoxification of cyanide is biotransformation by enzymatic conversion to thiocyanate (SCN^-). In humans, cyanide metabolism is complex and involves several enzymes that have multiple biochemical functions in addition to catalyzing formation of SCN^- and other minor metabolites (Figure 4.1). These enzymatic pathways account for 60–80% of administered cyanide (McMahnon & Birnbaum, 1990). The biochemical and molecular characteristics of these pathways have been reviewed in detail (Isom *et al.*, 2010).

Sulfuration to SCN^- is the main *in vivo* biochemical pathway for cyanide detoxification (Way, 1984). The pathways for cyanide transsulfuration involve both non-enzymatic and enzymatic reactions. Formation of thiocyanate is catalyzed directly by two sulfurtransferase enzymes, rhodanese (thiosulfate: cyanide sulfurtransferase, EC *2.8.1.1*) and 3-mercaptopyruvate sulfurtransferase (EC *2.8.1.2*). Under certain conditions albumin may function as a sulfurtransferase. Two other enzymes, cystathionase γ-lyase and thiosulfate reductase, may participate in cyanide metabolism. Cystathionase γ-lyase (γ-cystathionase, EC *4.4.1.1*) does not directly catalyze oxidation of cyanide to SCN^-, but produces sulfur donor molecules for the sulfurtransferases. Thiosulfate reductase (sulfane reductase) participates in cyanide sulfuration by generating a persulfide, followed by non-enzymatic transfer of sulfur to cyanide. Even though the sulfurtransferases can metabolize cyanide to SCN^-, the enzymes have different substrate specificity, organ distribution, and roles in regulation of cellular sulfur balance (Westley *et al.*, 1983; Cipollone *et al.*, 2007).

Cyanide can be converted to products other than thiocyanate through minor pathways that account for a small portion of administered cyanide (Boxer & Rickards, 1952). Cyanide reacts non-enzymatically with proteins and cellular structural components through carbonyl, aldehyde and keto constituents to form cyanohydrin intermediates, which can undergo degradation to form small molecule metabolites

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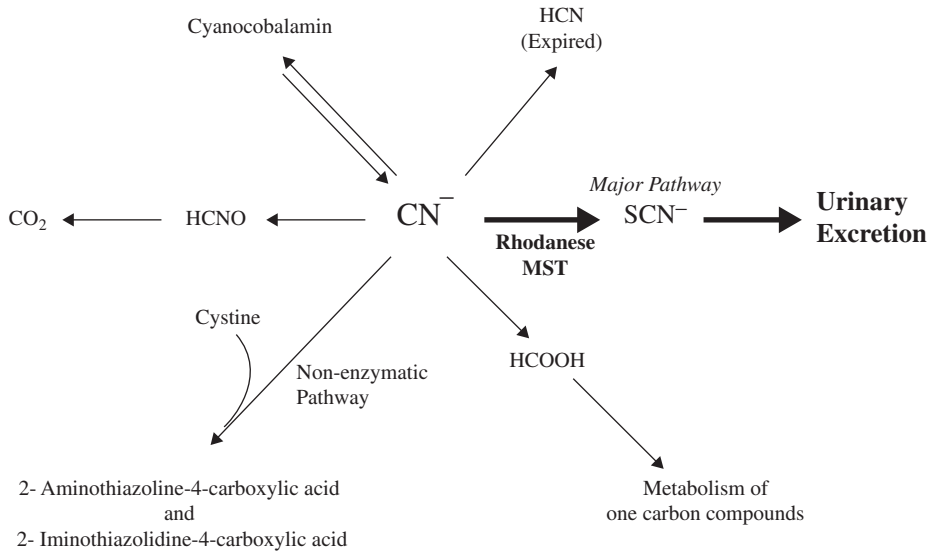


Figure 4.1 Summary of the primary routes of cyanide biotransformation and elimination. The enzymatic conversion of cyanide to thiocyanate (SCN^-), catalyzed by either rhodanese or 3-mercaptopyruvate sulfurtransferase (MST), is the major route of cyanide metabolism that accounts for up to 80% of cyanide elimination under normal conditions.

(Logue *et al.*, 2010). Protein-cyanide adducts have been detected in the plasma fraction of humans. Existence of such adducts in cell cytoplasm has not been demonstrated (Fasco *et al.*, 2007). The most notable non-enzymatic *in vivo* pathway of cyanide elimination involves reaction of cyanide with cystine to form 2-iminothiazolidine-4-carboxylic acid, which is either excreted by the kidneys without further metabolism or undergoes tautomerization to 2-aminothiazoline-4-carboxylic acid and then excreted (Wood & Cooley, 1956). Cyanide can also be oxidized to cyanate/formate and then enters the one carbon pool to form CO_2. Also, 1–2% of administered cyanide is eliminated as CO_2 or HCN by the respiratory tract and in body secretions (Johnson & Isom, 1985). Cyanide (HCN or CN^-) is excreted through three routes, expiration by the lungs, urinary elimination and in saliva. Under normal conditions, elimination of unmetabolized cyanide by these routes accounts for a small percentage of the systemic load.

4.2 Metabolism and toxicokinetics

The primary pathway of enzymatic-mediated detoxification is sulfuration of cyanide to SCN^- (McMahnon & Birnbaum, 1990; Ansell & Lewis, 1970). Transsulfuration of cyanide is rapid and availability of endogenous stores of sulfur donors (sulfane sulfur substrate) is rate limiting. The endogenous sulfur sulfane pool can be rapidly depleted to slow transsulfuration metabolism. The efficacy of sulfur sulfane antidotes, such as sodium thiosulfate, is thought to be the result of replenishing the pool or serving as an exogenous source of sulfane sub-substrate to accelerate cyanide conversion to SCN^- (Way, 1984). Cyanide and thiocyanate are normally in equilibrium and increasing systemic concentration of cyanide increases urinary excretion of thiocyanate. In humans and dogs, thiocyanate can be converted back to cyanide by erythrocytic thiocyanate oxidase (Goldstein & Reiders, 1953) and/or peroxidase (Chung & Wood, 1971).

The rate of detoxification varies by species and is dose-dependent since the endogenous sulfane sulfur co-substrate undergoes rapid depletion at high cyanide levels. In humans the elimination rate of cyanide after intravenous dosing with low cyanide doses is estimated at 0.017 mg/kg/min (Hinwich & Saunders, 1948; Leuschner *et al.*, 1991). Elimination follows first-order kinetics (Baskin *et al.*, 1992) with an elimination half-life varying from 1.2–66 hours, depending on

presence of antidotes and analytical methodology (Hall *et al*., 1987a; Baud *et al*., 1991).

Pharmacokinetic analysis shows that an intravenous bolus of cyanide rapidly distributes throughout the body and distribution fits a 3-compartment model (Djerad *et al*., 2001). In animals not undergoing antidote treatment, the conversion of cyanide to SCN^- is predominantly in the central compartment, with the volume of distribution approximating that of blood volume (Way, 1984). During the initial period (first 60–120 min) following a bolus dose of cyanide, elimination from the blood approximates first-order monocompartmental kinetics and half-life estimated at 18.4–24 min (Sylvester *et al*., 1983). This is followed by a second phase of cyanide elimination from the blood in which the elimination rate slowed to as long as 5.5 hours (Djerad *et al*., 2001). The reduced rate of elimination during the secondary phase is likely accounted for by depletion of sulfane sulfur cosubstrate.

Following acute exposure to cyanide, such as administration of a single bolus dose, elimination is predominantly via enzymatic conversion to SCN^- and dependent on the size of the cellular sulfane sulfur pool (Westley *et al*., 1983; Way, 1984). Under certain dietary deficiencies the sulfane sulfur pool can be reduced and hence cyanide metabolism is reduced. Rhodanese expression can undergo induction during repeated exposure to cyanide (Nandi *et al*., 2000). However pharmacokinetic analysis has shown that the rate of cyanide elimination is unchanged after chronic exposure to maximum tolerated doses (Leuschner *et al*., 1991). It appears that tissue levels of the enzyme are high under normal conditions and increased expression levels does not have a marked, overall influence on the rate of cyanide metabolism.

4.2.1 Toxicokinetics of cyanide

The toxicokinetics of cyanide are poorly understood, and what data are available mostly come from a few experimental animal studies or sparse anecdotal human case reports. *In vitro* studies with dog plasma showed that cyanide is about 60% protein bound (Christel *et al*., 1977). *In vivo*, cyanide concentrates in erythrocytes and whole blood cyanide levels may be four or more times greater than serum levels (Hall & Rumack, 1986).

In dogs, cyanide's volume of distribution (V_d) was 0.498 l/kg (Sylvester *et al*., 1983). In an anecdotal case of human ingestion potassium cyanide poisoning followed by treatment with the nitrite-thiosulfate antidote kit, a similar V_d of 0.41 l/kg was estimated (Hall *et al*., 1987a). Other estimated toxicokinetic parameters in this case were: area under the curve (AUC) 48 μg/ml, clearance 163 ml/min, initial phase half-life $\left(t_{1/2}\right)$ 20–30 minutes, terminal phase elimination $t_{1/2} = 19$ hours (Hall *et al*., 1987a). The terminal elimination phase $t_{1/2}$ is consistent with dog experimental data showing only minimal excretion of cyanide over the first 3 hours after oral administration, although about 95% of such doses were absorbed (Christel *et al*., 1977). Another patient who ingested potassium cyanide had a $t_{1/2}$ of 1 hour during the first 6 hours after ingestion and a $t_{1/2}$ of 6 hours thereafter (Selden *et al*., 1990). In smoke inhalation victims having a significant cyanide poisoning component, the $t_{1/2}$ of cyanide in whole blood was approximately 60 minutes (Baud *et al*., 1991).

The most frequent cyanide exposure route is currently inhalation as a component of enclosed space smoke inhalation (O'Brien *et al*., 2011). For this reason, it has become of interest to perhaps measure cyanide in exhaled breath, but as hydrogen cyanide is highly water-soluble it could be absorbed during inhalation and reabsorbed during exhalation, a so called "washin-washout effect" (Stamyr *et al*., 2008). In 10 normal human volunteers who had a 1 minute inhalation exposure to 10 ppm of HCN (the current US OSHA permissible exposure limit (PEL) for workplace exposures) (NIOSH, 2007), the average $t_{1/2}$ of cyanide in breath was 16 seconds (range: 10–24 seconds), supporting the use of breath monitoring as a potential systemic cyanide poisoning indicator (Stamyr *et al*., 2008).

4.2.2 Toxicodynamics of cyanide

In one of the 1982 Chicago acetaminophen-potassium cyanide tampering victims who was not administered any specific antidotes, the average urinary cyanide elimination over approximately 40 hours was 0.64 mg/hour following a probable ingestion of somewhere between 117 and 511 mg (Hall *et al*., 1987b). In this patient, the mean whole blood cyanide level at 1 hour post-ingestion was 8.2 mg/l, and increased to a mean level of 19.7 mg/l at 3 hours and then to 23.4 mg/l at 9 hours. Although intensive supportive care was provided, no specific cyanide antidotes were administered and the patient died approximately 40 hours after ingestion (Hall *et al*., 1987b).

In an anecdotal case report, a patient who survived a suicidal ingestion of 1 g of potassium cyanide following nitrite-thiosulfate antidotal treatment had a highest measured whole blood cyanide level of 16.58 mg/l at 1.75 hours, which then decreased to 0.82 mg/l at 5 hours (Hall *et al*., 1987b). A patient who survived cyanide poisoning from the cyanogenic nitrile compound, propionitrile, by the inhalation and dermal exposure routes was treated with a combination of hydroxocobalamin and sodium thiosulfate (Bismuth *et al*., 1987). In this case, antidotal treatment was associated with a highest measured whole blood cyanide level of 5.71 mg/l at 2 hours after exposure, decreasing to 0.93 mg/l 30 minutes later. Thus, in anecdotal case reports, specific antidotal treatment has been associated with more rapid decreases in whole blood cyanide levels than in cases when patients have not been administered specific antidotes (Hall, 2008; Hall & Rumack, 1986; Hall *et al*., 1987a; Bismuth *et al*., 1987).

4.2.3 Enzyme-mediated cyanide biotransformation

Early studies demonstrated that administration of cyanide or aliphatic nitriles increased excretion of thiocyanate in the urine (Lang, 1894) and it was subsequently shown that cyanide biotransformation was enzymatically mediated (Lang, 1933). Biochemical characterization of the reaction showed that a heat labile enzyme, with a pH optimum between 8 and 9, catalyzed the reaction. Since the German name for thiocyanate was rhodanid, the enzyme was named rhodanese, in which the *-ese* ending reflects sulfur acceptance. The more conventional enzymatic ending of *-ase* refers to sulfur donation.

Rhodanese and 3-mercaptopyruvate sulfurtransferase (MST) make up the two subfamilies of sulfurtransferase enzymes known to catalyze cyanide metabolism (Sörbo, 1975; Spallarossa *et al., 20*04). Rhodanese is localized to the mitochondrial matrix, whereas MST is both cytosolic and mitochondrial. These enzymes use or produce sulfane sulfur, which is defined as divalent sulfur covalently bonded only to other sulfurs. Rhodanese catalyzes transfer of sulfur from a sulfane sulfur compound to cyanide or other sulfur acceptors (thiophile). MST catalyzes transfer of sulfur from the methyl carbon of 3-mercaptopyruvate to several sulfur acceptors, including cyanide, thiols, sulfite, and sulfinates (Spallarossa *et al*., 2004). MST catalyzed sulfur transfer involves breaking a carbon-sulfur bond, instead of cleavage of the sulfur-sulfur (sulfane) bond by rhodanese. The result is that both enzymes form thiocyanate as the product of cyanide sulfuration, even though both utilize different sulfur donor substrates and catalysis mechanisms.

The comparative involvement of the two enzymes in *in vivo* cyanide metabolism under either basal conditions or following administration of sulfur donor antidotes has not been fully clarified. Traditionally, rhodanese has been considered the primary enzyme responsible for cyanide detoxification (Way, 1984). Recently, it has been suggested that MST and rhodanese function in concert, in which sulfane sulfur substrates are generated with MST-mediated catalysis, followed by transfer of sulfur to cyanide via the rhodanese-mediated reaction to form thiocyanate (Wing *et al*., 1992). Administration of exogenous sulfane sulfur substrates, such as the conventional antidote thiosulfate, increases the substrate pool to facilitate the rhodanese-mediated reaction. Since these enzymes have different tissue and subcellular distribution, it has been proposed that MST may mediate cyanide metabolism in the cytosolic fraction and rhodanese in mitochondria (Nagahara *et al*., 1999).

Alternative routes to sulfuration of cyanide by sulfane sulfur compounds have been characterized, but their overall role in *in vivo* detoxification has not been established. Westley *et al.* (1983) showed that albumin in the blood can transfer sulfane sulfur to cyanide to form thiocyanate. The protein appears to function as an elemental sulfur carrier in which the sulfur-albumin complex can readily react with cyanide. It was proposed that under conditions in which the sulfane sulfur pool is depleted, the sulfur-albumin complex may be the primary route of cyanide detoxification. Other studies have shown that thiosulfate reductase and cystathionase γ-lyase are involved in cyanide metabolism, but their *in vivo* role is questionable (Westley *et al*., 1983).

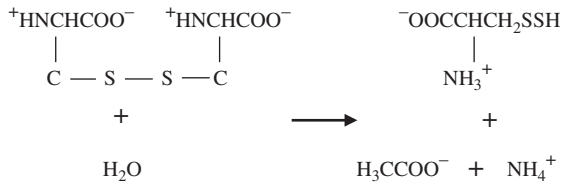
4.2.4 Sulfurtransferase substrate pool

Numerous endogenous compounds can function as sulfane sulfur substrates for the rhodanese-catalyzed reaction, whereas only one compound, 3-mercaptopyruvate, is a substrate for MST (Spallarossa *et al*., 2004). Sulfane sulfur compounds contain a divalent sulfur bonded to another sulfur and exist as organic polysulfides (R^1-S-S_x-S-R^2), organic persulfides (R^1-S-S-),

thiosulfate ($S_2O_3^{2-}$), organic thiosulfonates (RSO_2S^-), polythionates ($O_3S\text{-}S_x\text{-}SO_3^{2-}$) and elemental sulfur in the form of a staggered eight-membered ring (Westley *et al.*, 1983). In attempts to enhance the efficacy of sulfur-based antidotes, a variety of sulfane sulfur derivatives have been synthesized and tested as substrates (Isom & Johnson, 1987).

Tissue levels and chemical forms of endogenous sulfane sulfur undergo dynamic regulation. In humans, the primary site of sulfane sulfur formation is the liver and following synthesis, transport to peripheral tissues occurs by complexation with albumin in the blood. At peripheral sites sulfur compounds are then utilized in the synthesis of iron/sulfur centers (Sörbo, 1955; Westley *et al.*, 1983). In addition to catalyzing metabolism of cyanide, rhodanese is thought to participate in cellular sulfane sulfur pool regulation by rapidly equilibrating the interconversion of sulfanes to form a physiological sulfane pool for utilization in cellular metabolism (Westley, 1981a). The size of the endogenous sulfane sulfur pool determines the rate and extent of cyanide metabolism when antidotes are not administered.

γ-Cystathionase may be indirectly involved in cyanide metabolism by generating cellular sulfane sulfur for the substrate pool. γ-Cystathionase catalyzes cysteine persulfide formation through the asymmetric hydrolytic cleavage of cystine (4.1)



(4.1)

Cysteine persulfide then enters the sulfane sulfur pool to enhance the levels of the rhodanese co-substrate (Flavin, 1962). The physiological significance of the γ-cystathionase-mediated reaction in overall cyanide detoxification is questioned since the tissue concentrations of cystine are normally too low to generate significant levels of sulfane sulfur (Westley, 1981a).

4.2.5 Cyanide metabolism by rhodanese

Rhodanese (thiosulfate: cyanide sulfurtransferase) catalyzed conversion of cyanide to SCN^- is considered the primary enzyme-mediated route for biotransformation under endogenous conditions (without antidotes to accelerate cyanide elimination). Under normal conditions, it is estimated that this route accounts for as high as 80% of the conversion of cyanide to SCN^- (Sousa *et al.*, 2003). However, it is likely that a significant portion of the SCN^- is generated through the MST pathway. Rhodanese has multiple physiological functions in addition to serving as a biotransformation route for cyanide. It is involved in regulation of cellular sulfur flux (Toohey, 1989) and synthesis of iron-sulfur centers in the electron transport chain (Ogata *et al.*, 1989). Rhodanese has been used as a model for enzymatic catalysis and the catalytic mechanism has been characterized in detail (Spallarossa *et al.*, 2004).

Rhodanese catalyzes reactions with the following generalized reaction in which a number of thiophiles (sulfur attracting compounds) in addition to the cyanide anion (CN^-) can serve as substrates (4.2):

$$[RSO_xS^-]^- + \text{thiophilic anion} \rightarrow [RSO_x]^- + \text{thiolated anion} \quad (4.2)$$

For the sulfane sulfur co-substrate, the R is an aryl, or O group; X is an integral from 0 to 2. In the case of cyanide in which thiosulfate serves as the sulfane sulfur donor, the reaction is (4.3):

$$S_2O_3^{2+} + CN^- \rightarrow SO_3^{2+} + SCN^- \quad (4.3)$$

Rhodanese is a ubiquitous enzyme found in both animal and plant kingdoms (Westley, 1981a). Comparative species studies of rhodanese activity show it to be expressed in all phyla of the animal kingdom and in plants, bacteria, and fungi (Westley *et al.*, 1983; Aminlari *et al.*, 2007). Tissue levels of rhodanese are important when evaluating elimination of cyanide and efficacy of sulfane sulfur antidotes in different species. In mammals, rhodanese is generally found in highest concentration in the liver, with significant amounts in kidney, adrenals, and thyroid glands (Drawbaugh & Marrs, 1987; Cipollone *et al.*, 2007). Rhodanese activity in the livers of different species varies in the following order: sheep > cattle > horse > pig > dog ≥ human and is localized in hepatocytes in close proximity to blood vessels (Sylvester & Sander, 1990). It is also localized in epithelial cells around bronchioles of the lungs and in proximal tubules of the kidney. Adult human liver rhodanese activity is lower than most other mammalian species, whereas the dog kidney has similar activity levels to

humans (Aminlari *et al.*, 2007). In humans, the highest rhodanese activity is in kidneys, which is twice that of the liver, followed by lung > brain > stomach > muscle.

Since inhalation is a major route of exposure to HCN, such as in tobacco smoke, activity of rhodanese in the upper respiratory tract has been studied in detail (Dahl, 1989). In rats the nasal maxilloturbinates and ethmoturbinates expressed greater rhodanese activity than the liver. In a comparative study, human nasal epithelium rhodanese exhibited a higher cyanide K_m and a lower V_{max} than the rat nasal enzyme (Lewis *et al.*, 1991). Interestingly, rhodanese activity in maxilloturbinates from non-smokers was higher than smokers. Smoking appears to lower rhodanese activity in the nasal epithelium. Enzyme activity was concentrated in the mucosa of the respiratory tract of the dog, with highest enzyme activity in the larynx, trachea, and bronchioles (Aminlari *et al.*, 1994).

Rhodanese activity of the brain is of interest since this organ is a primary target organ of toxicity and several disease states are associated with deficiencies of brain rhodanese. In comparison to the liver and kidney, human brain rhodanese activity is relatively low and is not considered a major site of cyanide metabolism (Sylvester & Sander, 1990). In a postmortem study of human brain, rhodanese activity was the highest in the thalamus, amygdala, centrum semivovale, colliculus superior, and cerebellar cortex (Mimori *et al.*, 1984). In bovine cerebral cortex, rhodanese immunoreactivity was localized to fibrous astrocytes (Sylvester & Sander, 1990).

4.2.6 Biochemical characterization of rhodanese

Subcellular studies in mammalian liver show that rhodanese is localized to the mitochondrial matrix (Koj *et al.*, 1975; Westley *et al.*, 1983). Localization in the mitochondrial matrix has important implications for substrate availability. Charged sulfane sulfur compounds have low lipid solubility and limited ability partition to the enzyme in the matrix (Isom & Johnson, 1987). It appears that sulfane sulfur compounds are transported into the mitochondrial matrix or in the case of endogenous compounds, synthesized at this site.

Rhodanese is encoded by a nuclear gene and translated on cytoplasmic ribosomes (Sloan *et al.*, 1994). The human gene has been mapped to chromosome 22q13.1 and is made up of three exons with exons 2 and 3 supporting the coding sequence (Billaut-Laden *et al.*, 2006). The protein is imported into the mitochondrial matrix and the NH_2-terminal sequence is necessary for folding and import into the mitochondrial matrix (Trevino *et al.*, 1999). Little is known about post-translational regulation of this enzyme. Studies in human hepatoma cell lines suggest that unlike other mitochondrial proteins, rhodanese is not synthesized as a higher molecular weight precursor (Pallini *et al.*, 1990). Protein kinase C may phosphomodulate rhodanese since the phosphorylated enzyme is inactive and dephosphorylation activates the enzyme (Maduh & Baskin, 1994).

Four isozymes of rhodanese have been characterized electrophoretically (Blumenthal & Heinrikson, 1971; Cannella *et al.*, 1981). Red cell and tissue rhodanese are encoded by separate genes and heterogeneous tissue isozymes may have more than one locus (Whitehouse *et al.*, 1988). Ogata *et al.* (1989) proposed that interconversion of variants by phosphorylation reactions may be involved in regulation of mitochondrial function.

Polymorphism of the human rhodanese gene has been identified in select populations. Scott and Wright (1980) found a high prevalence of an inherited rhodanese variant in two linguistic groups of Athabaskan Indians in Alaska. The variant gene locus was determined to be autosomal. In a study of 50 individuals of French Caucasian origin, eleven polymorphisms were identified of which four mutations were located in the coding sequence of rhodanese (Billaut-Laden *et al.*, 2006). The effect of the polymorphisms on transcriptional regulation and kinetic parameters was moderate; however, due to the limited number of individuals tested, it was difficult to make a definitive conclusion about the *in vivo* functional significance of the polymorphism. Based on these observations, it is possible that in the general population, individuals may display genetic derived deficiencies of rhodanese activity that could lead to altered cyanide metabolism, such as that observed in select disease states or extreme sensitivity to cyanide.

Crystalline rhodanese is a small monomeric sulfurtransferase with a molecular weight of ~32 kDa containing one reaction site per molecule (Hol *et al.*, 1983). The bovine mitochondrial enzyme (Rhobov) is composed of a single polypeptide chain containing 293 amino acid residues (Spallarossa *et al.*, 2004; Cipollone *et al.*, 2007). The primary structure of rhodanese is

highly conserved across species (Miller *et al.*, 1991). The human enzyme has 89.9% and 91.2% sequence similarity with bovine and rat rhodanese. The rat enzyme is 91% identical to bovine and when considering conservative substitutions, they are 98% identical (Weiland & Dooley, 1991).

Based on detailed kinetic analysis, Westley and colleagues (1983) proposed a double displacement mechanism in which the free enzyme reacts with the sulfane sulfur donor to cleave the S-S bond, forming a persulfide-substituted enzyme (ES). The sulfur-substituted enzyme then reacts with the sulfur acceptor substrate (CN^-) to produce the product (SCN^-) and regenerate the enzyme (Figure 4.2). This mechanism was determined by steady-state kinetic studies in which ping-pong initial velocity patterns were observed. The sulfur-substituted form of the enzyme (ES) has been isolated and characterized (Chow *et al.*, 1985).

Kinetically, at pH 5.0 and in phosphate buffer, the rate constant that limits maximum reaction velocity is 300 s^{-1} and corresponds to a molar activity of $1.8 \times 10^4 min^{-1}$ (Westley, 1981a). The K_m for thiosulfate is 4 mM and is equal to the K_s. The equilibrium constant for the overall enzyme-catalyzed forward reaction is greater than 10^{10}. Crystalline rhodanese has a pH optimum between 8 and 9 and temperature optimum of 38–40°C. At optimum pH and temperature, the turnover number is about 2×10^4 molecules of thiocyanate formed per minute per molecule of enzyme. Kinetic studies show that SCN^- can inhibit the enzyme (product inhibition). At pH 5.0, SCN^- is uncompetitive with respect to $S_2O_3^-$ and competitive with cyanide (Chow *et al.*, 1985). Rhodanese is activated by Ca^{2+} and inhibited by di- and tri-carboxylates, α-keto acids, iodine, hydrogen peroxide, zinc, nickel, iron, and ferrocyanide (Volini & Alexander, 1981).

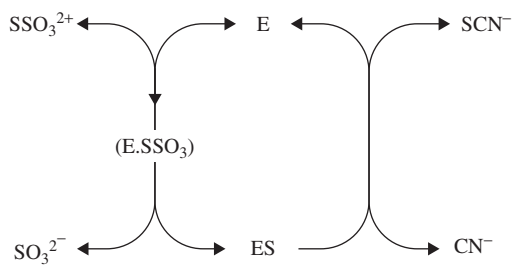


Figure 4.2 Formal kinetic mechanism of rhodanese mediated catalysis with thiosulfate as the sulfane sulfur donor. E, free enzyme; (E-SSO3), enzyme-thiosulfate complex (noncovalent); ES, sulfur-substituted enzyme (reproduced by permission of Academic Press (Westley, 1983: 378).

4.2.7 Exogenously administered rhodanese as a cyanide antidote

In acute cyanide intoxication, a mainstay of treatment is administration of a sulfane sulfur substrate, such as thiosulfate, to accelerate biotransformation of cyanide to thiocyanate. The relative efficacy of exogenous sulfane sulfurs as substrates is lower than expected since these compounds have limited distribution to rhodanese located intracellularly in the mitochondrial matrix. Since rhodanese is localized to the mitochondrial matrix, inorganic, ionized sulfur donor substrates, such as thiosulfate, have a low accessibility to the enzyme. To overcome the pharmacokinetic limitations of the sulfane sulfur substrates, crystallized rhodanese has been administered directly into the bloodstream. The rationale is that both substrate and rhodanese would be in the same compartment (blood) and the efficacy of cyanide detoxication would be enhanced.

Early studies showed that concurrent administration of sodium thiosulfate and purified bovine liver rhodanese significantly decreased the lethality of cyanide in rabbits (Clemedson *et al.*, 1954). Frankenberg (1980) tested the efficacy of combination therapy of rhodanese with two additional synthetic substrates, ethane thiosulfonate, and propane thiosulfonate. The antidotal efficacy was much greater than thiosulfate combined with rhodanese; however, the duration of action was short, thereby limiting the usefulness of this approach. Presumably rhodanese is cleared rapidly from the blood.

To increase duration of action of exogenously administered rhodanese, Way and colleagues (1991) encapsulated bovine rhodanese in resealed, annealed murine erythrocytes. Intravenous administration of encapsulated rhodanese with thiosulfate markedly antagonized cyanide. The duration of this antagonism was longer than that observed following administration of rhodanese directly into the blood. The limitation of this approach appeared to be depletion of thiosulfate over time. In order to extend the duration of activity of the encapsulated rhodanese, Petrikovics *et al.* (1995) used butanethiosulfonate as an alternative sulfur donor to be administered after dosing with erythrocyte encapsulated rhodanese. The rationale was this compound would replenish the sulfur pool in

erythrocytes containing rhodanese since it has higher lipid solubility than inorganic thiosulfate. This approach enhanced the protection from cyanide in mice and appeared to extend the use of exogenous rhodanese as a cyanide detoxification system. The usefulness of exogenously administered rhodanese remains to be determined in humans. A potential limitation of this approach is that the use of bovine rhodanese in humans may produce hypersensitivity reactions, which perhaps could be overcome by the use of recombinant human rhodanese. Recent studies have explored the use of liposome vesicles encapsulating rhodanese as a cyanide antidote (Petrikovics *et al.*, 2009). The antidotal efficacy of this delivery systems has not been reported.

4.2.8 Cyanide metabolism by 3-mercaptopyruvate sulfurtransferase

3-Mercaptopyruvate sufurtransferase (MST, EC 2.8.1.2) catalyzes sulfuration of cyanide with 3-mercaptopyruvate serving as the sulfur donor, yielding pyruvate and thiocyanide (4.4).

$$^{-}OOCCCH_2SH \quad (=O) \quad + \quad CN^{-} \quad \rightarrow \quad SCN^{-} \quad + \quad ^{-}OOCCCH_3 \quad (=O) \qquad (4.4)$$

In addition to metabolizing cyanide, MST is involved in regulation of cysteine and metahionine and post-transcriptional tRNA sulfuration (Spallarossa *et al.*, 2004). Phylogentically, it is widely distributed in eukaryotes and prokaryotes (Westley *et al.*, 1983). In rats, the highest levels are in the liver and kidney, whereas the heart, brain, and lungs express only moderate MST activity.

The enzyme is localized intracellularly in both cytosol and mitochondria (Nagahara *et al.*, 1999), in which cytosolic activity is approximately twice that of mitochondria. This is in contrast to rhodanese, which is localized to the mitochondrial matrix. Rat liver MST consists of 295 amino acids and shares 66% sequence homology with rhodanese (Spallarossa *et al.*, 2004). The formal catalytic mechanism, with 3-mercaptopyruvate as the donor substrate, is a sequential kinetic pattern with a ping-pong mechanism (Westley *et al.*, 1983).

The difference in subcellular distribution of MST and rhodanese likely accounts for their relative roles in cyanide metabolism. Nagahara *et al.* (1999) proposed that upon entry into a cell, cytosolic MST can rapidly metabolize cyanide to SCN^{-} and upon movement of cyanide into the mitochondrion, both MST and rhodanese detoxify cyanide to SCN^{-}. Dual mitochondrial pathways provide for increased protection against inhibition of cytochrome c oxidase, the primary toxicity target of cyanide. In tissues that express low rhodanese levels, MST may serve as the primary route of cyanide metabolism.

4.2.9 MST substrates as cyanide antidotes

Since MST has a more favorable tissue and subcellular distribution than rhodanese, it has been proposed that MST sulfur donor substrates could function as effective cyanide antidotes (Porter & Baskin, 1995). Both 3-MP and thiosulfate can serve as substrates for MST, with K_ms for recombinant rat MST of 1.2 and 73 mM, respectively (Nagahara *et al.*, 1995). Thus, thiosulfate is not a suitable substrate due to the high K_m value for 3-MP. 3-Mercaptopyruvate, the endogenous substrate, was not effective as an antidote when administered intravenously due to rapid degradation in the blood (Nagahara *et al.*, 2003). To increase the half-life, a series of prodrugs that liberate 3-MP have been evaluated (Nagasawa *et al.*, 2007). These compounds were effective by both parenteral and oral routes of administration. Interestingly, the prodrugs that initially liberated the ethyl ester of 3-MP were effective antidotes, suggesting ethyl 3-mercaptopyruvate may be a substrate for MSP. 3-Mercaptopyruvatedithiane (sulfanegen sodium), a prodrug that can continuously generate substrate, has been shown to be an effective antidote in animal models of cyanide toxicity and is presently a candidate for commercial development (Brenner *et al.*, 2010). It is likely that new, highly effective cyanide antidotes can be developed by utilizing the MSP sulfur substrate prodrug design, linked with strategies to optimize toxicokinetics.

4.2.10 Minor enzymatic pathways of cyanide metabolism

A number of enzyme-mediated pathways can metabolize cyanide to less toxic products and account for degradation of low amounts of cyanide under normal physiological conditions. The utilization of these pathways may increase under certain dietary conditions in which sulfur substrate availability for rhodanese and/or MST becomes reduced.

4.2.11 Thiosulfate reductase

Thiosulfate reductase (sulfane reductase) is a sulfurtransferase that does not use cyanide as an acceptor substrate. The enzyme generates persulfides and then transfers sulfur to cyanide (Westley *et al.*, 1983). The enzyme has broad tissue distribution in mammals, with highest activity in liver, kidneys, and heart (Westley, 1981b). Subcellular distribution parallels that of MST with the highest activity found in mitochondrial matrix. The physiological function of the reductase may be formation of sulfide for synthesis of iron-sulfur enzymes. The enzyme catalyzes cleavage of the sulfur-sulfur bond of thiosulfate or an organic thiosulfonate and then transfers the sulfane sulfur to a sulfhydryl nucleophile (glutathione or cysteine). In the presence of excess glutathione, the persulfide product can react with glutathione to form a sulfide. Cyanide can directly react with the persulfide product to generate thiocyanate (Westley, 1981b). The role of this enzyme-mediated reaction in overall *in vivo* biotransformation of cyanide has not been characterized. Since this enzyme exists in high concentration in some tissues, it likely participates in cyanide metabolism, however, further studies are needed to establish its relative role in the overall biotransformation.

4.2.12 Cystathionase γ-lyase

Cystathionase γ-lyase (γ-cystathionase: EC *4.4.1.1*) participates in endogenous detoxification of cyanide through a coupled *in vitro* enzyme system of γ-cystathionase and rhodanese. γ-Cystathionase enhances transsulfuration of cyanide to SCN^- and a co-product of this reaction, bis(2-amino-2-carboxyethyl)trisulfide (thiocystine), is a sulfur donor substrate for rhodanese (Szczepkowski and Wood, 1967). Thiocystine is sevenfold more effective than thiosulfate as a sulfur substrate in the rhodanese-catalyzed transsulfuration of cyanide to SCN^-. In a study of thiocystine as a cyanide antidote, it was shown that thiocystine protected rats from 1 to $2 \times LD_{50}$ of cyanide (Wood, 1980).

Another product of the γ-cystathionase reaction, 3-(thiosulfeno)-alanine (thiocysteine), may be involved in γ-cystathionase-mediated cyanide detoxification. Thiocysteine transsulfurates hypotaurine (2-aminoethanesulfinic acid) to thiotaurine (2-aminoethanethiosulfonate) (Cavallini *et al.*, 1960). Thiotaurine is an excellent substrate for rhodanese (Luo & Horowitz, 1994) and increases survival of mice following a lethal dose of cyanide (Dulaney *et al.*, 1989). These studies show that γ-cystathionase participates in endogenous cyanide detoxification, but the relative role of this enzyme in *in vivo* cyanide metabolism has not been established.

4.2.13 Albumin

Early reports showed that a serum enzyme labeled "rhodanese S" catalyzed the cyanolysis of colloidal elemental sulfur to form SCN^- (Sörbo,1955). Serum albumin forms a complex with sulfane sulfur in a form that can react directly with cyanide to generate SCN^-. Albumin may function as a sulfane carrier for transport of sulfur from liver to other tissues for incorporation into iron/sulfur centers. In addition to the sulfur carrier function, Westley *et al.* (1983) proposed that albumin may also be involved in *in vivo* biotransformation of cyanide.

Bovine serum albumin binds four sulfur atoms per molecule, whereas human serum albumin binds five to six sulfurs per molecule (Westley *et al.,* 1983). The albumin bound sulfane sulfur can readily undergo nucleophilic attack by cyanide to form SCN^- (Jarabak & Westley, 1991). At steady-state, each sulfane binding site displays different reactivity for cyanide, thereby producing complex initial kinetics. Interestingly, the cyanolysis can be influenced by exogenous compounds; it is inhibited by short-chain fatty acids and activated by *p*-nitrophenyl acetate.

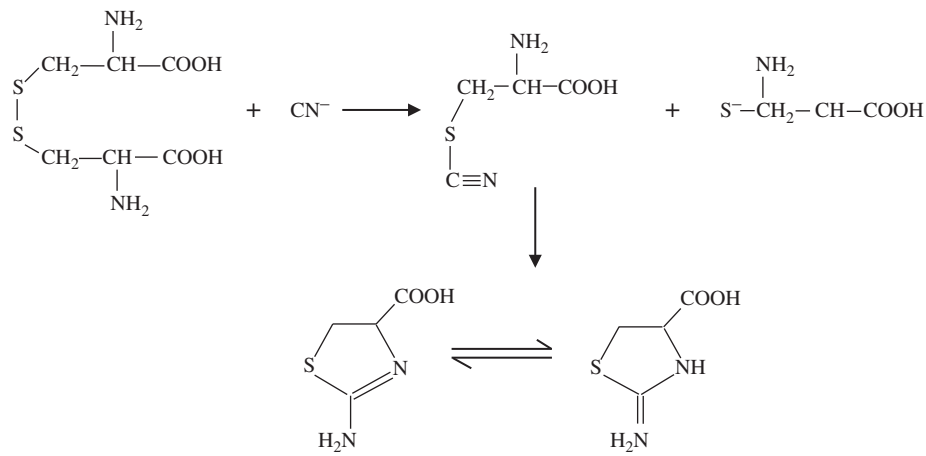
The physiological and toxicological significance of the cyanolysis role of serum albumin has not been determined. Westley *et al.* (1983) proposed that in the presence of an adequate sulfane sulfur pool, this reaction may be a primary route of cyanide detoxification. However, *in vivo* analysis shows that albumin appears to play a minor role in cyanide metabolism. In bloodless rats in which the albumin was removed by exchange transfusion with a perfluorochemical emulsion, sodium thiosulfate efficiently antagonized cyanide (Piantadosi & Sylvia, 1984). In the virtual absence of blood (serum), thiosulfate's detoxification role would be accounted for by sulfurtransferase-mediated reactions in tissues other than blood. There is also evidence that both erythrocytes and albumin can sequester cyanide to inactivate it biologically (McMillan & Svoboda, 1982). This process would have limited cyanide binding capacity and most likely plays a minimal role as an endogenous cyanide detoxification process.

4.3 Non-enzymatic detoxification of cyanide

Cyanide is a reactive chemical that can undergo several reactions at concentrations generated endogenously or at elevated tissue levels observed in acute toxicity. The rate and extent of these reactions are concentration-dependent (Logue *et al.*, 2010; Fasco *et al.*, 2007). Cyanide can directly react with protein components of enzymes or cellular structure. Cyanide is a strong nucleophile that readily reacts with organic aldehydes and ketones (carbonyl groups) to form cyanohydrins (Dzombak *et al.*, 2006). The chemical reactions remove (scavenges) cyanide to reduce its availability for interaction with cytochrome c oxidase, thereby reducing toxicity attributed to blockade of oxidative phosphorylation. Formation of cyanohydrins serves as the basis of cyanide antagonism by α-ketocarboxylic acids (α-ketoglutaric) (Moore *et al.*, 1986). On the other hand, formation of cyano intermediates may account for components of the toxic syndrome attributed to enzyme/structural changes, as is the case of altered NMDA-gated ion channel function leading to neurological dysfunction (Sun *et al.*, 1997).

The cyanide ion is an ambidentate ligand that readily forms neutral or anionic complexes with transition heavy metals in biological systems, most notably with iron in heme prosthetic groups of metalloproteins and with cobalt in protein complexes. Formation of a cyano-iron complex disrupts function of heme proteins, with the most notable being cytochrome c oxidase. On the other hand, metalloprotein complexation effectively scavenges cyanide, and in effect cyanide is detoxified by forming the complex. Cyanide anion is a strong π acceptor (π acid) ligand that can form stable octahedral complexes with cobalt (III) cations or can substitute for aquo groups from cobalt complexes, such as cobalamin and Cobalt EDTA (McGuinn *et al.*, 1994). Formation of high affinity cyano complexes is the basis of the antagonistic action of hydroxocobalamin and cobinamide (Chan *et al.*, 2010). However, urinary excretion of cyano-cobalt complexes after treatment with hydroxocobalamin to form cyanocobalamin accounts for less than 1% of administered cyanide (Borowitz *et al.*, 1992).

A minor pathway of cyanide metabolism involves the non-enzymatic reaction of cyanide with cystine to form 2-aminothiazolidine-4-carboxylic acid (2-ATCA) or its tautomer 2-amino-4-carboxylic acid (2-ITCA), which is slowly excreted by the kidneys and in saliva without further metabolism (Wood & Cooley, 1956). This pathway has been reported to account for as high as 20% of cyanide elimination under nontoxic conditions and 5–15% of a delivered dose of cyanide (Borowitz *et al.*, 1992). Cyanide reacts with cystine to produce β-thiocyanolanine, followed by ring closure to form 2-ATCA (4.5). 2-ATCA undergoes rapid tautomization to 2-ITCA and in solution, the tautomers are in equal concentration. Formation of 2-ATCA is dependent on cyanide concentration and availability of cystine, which can be depleted after high concentrations of cyanide.



(4.5)

Even though 2-ATCA is a minor metabolite generated in low concentrations, it has biological actions that may contribute to the post-intoxication CNS syndrome associated with cyanide (Bitner *et al.*, 1995). In rodents, administration 2-ATCA intracerebroventrically produced wild-running seizures and selective loss of hippocampal neurons, similar to that observed with excitotoxins. Additional studies are needed to establish the toxicological implications of this pathway of cyanide metabolism.

Studies have shown that 2-ATCA formation may serve as a diagnostic biomarker of cyanide exposure (Petrikovics *et al.*, 2011). After it is formed, 2-ATCA is deposited in tissues, such as the liver, and is slowly eliminated. Lundquist *et al.* (1995) showed that the compound was detectable in urine by HPLC assay up to 4 weeks after administration of acetonitrile, a compound metabolized to cyanide. 2-ATCA can also be detected in the urine of smokers, attributable to cyanide inhalation in tobacco smoke. More detailed toxicokinetic analysis establishing 2-ATCA elimination rates and tissue deposition are needed to establish the usefulness of 2-ATCA as a cyanide biomarker.

4.4 Diseases associated with altered cyanide metabolism

A number of disease states are associated with exposure to cyanide in the presence of reduced cyanide metabolism and/or elimination (Table 4.1). In many of these conditions, it is presumed that metabolic pathways are normal, but the cyanide load exceeds availability of sulfane sulfur substrate or detoxification capacity. Several conditions are associated with nutritional deficiencies combined with low-level cyanide exposure. Konzo, a spastic paraparesis, and tropical ataxic neuropathy are associated with ingestion of cyanogenic foods (Wilson, 1987). In many tropical areas, cassava (*Manihot esculenta*), a cyanogenic tuber, is a staple in the diet. Improper curing of cassava to remove the cyanide by off-gassing as HCN can lead to chronic low-level cyanide exposure (Bonmarin *et al.*, 2002). These conditions are more frequent and severe in the presence of inadequate intake of dietary protein. In dietary deficiency, it appears that plasma concentrations of sulfur-containing amino acids may be depleted, thereby lowering the availability of the substrate pool for sulfurtransferases. Also, in deficiencies of sulfur amino acids, cyanide may be oxidized to cyanate (OCN^-), a known neurotoxin (Tor-Agbidye *et al.*, 1999). In tobacco amblyopia, chronic exposure to cyanide in tobacco smoke may deplete hydroxocobalamin (vitamin B_{12}) by forming cyanocobalamin, leading to amblyopia similar to that associated with vitamin B_{12} deficiency (Wilson, 1987).

Leber's optic atrophy is a rare hereditary blindness in which individuals display extreme sensitivity to cyanide in tobacco smoke (Wilson, 1987). Many of these patients express substantially lower rhodanese levels compared to normal individuals, suggesting an underlying mechanism of Leber's disease is defective cyanide metabolism

Table 4.1 Pathological conditions associated with cyanide exposure and abnormal cyanide detoxification activity.

Disease state	Relative detoxification (compared to normal)	Relative rhodanese activity
Encephalitis peraxialis diffusa (Schilder's disease)	Unknown	Unknown
Tobacco amblyopia	Low	Deficient substrate pool; low rhodanese activity
Tropical ataxic neuropathy	Low	Deficient substrate pool
Konzo	Low	Deficient substrate pool
Leber's optic atrophy	Low	Low rhodanese activity; mitochondrial defects
Amyotrophic lateral sclerosis	Low	Low rhodanese activity

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(Berninger *et al.*, 1989). In amyotropic lateral sclerosis (ALS), serum levels of cyanide are elevated in comparison to control individuals (Kato *et al.*, 1985). Rhodanese activity was lower in spinal cord of ALS patients compared to controls (Mimori *et al.*, 1984). The deficiency was localized in the posterior column of the spinal cord, the area where morphological changes in ALS are most severe.

The role of cyanide metabolism and rhodanese in these conditions is complex. It should be kept in mind that rhodanese is a multifunctional enzyme involved in other cellular and mitochondrial functions, in addition to cyanide metabolism. The contribution of altered cyanide disposition to the pathology needs to be addressed in detailed biochemical studies.

4.5 Metabolism and endogenous generation of cyanide

Cyanide is generated in white blood cells and neural cells at low levels by a myeloperoxidase enzyme-mediated reaction (Stelmaszyńska & Zgliczyński, 1981). In leukocytes hydrogen cyanide (HCN) generation occurs by a myeloperoxidase-hydrogen peroxide system in which N-chlorination of glycine is catalyzed by myeloperoxidase to form N-monochloroglycine which then undergoes acid-mediated dismutation to N-dichloramine (Figure 4.3). This product undergoes decomposition to form hydrogen cyanide (Cipollone & Visca, 2007). A similar enzymatic reaction occurs in neurons to generate cyanide in which glycine and hydrogen peroxide enhances cyanide production via a myeloperoxidase-mediated reaction (Borowitz *et al.*, 1997).

Normal blood levels of cyanide are ~0.22μM (Tsuge *et al.*, 2000). Smoking and dietary consumption of cyanogenic foods, such as cassava, can significantly increase this level. It has been proposed that low level, endogenous generation of cyanide is physiologically significant and HCN may function as a small molecule modulator, similar to NO, CO, and H_2S (Gunasekar *et al.*, 2000; Cipollone & Visca, 2007). Cytochrome oxidase is highly sensitive to cyanide at the concentrations generated endogenously and it appears that endogenous generation may serve to regulate oxidative metabolism. In subcellular fractions of neural tissue, cyanide is generated in mitochondria via a myeloperoxidase-mediated

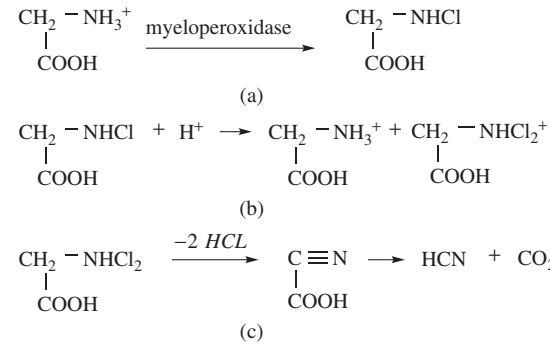

Figure 4.3 Hydrogen cyanide formation by the myeloperoxidase-H2O2-Cl^- system in leukocytes. (A) The N-chlorination of glycine catalyzed by myeloperoxidase results in conversion of glycine to N-monochloroglycine. (B) Acid-catalyzed dismutation of N-monochloroglycine results in N-dichloramine which is unstable and (C) decomposes to the corresponding nitrile and the degradation produce is cyanide. (reproduced by permission of IUBMB Life (Cipollone and Visca, 2007: 59; Cipollone *et al.*, 2007: 188).

reaction (Gunasekar *et al.*, 2004). In the brain, the generation of cyanide is region specific and mediated via opiate and NMDA receptors (Borowitz *et al.*, 1997).

The biological role of endogenous cyanide generation has not been established. It has been speculated that in mitochondria, endogenous generation of cyanide could modulate oxidative metabolism, since low-level HCN would bind to cytochrome oxidase to inhibit oxidative phosphorylation (Cipollone & Visca, 2007). Interestingly, rhodanese is present in the mitochondrial matrix of most tissues and would rapidly remove cyanide from its binding site on cytochrome oxidase to enhance oxidative metabolism. Similarly, cyanide may function as a neuromodulator via receptor-mediated biosynthesis. The gaseous compound could directly influence neuronal function by modulating ion channels (Sun *et al.*, 1997) or modulating oxidative metabolism. In brain tissue, the removal of cyanide appears to be mediated by rhodanese, which is expressed in the same brain areas that generate cyanide via receptor-mediated reactions (Wrobel *et al.*, 2006).

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CHAPTER 5
Biochemical mechanisms of cyanide toxicity

Gary E. Isom and Joseph L. Borowitz

At a Glance

- Cyanide produces a rapid inhibition of cytochrome oxidase to inhibit cellular utilization of oxygen and reduce ATP production.
- Cyanide forms a reversible coordination bond with the cytochrome oxidase binuclear heme center, resulting in inhibition of cellular oxidative metabolism.
- Tissues (heart and brain) that have limited anaerobic metabolism undergo rapid dysfunction to produce the manifestations of toxicity.
- Accompanying cytochrome oxidase inhibition is a marked cellular oxidative stress and changes in cellular calcium regulation which contribute to acute cyanide toxicity and post-intoxication lesions.

5.1 Introduction

Acute cyanide poisoning is characterized by a rapid onset of toxic manifestations attributed to a reversible, concentration-related inhibition of cellular oxidative metabolism. Organ systems that have limited anaerobic metabolism capability, including the myocardium and nervous system, undergo rapid dysfunction as a result of the reduced metabolism. Following the initial inhibition of aerobic metabolism, continued cyanide exposure leads to an irreversible stage in which damage to target organs results from activation of cell injury/death pathways.

The primary mechanism of toxicity results from binding of cyanide to cytochrome c oxidase (CcOX), the terminal oxidase of the oxidative phosphorylation chain. Binding of cyanide inhibits CcOX, thereby inhibiting mitochondrial utilization of molecular oxygen and reducing cellular uptake of oxygen. The toxicity is technically a histotoxic hypoxia in which oxygen delivery to the tissues and oxygen tension is normal, but utilization of oxygen via oxidation phosphorylation is blocked. The result is rapid depletion of cellular ATP producing a reduced energy charge and bioenergetic failure, followed by Ca^{2+} overload in target tissues and loss of cellular homeostasis (Maduh *et al*., 1991; Zhang *et al*., 2010). This impairs function of vital organ systems, and if the toxicity is not rapidly reversed, death occurs as a result of altered respiration, cardiovascular, and neurological function.

Traditionally, cyanide toxicity is attributed to inhibition of CcOX, however, to account for the wide spectrum of toxic manifestations, additional toxic biochemical pathways must be considered. Cyanide inhibits multiple enzymes and cellular processes that contribute to the loss of cellular homeostasis (Table 5.1). Several enzymes are more sensitive than CcOX to cyanide inhibition and their inhibition may not be reversible. Even though the initial event in acute toxicity is histotoxic hypoxia, other cellular actions of cyanide contribute to the rapid loss of cellular homeostasis in target organs. This includes rapid reduction of ATP, a rise in cytosolic free Ca^{2+}, excessive reactive oxygen species generation (ROS), and excess lactate production.

Toxicity of cyanide results from the two chemical species (HCN and CN^-) that exist under biological

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Table 5.1 Enzymes inhibited by cyanide at concentrations achieved in biological systems.

Catalase
Cytochrome c oxidase a_3
Glutamate decarboxylase
Glutathione peroxidase
Glutathione reductase
2-Keto-4-glutarate aldolase
Lipoxygenase
Nitrite reductase
Ribulose diphosphate carboxylate
Superoxide dismutase
Xanthine dehydrogenase
Xanthine oxidase

Table 5.2 Examples of chemical reactions by which cyanide interacts with enzymes and cellular components.

Complexation with metal ions
Complexation with metalloproteins containing Co^{2+} or Fe^{+3}
Nucleophilic reaction with aldehydes to form cyanohydrin
Nucleophilic reaction with ketones to form cyanohydrin
Scission of disulfide linkages
Cyanation of sulfhydryl groups
Cyanation of Schiff bases

conditions following exposure to cyanide salts or gas. HCN and the CN^- anion react with key metabolic enzymes at concentrations observed in acute intoxication (Table 5.2). The rate and extent of the interaction with enzymes and cellular components is concentration-dependent (dose of exposure) and the extent of ionization of HCN at physiological pH. The formation of cyanide adducts or binding to an enzyme inhibits enzyme function. The cyanide anion is a strong nucleophile that can react with aldehydes and ketones (carbonyl groups) to form cyanohydrin intermediates (Dzombak *et al.*, 2006). The reaction takes place in two steps as shown in Figure 5.1.

The stability of cyanohydrins formed is dependent on the structure of the specific carbonyl compound (protein). With stable cyanohydrin adducts, reversal of toxicity would be dependent on cellular repair mechanisms to remove the altered enzyme or cell component. Reaction of cyanide with aldehyde functional groups yields stable cyanohydrins, whereas aliphatic and aromatic ketones form less stable cyanohydrins that may decompose under biological conditions. Formation of the cyanohydrins can have additional biological consequences since biologically active intermediates can be formed during cyanide toxicity. Cyanide also reacts with sulfhydryl containing cellular components such as cystine to form stable adducts. For instance, cyanide reacts with cystine to form 2-aminothiazolidine-4-carboxylic acid, a biologically active compound which contributes to toxicity (Bitner *et al.*, 1995). Reaction of alpha-ketoglutarate with cyanide serves as the basis for scavenging of cyanide and is the basis of its use as a cyanide antidote (Moore *et al.*, 1986).

Cyanide also complexes with metalloproteins containing iron (Fe^{++} and Fe^{+++}) or cobalt (III) to form complexes. The cyanide ion is an ambidentate ligand that readily forms neutral or anionic complexes with transition heavy metals in biological systems, most notably with iron in heme prosthetic groups of metalloproteins and with cobalt in protein complexes. The complex can undergo dissociation to reactivate the free enzyme. Formation of a cyano-iron complex disrupts heme protein function, with the most notable being CcOX. On the other hand, metalloprotein complexation effectively scavenges cyanide, and in effect cyanide is detoxified by forming the complex. The cyanide anion is a strong π acceptor (π acid) ligand that can form stable octahedral complexes with cobalt (III) cations or can substitute for aquo/hydroxo groups from cobalt complexes, such as cobalamin and cobalt EDTA (McGuinn *et al.*, 1994). Formation of metal-cyanide complexes is the basis of the antidotal action of hydroxocobalamin and cobinamide (Chan *et al.*, 2010).

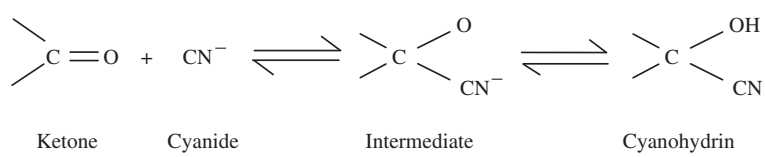

Figure 5.1 Hydrolytic degradation of nitriles.

The following discussion will focus on the primary biochemical pathways altered in cyanide toxicity and the biological manifestations attributed to their inhibition. Inhibition of CcOX is considered the primary initiating event, however, the toxic manifestations are attributed to the biological consequences of multiple toxic actions. The rapidity of action and multiple toxic pathways contribute to the extreme lethality of cyanide and the complexities of effectively reversing or treating the toxicity.

5.2 Cytochrome oxidase inhibition and mitochondrial dysfunction

The primary biochemical target of cyanide is CcOX, the terminal oxidase of the mitochondrial oxidative phosphorylation chain (Way, 1984). Early investigators made empirical observations on the apparent "arterialization" of venous blood during cyanide intoxication (Bernard, 1857). Partial explanation of the toxicity was provided by Hoppe-Seyler (1876, 1877), who demonstrated that cyanide poisoning inhibited tissue oxidative processes, thereby reducing oxygen extraction from arterial blood to result in increased oxygen content of venous blood. It was subsequently shown that the initial biochemical lesion produced by cyanide involved the inhibition of CcOX, the newly discovered terminal respiratory enzyme (Keilin, 1930; Warburg, 1931).

Cyanide reduces cellular aerobic metabolism by inhibiting the catalytic activity of CcOX located in complex IV of the mitochondrial oxidative phosphorylation chain (electron transport chain). This blocks intracellular utilization of oxygen (histotoxic hypoxia) and decreases ATP generation that eventually produces cellular bioenergetic failure (Leavesley *et al.*, 2008). The mechanism of CcOX inhibition was not well understood until the cytochrome respiratory enzyme system was characterized (Way, 1984). The mitochondrial respiratory chain, located in the inner mitochondrial membrane, is comprised of four electron-transporting protein complexes (I–IV) linked with production of ATP at ATP synthase making up complex V (Duchen, 2004). The transport of electrons from complexes I–IV is coupled to the efflux of protons from the mitochondrial matrix to the inner membrane space at complexes I, III, IV (Figure 5.2). The pumping of protons from the matrix into the inner membrane space creates a pH gradient and a mitochondrial membrane potential which is the proton motive force that drives phosphorylation of ADP at complex V. Since oxidative phosphorylation accounts for up to 95% of cellular energy production, mitochondrial dysfunction can disrupt electron transport and result in cellular energy failure.

CcOX is a large membrane bound, multicomponent protein made up of 13 subunits. The terminal oxidase contains four metal-redox centers, Cu_A, Cu_B, and two hemes (Figure 5.3). In the catalytic cycle, Cu_A accepts an electron from cytochrome c, then the electron is transferred to heme a and then to the binuclear a_3-Cu_B center, where the electron and protons are transferred to molecular O_2 to form H_2O. The cyanide anion (CN^-) preferentially forms a relatively stable coordination complex with the binuclear heme center of CcOX to inhibit the catalytic activity of the terminal oxidase. Recent studies show that CN^- binds with high affinity to the Fe a_3-Cu_B center to block diffusion of O_2 into a channel in the protein's tertiary structure in order to access the catalytic binuclear center, thus inhibiting oxidative phosphorylation (Parul *et al.*, 2010). The kinetics of the inhibition is complex (Leavesley *et al.*, 2008, 2010) Initially a competitive inhibition with O_2 is observed, followed by mixed inhibition due to CN^- binding to the other metal centers of the enzyme.

CN^- binding to CcOX is complex and dependent on the enzyme's catalytic turnover, electron flux rate, and binuclear center redox status (Nicholls & Soulimane, 2004). Under normal respiratory conditions, when the enzyme's heme Fea_3-Cu_B binuclear center is fully reduced (Fe^{2+} and Cu^+ states), oxygen binds to CcOX and complex IV transfers electrons from CcOX to molecular oxygen and a proton is transferred across the inner mitochondrial membrane. This generates a membrane potential, which then drives ATP synthesis at complex V (ATP synthase) and changes the redox status of complex IV (Leavesley *et al.*, 2008). The binuclear center can exist in reduced, oxidized, or partially reduced states and during the catalytic cycle in which an electron is transferred to molecular oxygen, the redox state cycles from fully reduced to oxidized states. When the binuclear center is fully reduced with iron in the ferrous (Fe^{+2}) state and copper as cuprous (Cu^+), oxygen binds (K_D = 1 μM) and then complex IV transfers electrons from cytochrome c to molecular oxygen. Although oxygen only binds to the fully reduced binuclear center, cyanide can interact with all redox states of

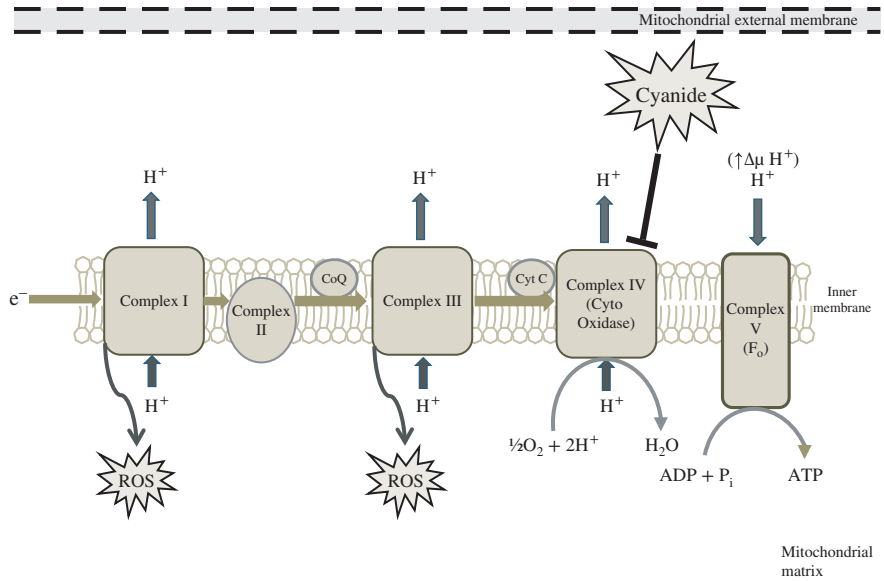

Figure 5.2 Diagram of the electron transport chain (ETC) and inhibition by cyanide. The five complexes of the ETC are located in the cristeae of the inner mitochondrial membrane. NADH + H^+ delivers electrons and protons to complex I and then electrons are transported down complexes I–IV. At complex IV (cytochrome oxidase) molecular oxygen is reduced to form H_2O. As the electrons are transported down the ETC, at complex I, III, and IV protons are pumped to the cytosolic side of the inner-membrane to produce a proton gradient across the mitochondrial inner membrane. The proton gradient generates a membrane potential across the inner membrane which drives complex V (ATP synthase) to phosphorylate ADP to form ATP. Normally, a leakage of electrons at complexes I and III results in formation of low levels of reactive oxygen species (ROS) which are rapidly removed by enzymatic metabolism. Cyanide binds to the redox-active reaction center of cytochrome oxidase to inhibit oxidative phosphorylation resulting in a marked reduction of ATP generation. As a result of the blockade of the terminal oxidase, electrons back up in the ETC, thus markedly enhancing generation of ROS which can produce mitochondrial and cellular oxidative damage.

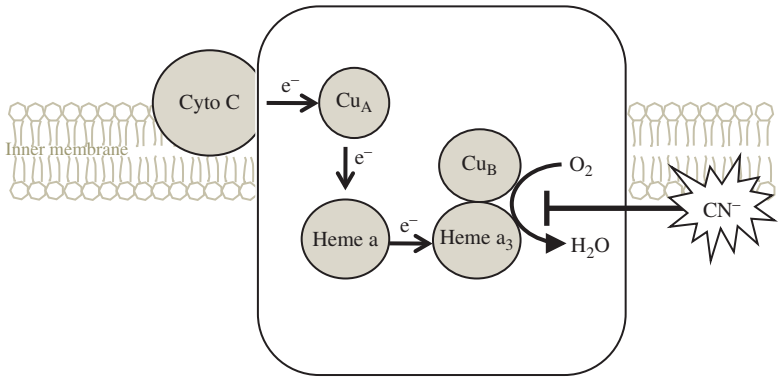

Figure 5.3 Diagram of the redox catalytic site of cytochrome c oxidase (CcOX) and inhibition by cyanide. CcOX contains four redox active metal centers which undergo changes in redox state as an electron is sequentially transferred from cytochrome c to CuA, heme a, and the cytochrome a_3-Cu_B binuclear center. At the binuclear center O_2 is rapidly reduced to H_2O. The cyanide anion (CN^-) forms a high affinity coordination complex with the binuclear center to block a channel that molecular O_2 must diffuse in order to access the redox site in the binuclear center. This is followed by slow binding of CN^- to the reduced state of the other two metal sites (Cu_A and heme a). The slow binding is due to lower binding affinity, a reduced catalytic turnover and electron flux required to reduce the Cu_A and heme a centers. The result is blockade of oxidative phosphorylation which has mixed inhibition kinetics.

the enzyme to produce a concentration-dependent inhibition. Cyanide complexes tightly to the fully oxidized state ($Fe_{a3}^{3+}-Cu_B^{2+}$) at a rate constant $K = 10\ M^{-1}\,s^{-1}$ and binding constant $K_D = 1\ \mu M$, whereas binding to the fully reduced binuclear is weaker ($K = 1.3 \times 10^2\ M^{-1}\,s^{-1}$, $K_D = 500\ \mu M$). Cyanide has the highest affinity ($K = 2 \times 10^6\ M^{-1}s^{-1}$, $K_D = 20\ nM$) for the partially reduced state ($Fe_{a3}^{3+}-Cu_B^{+}$).

Complexation of cyanide to CcOX is reversible and dissociation of cyanide from the binuclear center restores the catalytic activity of the native enzyme to reactivate oxidative metabolism. The rate of dissociation is dependent on concentration of cyanide in the mitochondrial inner membrane. Following binding and inhibition of CcOX, a reduction of cyanide's free concentration within the mitochondrion will drive dissociation of the anion from the enzyme and gradually reactivate catalytic activity. CN^- dissociation from CcOX can be accelerated to reverse toxicity by scavenging free cyanide by antidotes that have a high affinity for cyanide (Hall *et al*., 2009; Leavesley *et al*., 2010).

Kinetic analysis shows that inhibition of CcOX by cyanide is non-competitive with respect to cytochrome c-mediated reduction of the enzyme and interaction of cyanide with the catalytic binuclear center competes with oxygen for binding to the enzyme (Leavesley *et al*., 2008). This provides a partial explanation for the antidotal action of hyperbaric oxygen since increased oxygen tension would facilitate displacement of cyanide to reactivate the enzyme (Way, 1984). Also, this observation explains in part why cyanide is such a rapid acting and potent intoxicant. In the brain, the primary target organ, aerobic metabolism is extremely sensitive to cyanide since the normal oxygen tension present in brain mitochondria is lower (< 15 μM) than peripheral tissue. Brain mitochondria function *in situ* at a lower oxygen tension as compared to that of peripheral tissue (Cooper & Giulivi, 2007). This would account for the lower cyanide IC_{50} of brain CcOX, resulting in a rapid onset and potent action in brain. Similarly, myocardial CcOX also displays a high sensitivity to cyanide (Ballantyne, 1987).

Analysis of cyanide's action in isolated tissue shows that cyanide rapidly inhibits CcOX to reduce cellular oxygen consumption (Mazat *et al*., 1997). In isolated cell models, KCN produces a concentration-dependent inhibition of ADP-stimulated (state 3) respiration (oxygen uptake) with an IC_{50} of ~ 13.2 μM (Leavesley *et al*., 2008). It should be noted that cyanide inhibition of oxygen uptake is different from that of the classical mitochondrial uncouplers which produce an apparent increase of oxygen uptake under state four respiratory conditions (absence of ADP), but as a result of uncoupling oxygen utilization from CcOX activity, the uncouplers decrease ATP synthesis. Cyanide inhibition of oxygen utilization is followed within seconds by reduction of ATP synthesis. At lethal cyanide levels, cellular ATP rapidly decreases to 50–60% of normal cellular levels. Tissue with limited anaerobic capacity and high energy demand, such as brain and myocardium, experience a more rapid and greater reduction in ATP. At the same time, the shift from aerobic, oxidative phosphorylation-driven metabolism to anaerobic glycolytic metabolism increases plasma and tissue lactate (acidosis) and changes cellular redox status by increasing the levels of reduced adenine nucleotide (Maduh *et al*., 1991).

Extensive studies have been conducted on cyanide inhibition of CcOX in *in vitro* systems and purified enzymes, however, a limited number of studies have been reported on *in situ* CcOX activity and energy status of the target tissue (Ballantyne, 1987). The noninvasive *in vivo* diffuse optical spectroscopy system has been used to monitor changes in the redox state of CcOX on a real-time basis during cyanide infusion in rabbits (Armstrong *et al*., 2007). During the administration of cyanide, a concurrent increase in reduced CcOX and a reduction in the oxidized CcOX state were observed in hind leg tissue. Additional studies on analysis of brain CcOX activity and ATP levels in rodents showed a correlation between ATP levels and inhibition of aerobic metabolism in the primary toxic target organs, brain, and myocardium (Petterson & Cohen, 1993). Within seconds lethal doses reduce high-energy phosphate compounds to levels similar to those observed with ischemia (Decorps *et al*., 1984). Cyanide inhibits brain CcOX in a dose-related manner (Isom *et al*., 1982). It appears that at least a 50% inhibition of brain CcOX is necessary for changes in EEG activity and functional impairment of neurological activity (Piantadosi & Sylvia, 1984). As in the brain, myocardial CcOX is significantly inhibited following acute lethal cyanide poisoning in rabbits, with a lower IC_{50} in myocardium as opposed to cerebral CcOX (Ballantyne, 1987). It was concluded that in this species, the myocardial CcOX is more sensitive to cyanide than the brain, but the

toxic manifestations observed following lethal doses results from dysfunction of both the neurological and cardiovascular systems.

Studies in animals have questioned the unique mechanistic role of CcOX inhibition in acute cyanide toxicity. Detailed analysis of brain CcOX activity following high doses of cyanide in mice showed that brain CcOX exists in excess and that at least a 50% inhibition was necessary to decrease brain respiratory activity (oxygen uptake) (Petterson & Cohen, 1993). The functional excess of CcOX in brain may compensate to diminish the respiratory chain's vulnerability to cyanide. As a result of these observations, it was concluded that cyanide produces actions independent of CcOX and acute toxicity is attributed solely to inhibition of aerobic metabolism.

5.3 Oxidative stress and inhibition of cellular oxidative defense

Johnson, Conroy, Burris, *et al.* (1987) were the first to report that cyanide caused a dose and time-dependent lipid peroxidation in a variety of tissues, including brain and heart. In mice, lethal doses of KCN produced detectable levels of conjugated dienes within 10 minutes of exposure. Conjugated dienes are generated when reactive oxygen species (ROS) react with membrane lipids and are used as markers of membrane peroxidation and oxidative damage. In neural tissue, oxidative damage was linked to altered neuronal Ca^{2+} homeostasis, neurological dysfunction, and cell injury (Gunasekar *et al.*, 1996; Shou *et al.*, 2000). Biochemical analysis of tissue from animals treated with lethal doses of cyanide salts show that oxidative stress and subsequent oxidative tissue damage play a role in the toxicity.

Under basal aerobic respiration, transfer of electrons down the electron transport chain generates a low level of ROS (superoxide, hydroxyl radical, peroxides) at complexes I and III (Chen *et al.*, 2003). The low levels of ROS generated under normal respiratory conditions produce minimal cellular damage since the cellular antioxidant defense rapidly metabolizes the ROS. Mechanisms by which cyanide produces high levels of oxidative stress have been studied in detail (Borowitz *et al.*, 2001). Inhibition of CcOX at complex IV blocks transfer of electrons to molecular oxygen at complex IV, resulting in backup of electrons down the oxidative phosphorylation chain that eventually produces excess ROS generation at complexes I and III (Figure 5.2). Real-time analysis of single cells by microfluorescence spectroscopy showed that within seconds of cyanide exposure, an intense burst of ROS is produced in mitochondria and this high level of generation continues for the duration of cyanide exposure (Kanthasamy *et al.*, 1997; Jones *et al.*, 2000). Cyanide stimulates generation of superoxide, peroxides, and peroxynitrite, which are highly cytotoxic radicals (Gunasekar *et al.*, 1998). Elevated levels of ROS then promote oxidative damage to mitochondrial membranes and further mitochondrial dysfunction. Importantly, the oxidative stress, coupled with cyanide-induced ATP depletion, results in loss of cellular homeostasis within seconds of cyanide exposure (Prabhakaran *et al.*, 2004). Thus the primary source of oxidative stress after cyanide exposure is the mitochondrion where inhibition of CcOX stimulates production of reactive oxygen species.

In the presence of cyanide, cellular conditions are ideal for production of significant oxidative damage to cellular membranes in brain and myocardium. During cyanide toxicity, oxygen tension in mitochondria remains high since only oxygen use is impaired, not its availability. This insures ample oxygen is present to react with electrons as they are transferred down the oxidative phosphorylation chain. Additionally antioxidant defense enzymes that catalyze metabolism of oxygen radicals and peroxides are inhibited by cyanide (Ardelt *et al.*, 1989). This includes the metalloenzymes which make up the antioxidant defense – superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Hariharakrishnan *et al.*, 2009). Related, cyanide also stimulates nitric oxide production in mitochondria. Excess generation of nitric oxide in the presence of superoxide leads to formation of the highly cytotoxic peroxynitrite anion (Gunasekar *et al.*, 1996). Under these cellular conditions, inhibition of CcOX by cyanide results in rapid generation of ROS which in turn promotes peroxidation of membrane lipids, followed by activation of redox-sensitive cellular regulatory factors (Ardelt *et al.*, 1994; Müller & Krieglstein, 1995). Oxidative damage observed during the acute phase of toxicity is greater than that observed in hypoxic, ischemic conditions and the brain is the main tissue in which lipid peroxides are formed after cyanide exposure (Borowitz *et al.*, 1992).

Even though the primary mechanism underlying the acute toxic syndrome results from CcOX inhibition, the accompanying oxidative stress produces peroxidation of cellular membranes which rapidly initiates changes in neuronal morphology and impaired ion handling mechanisms (Isom *et al*., 1999). Oxidative stress can also lead to changes in cellular redox status which has been linked with excessive activation of NMDA receptors (Sun *et al*., 1999). These effects appear to contribute to global activation of neural function, resulting in seizures and impaired regulation of vital function (respiration and cardiovascular system). Recent studies have shown that the oxidative stress may be the primary route for activation of cell death pathways and eventually produces tissue damage observed following the immediate toxic syndrome (Mills *et al.,* 1999). Studies in cultured cells have shown ROS generation is a primary factor in cyanide-induced cell death (Watabe & Nakaki, 2007). In cultured cells, the cell death produced by cyanide can occur without significant ATP depletion (CcOX inhibition) and under the experimental conditions oxidative stress is the initiating factor of cell death. However, it should be noted that studies in isolated cells must be interpreted with caution since cultured cell lines generally have a high anaerobic respiratory capacity and can survive significant inhibition of aerobic metabolism by cyanide.

5.4 Cyanide-induced changes in cellular Ca^{2+} regulation

Cyanide-induced alterations in ion handing mechanisms are related temporally to a number of the acute toxic responses (Johnson, Conroy, and Isom, 1987; Maduh, Borowitz, Turek, *et al*., 1990; Carroll *et al*., 1992). A rapid, sustained elevation of cytosolic free Ca^{2+} occurs within seconds of cyanide exposure and is an initiating event in a number of toxic actions in target organs. The uncontrolled rise in Ca^{2+} is due to both sudden increase of influx of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+} stores (Rajdev & Reynolds, 1994; Kaplin *et al*., 1996). In electrically excitable cells, cyanide produces a sustained elevation of cytosolic free Ca^{2+} by activating multiple mechanisms – increasing Ca^{2+} conductance by activating voltage sensitive Ca^{2+} channels (Patel *et al*., 1992), reversal of the Na/Ca exchanger (Kiang & Smallridge, 1994), and activation of NMDA receptors (Patel *et al*., 1993). Intracellular stores of Ca^{2+} contribute to the rise in cytosolic Ca^{2+} as a result of stimulating inositol trisphosphate (IP_3) generation (Kaplin *et al*., 1996). Release of Ca^{2+} from intracellular storage sites also amplifies receptor signal transduction and contributes to a sustained loss of Ca^{2+} homeostasis. Cells also have an impaired ability to buffer the excessive load of Ca^{2+} since reduction of ATP synthesis decreases activity of ATP-driven Ca^{2+} extrusion pumps and impairs sequestration of Ca^{2+} by endoplasmic reticulum and mitochondria (Borowitz *et al*., 1988; Ray *et al*., 1994).

As toxicity progresses, the cyanide-induced sustained rise in excessive levels of cytosolic Ca^{2+} activates several critical membranous and cytoplasmic events in concert that lead to cell dysfunction and eventually, if not controlled threatens survival of the cell by stimulating cell death pathways (Tymianski & Tator, 1996). Since the response to Ca^{2+} is pleiotropic, multiple, diverse biochemical cascades are activated following cyanide-induced elevation of the ion. In a variety of tissues, a number of Ca^{2+}-dependent endonucleases, phospholipases, kinases, and proteases induce morphological and genomic damage (Shou *et al*., 2004). Recent studies have shown that activation of these pathways contributes to the tissue injury observed in both acute and chronic exposures (see next section) (Zhang *et al*., 2007). Dysfunction of cardiac and nervous systems observed following acute exposure may in part be attributed to the loss of ion homeostasis. The sustained rise in cytosolic Ca^{2+} stimulates release of neurotransmitters and is linked with increased levels of catecholamines in blood and increased levels of glutamate in the brain (Kanthasamy *et al*., 1991). These actions appear to explain a number of the manifestations of acute toxicity, including changes in blood pressure and seizures.

The rise in cytosolic Ca^{2+} is an important biochemical event in overall cyanide-induced toxic responses and modification of these effects can alter the duration and severity. Changes in neuronal cytosolic Ca^{2+} handling mechanisms account for many of the neurochemical events associated with cyanide toxicity. For instance, pretreatment of laboratory animals with calcium channel blockers prevents elevation of brain Ca^{2+} and the subsequent cyanide-induced tremors and seizures (Johnson, Conroy, and Isom, 1987). Diltiazem, a blocker of the L type of Ca^{2+} channels, prevented

both brain accumulation of Ca^{2+} and cyanide-induced tremors. Also, treatment with flunarizine, a calcium channel blocker that can cross the blood brain barrier, protected mice against cyanide lethality (Dubinsky *et al*., 1984). In mice treated with cyanide, a dose-dependent peroxidation of brain lipids was blocked by diltiazem pretreatment, indicating that a Ca^{2+}-sensitive process mediated peroxidation of brain lipids (Johnson, Conroy, Burris, *et al*., 1987). In morphological studies, incubation of neuron-like cells with cyanide produced retraction of cell surface microspikes, swollen mitochondria, and bleb formation. Voltage-sensitive Ca^{2+} blockers attenuated these effects as well as the cyanide-induced decrease of cell viability. Cyanide also impaired anionic and cationic transport systems controlling intracellular pH resulting in cytosolic acidosis (Maduh, Borowitz, and Isom, 1990). Pretreatment with diltiazem or exclusion of extracellular Ca^{2+} delayed the onset of cellular acidification. Since the alteration of Ca^{2+} homeostasis is a global effect in cyanide toxicity, treatments that either prevent or reverse the effect on the ion's handing mechanisms may be an effective antidotal therapy and warrant additional evaluation.

5.5 Cyanide-induced cell death and post-intoxication lesions

Following acute intoxication, lesions of the CNS have been reported and a number of individuals developed a post-intoxication sequelae characterized by a Parkinson-like syndrome and cognitive impairment (Di Filippo *et al*., 2008). Similar neurological damage has been noted in animals administered cyanide. In mice administered multiple doses of cyanide, both necrotic and apoptotic neuronal death in select regions of the brain has been reported. Biochemical studies show that the neuronal loss involves divergent biochemical pathways activated by initiation signals enhanced by cyanide (Mills *et al*., 1999; Zhang *et al*., 2010). Initiation signals and execution pathways underlying the two modes of death have been characterized in *in vitro* analysis (Prabhakaran *et al*., 2004, 2005). The primary event is inhibition of CcOX, which then stimulates oxidative stress, elevates cellular Ca^{2+}, and alters mitochondrial function (reduced ATP, reduced mitochondrial membrane potential and opening of the mitochondrial transition pore). These events then serve as a stimulus for execution of the apoptotic or necrotic cell death pathways (Li *et al*., 2006). In cyanide toxicity, the pathways share common initiator stimuli, but one pathway will dominate in select cell types to produce the characteristic mode of death observed in tissue responding to cyanide with pathological injury.

The apoptotic cell death pathway stimulated by cyanide is initiated by an increase of cytosolic Ca^{2+} and excess ROS generation (Prabhakaran *et al*., 2006; Zhang *et al*., 2010). This pathway is complex and dependent on release of cell death factors from mitochondria, thus cyanide produces a mitochondrial-dependent apoptotic death. The cell death cascade is initiated by excess generation of ROS and the elevated Ca^{2+} levels though activation of NF*k*B (redox-sensitive transcription factor), which then up-regulates Bax expression (Shou *et al*., 2000, 2003). Bax is a cytosolic kinase that normally is localized in the cytosolic cell compartment in an inactivated state. Following activation, Bax translocates from the cytosol to mitochondria, to stimulate release of cytochrome c into the cytosol. Then, cytochrome c activates the well characterized caspase cell death cascade (Shou *et al*., 2004).

In cells undergoing necrosis, it appears that excess oxidative stress and the rapid breakdown of mitochondrial function (onset of mitochondrial permeability transition, ATP depletion and reduced mitochondrial membrane potential) initiate the necrotic cell death pathway by producing a loss of ionic homeostasis and damaged cell membranes (Prabhakaran *et al*.,2002, 2006). Interestingly, studies have shown that in cyanide exposure either the necrotic or the apoptotic pathway may be selectively activated, resulting in different lesion types in different brain regions. For instance, cyanide induces apoptotic cell death in cortical cells as opposed to necrosis in mesencephalon dopaminergic cells (Prabhakaran *et al*., 2002). In cyanide toxicity, dopaminergic pathways in the brain are extremely sensitive to the oxidative stress and undergo necrotic death. A similar action has been noted in humans undergoing severe, acute intoxication in which there is a selective loss of dopaminergic pathways in the basal ganglia, resulting in Parkinson-like manifestations (Rosenberg *et al*., 1989).

5.6 Alteration of intermediary metabolism and lactic acidosis

Lactic acidosis is an important factor in cyanide toxicity that results primarily from metabolic alterations induced by CcOX inhibition. Increased blood lactate production is one of the earliest and most sensitive indicators of cyanide exposure, and the severity of the lactic acidosis can provide a prognostication of the clinical course of the toxicity (Vogel, 1987). Cyanide produces an immediate reduction of energy metabolism resulting in marked alterations in the intermediary metabolism pathways utilized for production of ATP and maintenance of cellular redox balance (Way, 1984). The metabolic component of toxicity and the resultant clinical manifestations are well characterized and have been studied in isolated cell models, intact animals, and humans (Vogel, 1987).

Immediately after exposure, cyanide inhibits CcOX resulting in a shift from aerobic to anaerobic metabolism. When this occurs, lactic acid is formed from pyruvate (Borowitz *et al*., 1992). As the anaerobic metabolism progresses, lactate accumulates intracellularly and eventually diffuses from cells into plasma. The result is a progressive fall in plasma bicarbonate and lactic acidosis (Ballantyne, 1987). Studies in cultured cells and intact animals show that changes in metabolism are compensatory mechanisms activated by reduced mitochondrial oxygen utilization, altered redox status (when mitochondria pathways cease to generate NAD^+) and decreased ATP levels (Isom *et al*., 1975). The shift from aerobic to anaerobic metabolism results in an increase of inorganic phosphate levels, accompanied by reduction of the ATP/ADP ratio, phosphocreatine, glycogen, and glucose (Maduh *et al*., 1991). Since NAD^+ generation by mitochondrial aerobic metabolism is blocked by cyanide, cells compensate by increasing oxidation of pyruvate to lactate, eventually resulting in lactic acidosis. As toxicity progresses, the shift to an anaerobic metabolism drives an imbalance of cellular redox status (depletion of NAD^+) which then initiates a complex series of oxido-reductive reactions (Maduh *et al*., 1991). Radiorespirometric analysis in intact animals showed that there is a shift from the anaerobic glycolytic pathway to the pentose phosphate pathway in order to maintain cellular redox status (Isom *et al*., 1975).

Lactic acid plasma levels are directly related to the severity of the poisoning and are used as an indicator of the progression of the toxicity (Baud *et al*., 2002). In combination with failure of normal cellular energetic generation, the acid-base imbalance that results from excessive production of lactate plays a pivotal role in the toxicity. Intracellular acidosis is associated with collapse of mitochondrial membrane potential, stimulation of inositol triphosphate-induced intracellular Ca^{2+} mobilization and impaired activity of the hydrogen ion exchanger in cell membranes (Borowitz *et al*., 1992). Physiologically these actions lead to impaired cell function and eventually if not reversed, a cell death pathway will be executed.

5.7 Conclusion

Acute cyanide intoxication is complex, resulting from concurrent disruption of multiple biochemical pathways and activation of cell injury/death cascades. Inhibition of CcOX is an initiating event that stimulates a series of toxic pathways producing death if not rapidly reversed. Onset of toxicity occurs within seconds of exposure and is attributed to rapid inhibition of oxidative metabolism, followed by intense oxidative stress that accelerates mitochdondrial dysfunction resulting in loss of basal aerobic metabolism and ionic homeostasis in susceptible tissues. Neuronal dysfunction and characteristic lesions associated with the post-intoxication sequelae can be attributed to activation of cell injury/death pathways as a result of the metabolic crisis and subsequent changes in critical ion handling mechanisms induced by cyanide.

The brain and heart are the primary toxicity targets since they are highly dependent on aerobic metabolism and are extremely sensitive to the energy deficit produced by cyanide. Decreased ATP generation, cellular redox imbalance and cellular/systemic lactic acidosis results in severe physiological consequences. In acute intoxication, the marked shift in metabolism produces a catastrophic metabolic crisis manifested as progressive decline in homeostasis and eventually loss of vital function. The course of the toxicity is generally reflected by the duration and severity of the cyanide-induced metabolic deficit and prognosis for a favorable outcome is reduced if the metabolic consequences are not rapidly reversed.

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CHAPTER 6
Environmental toxicology of cyanide

Samantha L. Malone, Linda L. Pearce, and Jim Peterson

At a Glance

- From a Public Health perspective, the general population are exposed to environmental cyanide from two main sources: 1) contaminated air, and 2) consumption of foodstuffs containing cyanogenic glycosides.
- Cyanide and cyanogenic nitrile compounds are efficiently degraded by soil microorganisms, such that filtration into the subsurface soil layers is usually insignificant.
- An exception to the above point is cyanide in landfills, tailing ponds, and spills where high levels of cyanide-containing waste may have been released.
- Cyanogen chloride may be formed when cyanide-polluted water is treated by chlorination.
- Fire and cigarette smokes both contain cyanide.
- A parsimonious global cyanide cycle is proposed in this Chapter.

6.1 Introduction

From a public health perspective, the available data (ATSDR, 2006) indicate that the general population is primarily exposed to cyanide in two ways worldwide. First, through inhalation of contaminated air including tobacco smoke, and, second, by ingestion of foods derived from cyanogenic plants. Air exposure is an essentially continuous, low-dose (i.e., chronic) process, with the exception of exposure during fires (see Fire Smoke section). Consumption of cyanogenic plant materials, especially by livestock (Merk Veterinary Manual, 2005), can result in symptoms of acute and chronic cyanide poisoning (ATSDR, 2006). While the deliberate consumption of cyanide-laced foods and beverages can be an effective method for murder/suicide (Bebarta *et al.*, 2011; Hall, 1979), accidental exposure from contaminated drinking water is of relatively low concern. The pK_a of HCN, ~9.24 at 25°C (Ghosh *et al.*, 2006), ensures that the toxic anion (CN^-) readily becomes protonated in aqueous media around neutral pH and, subsequently, the uncharged HCN molecule is rapidly lost to the atmosphere.

There are numerous routes by which cyanide may be released into the environment, but monitoring data suitable for quantifying the relative importance of the sources worldwide are scarce. Available data indicate that industrial manufacturing of cyanide may total approximately 2.3 million metric tonnes every year (Baskin *et al.*, 2009). While the estimates vary between $0.5 - 12.9 \times 10^{12}$ g of N/year emitted, the principal source of "environmental cyanide" (i.e., atmospheric HCN) is thought to be biomass burning (Crutzen & Carmichael, 1993; Flematti *et al.*, 2011; Li *et al.*, 2003; Lupu *et al.*, 2009), followed by – in no particular order – automobile emissions, volcanic activity, and loss of industrial containment, especially in association with mining operations (ATSDR, 2006). Deliberate releases of cyanide during activities such as "cyanide fishing" (Mak *et al.*, 2005) and fumigation (ATSDR, 2006) can be locally devastating to the wildlife targeted, but likely account for an insignificant addition to the total environmental cyanide burden.

The cyanide anion is a potent inhibitor of mitochondrial cytochrome *c* oxidase (respiratory complex IV) resulting in the observed acute toxicity toward the

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central nervous system and death by pulmonary failure (ATSDR, 2006). Many other enzyme systems are also subject to inhibition, but only at significantly higher cyanide concentrations (Ballantyne, 1987, Ballantyne and Salem, 2006). It is less widely appreciated that at lower cyanide concentrations, there are some intriguing non-toxic biological effects. For example, it has been independently verified in rats that potassium cyanide is radioprotective (Schubert and Markley, 1963, Strelina, 1970, van der Meer *et al.*, 1961) and metabolic cyanides appear to have multiple, beneficial effects in some plants (Xu *et al.*, 2012). More recently, it has emerged that nitric oxide is able to reverse the inhibitory action of cyanide at cytochrome *c* oxidase (Cambal *et al.*, 2011; Pearce *et al.*, 2008), thereby affording protection in the form of an endogenous antidote. However, the extent to which our tolerance of normal environmental (and dietary) cyanide levels depends (or not) upon endogenous nitric oxide is presently unclear.

In this chapter, covering the literature up to the end of December 2012, we review the major cyanide sources/sinks in relation to the environment and human exposure, and, so far as may be possible, assess the limits of what may be considered "normal" environmental cyanide levels.

6.2 Environmentally relevant chemistry of cyanides

Hydrogen cyanide is the IUPAC-approved name for the molecular compound HCN, a colorless liquid having the odor of bitter almonds. Aqueous solutions and their vapors are now known as hydrocyanic acid, having previously been called prussic acid. The HCN molecule is soluble in alkaline aqueous media due to its ability to ionize to the cyanide anion (CN^-) and hydronium ion. However, the pK_a of this weak acid is > 9, so that in mildly acidic-to-neutral natural waters the cyanide anion becomes protonated to the less soluble molecular acid – with a Henry's law constant favoring loss of HCN to the atmosphere (Ma *et al.*, 2010) (Table 6.1).

Large amounts of HCN are produced industrially – approximately 750,000 tons were produced in 2001 in the United States – and it is a highly valuable precursor to many chemical compounds ranging from polymers

Table 6.1 Physical properties of common cyanide compounds.[a]

Property	Hydrogen cyanide	Cyanogen chloride	Acetonitrile	Sodium cyanide	Potassium cyanide
Chemical formula	HCN	CNCl	CH_3CN	NaCN	KCN
CAS registry	74-90-8	506-77-4	75-05-8	143-33-9	151-50-8
Formula weight	27.03	61.47	41.05	49.01	65.12
Odor	Bitter almonds[b]	Pungent	Faint but distinct	Odorless if dry[c]	Odorless if dry[c]
Appearance[d]	Colorless	Colorless	Colorless	White	White
Physical state (STP)	Volatile liquid	Gas	Liquid/solvent	Solid/crystals	Solid/crystals
Melting point (°C)	−13.4	−6.0	−46.0	563.7	634.5
Boiling point (°C)	25.7	12.7–13.8	81.6	1496	Not available
Solubility (water)	Miscible	28 mg/l (25°C)	Miscible	480 g/l (10°C)	716 g/l (25°C)
Solubility (organic solvents)	Diethyl ether, ethanol	Diethyl ether, ethanol	Miscible	Ethanol, formamide[e]	Ethanol, methanol[e]
Log K_{ow}	0.66	Not available	−0.34 [f]	0.44	Not available
Henry's law constant	5.1×10^{-2} atm · m^3/mol	3.2×10^{-3} atm · m^3/mol	Not applicable	Not applicable	Not applicable

[a]Data obtained from ATSDR (2006) and references cited therein.
[b]Faint smell not detectable by everybody.
[c]Bitter almond smell of HCN apparent if wet.
[d]Pure compounds, aqueous solutions are colorless.
[e]Sparingly soluble in organic solvents.
[f]Data obtained from International Programme on Chemical Safety (1993) and references cited therein.

to pharmaceuticals (Wong-Chong *et al.*, 2006). There are two common manufacturing routes both involving the reaction of methane and ammonia at elevated temperature over a platinum catalyst (Housecroft & Sharpe, 2008), but the first of these continues to be the more important:

$$\begin{aligned} 2CH_4 + 2NH_3 + 3O_2 &\rightarrow 2HCN + 6H_2O \\ CH_4 + NH_3 &\rightarrow HCN + 3H_2 \end{aligned} \qquad (6.1)$$

A number of industrially important organic compounds are prepared by reaction of precursors with HCN including acetone → methyl methacrylate, used to form many resins and polymers, and butadiene → adiponitrile, the precursor to 1,6-diaminohexane used in the synthesis of Nylon 66 (Fox & Whitesell, 2004). (Acrylonitrile, a component of ABS plastics, is usually manufactured from propene and ammonia, not HCN.) The cyanide anion is a good nucleophile, which explains its use in organic chemistry as an attacking agent of partially positive carbons and its use in inorganic chemistry as a complexing agent for metal ions. Many industrial applications of cyanide make use of its complexing properties in various processes where metal surfaces are chemically modified, or metal mining operations. For example, the extraction of gold and silver during the refining of some ores utilizes the following chemistry (Housecroft & Sharpe, 2008):

$$\begin{aligned} Ag_2S + 4NaCN + H_2O &\rightarrow 2Na[Ag(CN)_2] \\ &\quad + NaSH + NaOH \\ 4Au + 8NaCN + O_2 + 2H_2O &\rightarrow 4Na[Au(CN)_2] \\ &\quad + 4NaOH \end{aligned} \qquad (6.2)$$

The organic chemistry of organo-cyanides, also referred to as nitriles, is in fact, somewhat similar to that of carboxylic acids. Both types of compounds have three carbon bonds to an electronegative atom and π bonding, which together render the carbon atom of the functional group somewhat positive and thus electrophilic. Consequently, common pathways to the degradation of nitriles (Fox & Whitesell, 2004) involve the acid-catalyzed addition of water to form an imine, followed by rearrangement to the amide, addition of a second water molecule, rearrangement and elimination of ammonium ion (Figure 6.1).

Thus, liberation of ammonia tends to be a feature of the environmental (and some biochemical) pathways to the degradation of nitriles, thereby linking environmental cyanide chemistry to the global nitrogen cycle.

Formation of nitriles is possible by several synthetic routes (Fox & Whitesell, 2004). For example, the addition of HCN to molecules containing carbonyl groups, forming hydroxynitriles, probably occurs in situations where inadequately contained cyanide waste comes into contact with organic matter (Figure 6.2, left). However, the dehydration of amides to nitriles is probably of greater biochemical importance (Figure 6.2, right):

The latter overall reaction is carried out by many plants in a series of steps to form cyanoglycosides (or cyanogenic glycosides) (Vetter, 2000). Cyanoglycosides contain a sugar ring connected by bridging oxygen to a nitrile bearing carbon. Thousands of these are known, prime examples being linamarin and dhurrin (Figure 6.3), the most prevalent cyanogenic glycosides found in, respectfully, cassava root (Nhassico *et al.*, 2008) and sorghum leaves (Busk & Møller, 2002).

Other examples of biological nitriles include those with anti-microbial and, in some cases, antitumor activities isolated from bacteria. For example, cyanocyclines, isolated from *Steptomyces*, are composed of an isoquinoline residue fused to a diazabicyclic core (Figure 6.4) (Arora & Cox, 1988). In addition, HCN is often "fixed" or combined with the amino acid alanine (Figure 6.5) where it may subsequently add water to form an amino carbonate. In summary, plants (algae, bacteria, cyanobacteria, fungi, and higher green plants) exhibit quite a diverse set of anabolic pathways leading to formation of nitriles, and we only present a few examples here.

Interestingly, many plants also produce HCN in small quantities (Peiser *et al.*, 1984). The plant hormone ethylene is generated by oxidation of aminocyclopropane carboxylic acid and HCN is released as a byproduct (see Figure 6.6).

Some microbes synthesize HCN, but a significantly greater number tend to biodegrade cyanide using a variety of pathways employing oxidative, reductive, hydrolytic, and group-exchange reactions (Ebbs, 2004). Pathways such as the cysteine → β-cyanoalanine → arginine conversion (Raybuck, 1992) can fix cyanide in the biosphere, but the vast majority lead to release of the nitrogen from cyanide as an ammonium ion (Ebbs, 2004). Cyanate and thiocyanate (excreted by animals) are intermediates in some of the microbial pathways and, consequently, the biosphere may be thought of as a

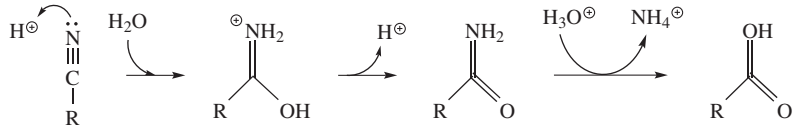

Figure 6.1 Hydrolytic degradation of nitriles.

Figure 6.2 Formation of nitriles.

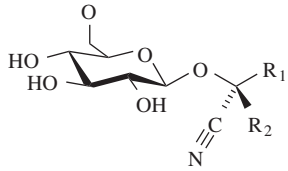

Figure 6.3 Linamarin (R1 ≡ R2 = −CH3) and dhurrin (R1 = *p*-hydroxyphenyl; R2 = −H).

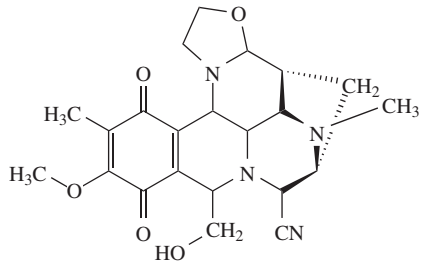

Figure 6.4 Cyanocycline A.

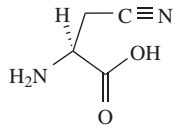

Figure 6.5 ß-cyanoalanine.

net converter of cyanide to ammonia thereby providing a link to the global nitrogen cycle. In most cases these reactions are carried out around neutral pH where cyanide is predominately protonated and the solubility of HCN is limited by Henry's law. However, recent studies searching for bacteria that could be useful in the remediation of highly contaminated (i.e., alkaline) soil have found and characterized a *Pseudomonas* strain of bacteria which degrades cyanide and its metal ion

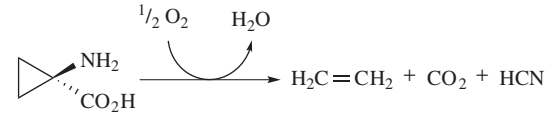

Figure 6.6 Release of HCN by plants.

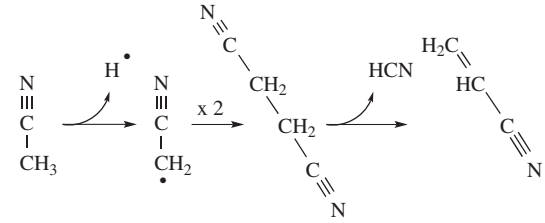

Figure 6.7 One mechanism of HCN release during pyrolysis of acetonitrile.

complexes at pH 11, while seemingly requiring only a carbon source (e.g., acetate) for cyanotrophic growth (Luque-Almagro *et al.*, 2011a; Luque-Almagro *et al.*, 2011b).

The combustion/pyrolysis of organonitriles and carbon-nitrogen containing polymers is complicated. The production of HCN from these materials is dependent on time-dependent temperature and oxygen concentration variations during the course of a fire. Detailed molecular studies are scarce, but the mechanism of combustion/pyrolysis of acetonitrile (CH_3CN), an important solvent and byproduct of acrylonitrile production, has been described in some detail (Britt, 2002). When oxygen is depleted and especially at temperatures below 1,000°C there is substantial formation of HCN due to pyrolysis of acetonitrile by radical mechanisms including the above process (see Figure 6.7).

When the relative amounts of fuel and oxygen are at least comparable, or oxygen is in excess, combustion

of CH_3CN producing CO and CO_2 results, and the oxidation of any HCN present to NO occurs. The extent to which these reactions of acetonitrile can be used as a model for combustion/pyrolysis of other nitrogen containing molecules, including polymers, is not entirely clear. However, it does seem reasonable to infer that low-temperature smoldering fires, particularly where oxygen is depleted, have the potential to produce the greatest amount of HCN – that is, during the salvage phase of firefighting operations.

Similarly, in natural fires the most important contributor to atmospheric HCN levels is thought to be biomass burning (Li *et al*., 2003) with the greatest production of HCN from brush fires occurring during the smoldering phases, and the most likely nitrogen source being amino acids (Lobert & Warnatz, 1993). A comparison of HCN production from the burning of different natural and synthetic materials is given in Table 6.2.

Recent work on modeling the persistence of atmospheric HCN suggests that in both the stratosphere and the troposphere, the major degradation pathway is via a reaction with hydroxyl radical, followed by a cascade of reactions dependent on oxygen-derived species, where the ultimate products, CO_2 and NO_x, feed into the global carbon and nitrogen cycles, respectively (see Figure 6.8).

In the stratosphere, HCN is thought to be a major trace gas at levels around 10 ppt and most likely degrades slowly with an average lifetime of 5–10 years per molecule (Kleinböhl *et al*., 2006). After the initial reaction with hydroxyl radical the product degrades in a very complicated fashion. A minor degradation pathway by initial reaction with singlet oxygen may also be of some significance. In the troposphere, HCN also predominately reacts with hydroxyl radical but the average residence time is less than 6 months per molecule. As the degradation pathway dependent on

Table 6.2 HCN produced by combustion of a variety of materials.[a]

Material	Temperature (°C)	Yield (g HCN produced/ gsample combusted)
Acrylonitrile	750	0.030[b]
	> 1,000 (low O_2)	0.590[b]
Acrylic fiber	800	0.095–0.193[c]
Nylon	650 (well-ventilated)	0.005[d]
	650 (ventilation limited)	0.018[d]
	800	0.0076–0.0700[c, e]
	900 (well-ventilated)	0.011[d]
Polyurethane	650 (well-ventilated)	0.003[d]
	650 (ventilation limited)	0.001[d]
	900 (well-ventilated)	0.0003[d]
Urea-formaldehyde foam	800	0.015–0.042[c]
Rigid urethane foam	800	0.008[c]
Silk	n/a	0.0222–0.0680[f]
	800	0.036[e]
Melamine	650 (well-ventilated)	0.001[d]
	900 (well-ventilated)	0.033[d]
Wool	350 (well-ventilated)	0.018[d]
	650 (well-ventilated)	0.002[d]
	900 (well-ventilated)	0.006[d]
	800	0.007–0.054[c, e]
	Not given	0.0126–0.0252[f]

[a]List not intended to be exhaustive.
[b]Data obtained from Britt (2002).
[c]Data obtained from Sumi and Tsuchiya (1973).
[d]Data obtained from Simonson *et al*. (2000).
[e]Data obtained from Hobbs and Patten (1962).
[f]Data obtained from Olsen et al. (1933).

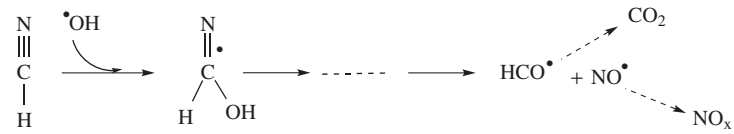

Figure 6.8 Atmospheric degradation of HCN.

hydroxyl radical is slow, the major sink is consensually argued to be the ocean (Lupu *et al.*, 2009; Li *et al.*, 2003). Presumably this is tied to microbial degradation, the algal and cyanobacterial populations of the ocean almost certainly being large enough to support this idea (Dzombak *et al.*, 2006).

6.3 Occupational concerns

Some of the cyanides commonly employed in industrial processes are either volatile themselves, or unavoidably converted to HCN upon contact with water (Table 6.1) – worrisome properties that facilitate dissemination of their toxic consequences. Despite this concern, the manufacture, commercial transport, and industrial use of these compounds make very little contribution (TRI03, 2005) to the overall cyanide content of the environment based on available data. More importantly, incidents similar to the Bhopal disaster in which the accidental release of methyl *isocyanate* from a manufacturing facility eventually resulted in 20,000 human casualities in India (Varma & Varma, 2005) have, to date, not occurred with cyanide compounds. Where large-scale spills of cyanide have occurred and been well documented, wildlife has sometimes been decimated, but there have been relatively few human fatalities reported (Table 6.3).

Workers may be exposed to cyanide on the job if they use cyanide compounds. According to the National Occupational Exposure Survey, the number of workers exposed to cyanides in the United States total 165,295 (ATSDR, 2006). Dermal and inhalation are the main routes of exposure for this population (Baskin *et al.*, 2009). While measured data are limited, the professions where a risk of being exposed exists include: cassava processing, factory work, electroplating, metal mining processes, metal finishing and plating, metallurgy, metal cleaning, pesticide application, leather tanning, photography and photoengraving, firefighting, gas works operations, and dye/pharmaceutical industries (ATSDR, 2006). NIOSH reports that workers who have been exposed to cyanide over time may experience symptoms ranging from headache, palpitations, loss of appetite, nausea, and irritation of the upper respiratory tract and eyes (NIOSH, 2011).

6.4 Ground/surface water

Cyanides/nitriles in soil are efficiently biodegraded by microorganisms (Ebbs, 2004) so that their infiltration into the subsurface layers is usually insignificant and aquifers do not become contaminated (ATSDR, 2006). The exception to this is in landfills, tailings, ponds, and spills where high levels of cyanide-containing waste may have been released (Mudder *et al.*, 2001). The concentration of cyanide in landfill leachates can be high enough to kill the microorganisms normally responsible for their degradation (Lagas *et al.*, 1982). Consequently, drinking water wells sunk in the vicinity of these incidents could conceivably become contaminated. Approximately 14% of households in the United States depend upon private wells for their domestic supplies (U.S. Census Bureau, 2008) – essentially closed systems delivering water directly into homes that potentially could result in the release of HCN gas in enclosed spaces like bathrooms, kitchens, laundries, and so on. Fortunately, to date, there seem to have been no such occurrences reported.

In the United States, 0.9 tons of pollutants per year were released into surface waters from registered industrial processes that use hydrogen cyanide. In comparison, 570 tons were released into the air and 779 tons placed into underground injection wells (TRI03, 2005). Free cyanide ($HCN + CN^-$) has been found in Canadian lakes at up to 19 ppb (μg HCN/l water) (Sekerka & Lechner, 1976) and measured in municipal drinking water at up to 11 ppb in Canada and the United States (ATSDR, 2006). At the mean environmental temperature of ~15°C (WMO, 2012), a reasonable estimate for the dimensionless form of Henry's law constant for the

Table 6.3 Major reported incidents of cyanide spills and leaks.[a]

Site/Operator/Location	Release Period	Type of Spill/Media	Quantity Spilled	Environmental Consequences	Human Causalities
Summitville gold mine, Summitville Consolidated Mining Co., Inc., Colorado, United States[b]	1986–1992	Cyanide, heavy metals and acid leached from the mine site into groundwater below heap leach pad and on several occasions leaked from transfer pipes into surface water	*unknown*	All stocked fish in nearby reservoir and in farm holding ponds died along 17 miles of river. Possible association with cyanide release; probable with acid and metals exposure.	0
Grouse Creek gold mining plant, Hecla Mining Co., Idaho, United States[c]	1994–1999	Several spills of cyanide solution containing sodium cyanide (NaCN)	> 18.93 m^3	Unknown. Closed site continues to leak. Fish kills reported.	0
Omai gold mine, Cambior Inc., Omai, Guyana[d]	1995	Walls of tailings pond were breached. Waste fluids containing cyanide leaked into surface waters	4, 200, 000 m^3	At least 20,000 steelhead fish died. Possible effects to nearby wildlife along 50-mile stretch of river.	0 – Human health effects reported
Aurul precious metals recovery plant, Esmeralda Exploration (Australian co.) and Romanian government, Baia Mare, Romania[e]	2000	Tailings dam broke, leaked cyanide and metal-rich liquid waste into surface waters	100, 000 m^3	Rapid death of aquatic organisms and animals living close to the polluted rivers. Disruption of drinking water supplies in 24 locations and for 2.5 million people.	0
Tarkwa gold mine, Gold Fields Limited, Tarkwa, Ghana[f]	2001	Pipe carrying cyanide solution broke, eventually reaching a nearby stream	900-650 m^3	Approximately 50 fish died from exposure. Additional distressed fish caught by residents.	0 – Human health effects reported
Granite mine transportation vehicle, Central Australia[g]	2002	Transportation accident spilled cyanide pellets (NaCN)	0.4 m^3	Killed > 500 birds and a dingo.	0
Phu Bia gold mine, Pan Australian Resources, Chai Somboun special zone, Laos[h]	2005	Heavy rainfall caused cyanide to leak from the mine into small nearby river	*unknown*	Killed fish in the nearby rivers, and impacted villagers within at least 3 km of the mine site.	0 – Human health effects reported
Lucebni Zavody chemicals plant, Kolin, Czech Republic[i]	2006	Cyanide-laced waste water overflowed into nearby river (CN^-)	600 $kgCN^-$ per 30 m^3 waste water	Contaminated 85 km of the river. 10 tons of fish died.	0

[a]Not meant to be an exhaustive list.
[b]Data obtained from USGS (2005).
[c]Data obtained from Cascadia Times (2000).
[d]Data obtained from Beebe (2001) and references cited therein.
[e]Data obtained from Soldán *et al*. (2001) and Bacsujlaky (2004).
[f]Data obtained from Amegbey and Adimado (2003).
[g]Data obtained from Wakeham and Blair (2002).
[h]Data obtained from Mineral Policy Institute (2005).
[i]Data obtained from Balej (2008) and European Rivers Network (2006).

partitioning of total cyanide between air and water is 4×10^{-3} (Dzombak *et al.*, 2006). Using the reported value of 11 ppb for cyanide ($HCN + CN^-$) in drinking water to calculate the predicted atmospheric concentration of HCN gives: $11\ ppb \times 4 \times 10^{-3} = 0.044\ ppb$. The analogous calculation for the Canadian lake data yields: $19\ ppb \times 4 \times 10^{-3} = 0.076\ ppb$. The background level of atmospheric HCN at sea level is seemingly around 0.1 ppb (Ambrose *et al.*, 2012; Li *et al.*, 2003). Therefore, the level of cyanide that has been found in oligotrophic lakes and processed drinking water is at, or just below, the level predicted by atmospheric exchange according to Henry's law.

In addition, cyanogen chloride, formed as a consequence of water treatment with chlorine, may also be present at up to 25 ppb (Zheng *et al.*, 2004). The molecular mass of cyanogen chloride (61.5) is about twice that of HCN (27) and so, there is up to ~22 ppb total cyanide concentration present in drinking water. The LD_{50} for orally administered cyanide in rats is ~3 mg/kg (ATSDR, 2006). Using this value to estimate the LD_{50} for 70 kg humans, one finds $3 \times 70 = 210$ mg. To achieve this LD_{50} dose by drinking water 22 ppb (0.022 mg/l) in cyanide would require the consumption of 9,545 liter – that is, at the average consumption rate of ~2 l/day, the amount of water that an adult person would normally consume in 13 years. Clearly, in the absence of any tampering, acute cyanide poisoning through drinking a properly managed public water supply should not be a concern. Of course, this statement does not directly apply to water drawn at private wells, where there may be additional sources of cyanide that are likely to persist without further processing.

6.5 Exposure to cyanogens through diet

Humans may also be naturally exposed to cyanide through their diet (Dolan *et al.*, 2010). Research indicates that cyanogenic β-glycosides (cyanides bound to sugar molecules containing a nitrile function) in plants help to protect them from being destroyed by pathogens and herbivores (Poulton, 1993), although the effectiveness of this strategy depends on the organisms that consume the plants (Jones, 1998; Møller & Siegler, 1999). In many animals, cyanide is metabolized into the less toxic thiocyanate (SCN^-), but a variety of foods also contain thiocyanate, including plants, dairy products, and meat. Thiocyanate is efficiently excreted by the body, and presently there is no concern that it may accumulate in humans, even though very little thiocyanate exposure data exist (ATSDR, 2006).

Approximately 2,650 identified plant species, including fruits, vegetables, and the pits of fruits and nuts, contain cyanogenic glycosides that release HCN upon hydrolysis. For humans, such hydrolysis occurs during digestion (ATSDR, 2006; Siegler, 1991; World Health Organization, 2007). In plants, cyanogenic glycosides are normally stored separately from the enzyme that converts them to cyanohydrins ($HO\text{-}C(R_2)\text{-}CN$), which are also readily hydrolyzed to produce cyanide (Selmar, 1993). This represents an exposure hazard to humans when the edible part of the plant contains high levels of these cyanogenic compounds and the rate of ingestion is faster than the rate in which the body detoxifies cyanide into thiocyanate (Donato, 2002; Jones, 1998; Westley, 1988). Newly germinated shoots typically contain the most cyanogenic potential (Busk & Møller, 2002; Chand *et al.*, 1992), particularly under drought conditions (Merk Veterinary Manual, 2005). This is why livestock cyanide intoxication due to grazing on the emerging shoots of cyanogenic, heat tolerant plants after a prolonged drought is a common scenario (Merk Veterinary Manual, 2005) – for example, there were 15 such cattle deaths recently reported in Texas (CBS News, 2012). Plant-derived foodstuffs may contain high levels of cyanide when the cyanogenic plants have not been properly prepared before consumption (ATSDR, 2006), and depending on the type of food, as summarized in Table 6.4.

For the United States population, the number of people exposed naturally to cyanogens in their food is not known (ATSDR, 2006), but accidental poisoning through the ingestion of cyanogenic food in industrialized countries is uncommon (Baud, 2007). A significant number of cyanide poisonings through ingestion in the United States (45%) occur as a result of swallowing a cyanide solution or cyanide salts to commit suicide (Bebarta *et al.*, 2011), as opposed to consuming naturally cyanogenic foods or through accidental occupational exposures (Baskin *et al.*, 2009; Gill *et al.*, 2004).

6.6 Dietary health hazards

While acute cyanide toxicity is known to be mediated through inhibition of mitochondrial cytochrome *c*

Table 6.4 Cyanide concentrations in food products.

Plant Type[a]	Releasable HCN (mg/kg or mg/liter)
Cassava – whole tubers (roots)	380–445[b]
Mash (sweet)	81[c]
Dried roots (bitter)	95–2,450[c]
Leaves (bitter)	347–1,000[b, c]
Dried root cortex (bitter)	2360[b]
Gari flour (Nigeria)	10.6–22.1[b]
Sorghum – whole immature plant	2400–2,500[b, c]
Leaves (wet weight) (CN^-)	192–1,250[b, d]
Bamboo – immature shoot tip	7,700–8,000[b, c]
Soy protein products (processed)	0.07–0.3[b]
Soybean hulls	1.24[b]
Lima beans from Puerto Rico (black)	2,900–3,000[b, c]
from Java (colored)	3,000–3,120[b, c]
from Burma (white)	2,000–2,100[b, c]
U.S. lima beans	100–170[b, c]
Commercial cherry juice (processed)	4.6[b]
Apricot pits (wet weight)	89–2,170[b]
Cereal grains and their products (processed)	0.001–0.45[b]

[a]Unprocessed unless otherwise indicated.
[b]Data obtained from WHO (2004) and ATSDR (2006) and references cited therein.
[c]Data obtained from Eisler (1991).
[d]Data obtained from Chand *et al*. (1992).

oxidase (Ballantyne, 1987, Ballantyne, and Salem, 2006), the molecular mechanism(s) involved in chronic (low-level) cyanide intoxication is (are) presently unknown. Human diets deficient in protein, sulfur, riboflavin (vitamin B_2) and hydroxocobalamine (vitamin B_{12}) show greater risks of health effects from consuming foods high in cyanide, especially cassava and sorghum (ATSDR, 2006; Oke, 1980; Speijers, 1993). In Africa, chronic cyanide poisoning has been attributed to consumption of cassava and nutritional deficiencies, resulting in spastic paraparesis or "konzo" (Howlett, 1994; Tylleskar *et al*., 1992) and implicated in tropical ataxic polyneuropathy and the stunting of children (Oluwole *et al*., 2003). People often experience significant effects on the central nervous system, including weakness in the fingers and toes, dimness of vision, and deafness. Impacts on the thyroid gland have also been linked to the consumption of highly cyanogenic cassava (ATSDR, 2006). It should be noted that consumption of cassava or its cyanogen may not be the only potential causes of these health effects. Interestingly, there is some evidence to suggest that low-level cyanide consumption and inhalation (10 ppm for 2 hours) can induce hearing deficiencies and loss through noise promulgation (Fechter *et al*., 2002). Concern regarding the level of cyanogens in cassava and sorghum is compounded by the sheer number of people whose diet is primarily made up of these foods – hundreds of millions across the globe (WHO, 2004).

6.7 Cassava consumption

Cassava, in particular, serves as a staple food for developing countries within Africa, South and Central America, Southeast Asia, and India. Other names for cassava include *Manihot esculenta*, tapioca, manioc, or yucca. The cyanogen of concern in cassava is linamarin (Figure 6.3). With proper processing – which involves drying, fermenting, soaking in water, rinsing and/or baking the cassava – toxic cyanogen levels can be decreased up to 97–99% (Burns *et al*., 2012; Ferreira *et al*., 1995; Ngudi *et al*., 2003). Unfortunately, during periods of food shortage, drought, or a rush to get the product to market, cassava may not be thoroughly processed (Nhassico *et al*., 2008). For example, as recently as 2011 there were reported cases of unsafe levels of cyanide being found in ready-to-eat cassava snacks (Miles *et al*., 2011).

The amount of cyanide actually consumed through cassava intake is difficult to gauge and varies by region and population. The worldwide average consumption of cassava from 2005–7 was 43 Calories (kcal)/person/day. Daily cassava consumption in some countries such as the Democratic Republic of Congo, Mozambique, and Ghana were as high as 843, 658, and 603 kcals, respectively (Food and Agriculture Organization, 2010). There have also been estimations regarding the average concentration of HCN within cassava that disagree with the more commonly accepted ranges reported in Table 6.4. Table 6.5 demonstrates the difficulty in estimating the average daily dose per kg body weight of HCN through the consumption of cassava due to this variability, differences in consumption rates per day, and the type of cassava product ingested.

In 1996, due to food scarcities in impoverished countries, the World Bank's Consultative Group on

Table 6.5 Estimating human exposure to HCN through cassava consumption.

		Estimated HCN intake mg/HCN/person/day[a]					
	kcal within edible portion/g[b]	**High concentration: 255 mg/HCN/kg[c]**		**Medium concentration: 38 mg/HCN/kg[d]**		**Low concentration: 0.1 mg/HCN/kg[e]**	
Daily consumption		*43 Cal*	*843 Cal*	*43 Cal*	*843 Cal*	*43 Cal*	*843 Cal*
Fresh cassava	1.46	0.1073	2.1034	0.016	0.3134	n/a	n/a
Meal/flour cassava (heated)	3.38	n/a [f]	n/a	n/a	n/a	0.0002	0.0036

[a]70 kg body weight assumed per person.

[b]Data obtained from World Health Organization (1972) and references cited therein.

[c]Assessed fresh cassava (not processed). Data obtained from the following sources: Yeoh and Sun (2001), Siritunga and Sayre (2003), Dufour (1988). HCN concentration results ranged from 10–500 mg cyanide equivalent/kg dry matter.

[d]Assessed fresh cassava (not processed). Data obtained from Yeoh and Sun (2001). HCN concentration results ranged from 15–61 mg HCN/kg.

[e]Data obtained from Emmanuel *et al*. (2012). HCN concentration results ranged from 0.08–0.12 mg/HCN/kg dry weight. List of studies and concentrations not meant to be exhaustive.

[f]N/A: Not measured in the referenced study.

Exposure Limits Comparison:

Oral LD_{50}: 3 mg HCN-kg (in rats non-fasting) according to ATSDR (2006).

NOAEL: 12.5–28.8 mg HCN/kg-day (mice and rats) according to ATSDR (2006).

Chronic Oral RfD for cyanogen: 0.001 mg HCN/kg-day (daily oral exposure to population and sensitive subgroups without appreciable risk during lifetime) according to U.S. EPA (2010).

International Agricultural Research recommended more cassava cultivation (Babaleye, 1996). Some researchers, however, recommend using caution when promoting cassava cultivation to countries where it was previously never used as this could increase the risk of cyanide poisoning due to improper cassava processing (Nhassico *et al*., 2008). Progress has been made to reduce the risk by creating a cassava strain that contains 60–94% less leaf linamarin and 99% less root linamarin (Siritunga & Sayre, 2003). Increased sorghum (a hardy cereal grain) production has also been recommended to provide food in places where it is difficult to grow most crops (International Fund for Agricultural Development, 2011). As shown in Table 6.4, however, sorghum has been found to contain higher levels of HCN compared to cassava according to data compiled by ATSDR (2006) and others. Alternatively, recent data using a chilling method indicate a much lower range of HCN concentrations in sorghum than was originally estimated: 6.65 – 1.68 mg/100 g (Prasad & Dhanya, 2011). In order to properly assess risk and make intelligent policy recommendations these data and measurement discrepancies should be addressed. They challenge the validity of present risk assessments and exposure limits, which were developed primarily from animal studies.

6.8 Fires and smoke

6.8.1 Fire smoke

Hydrogen cyanide is also a byproduct of the combustion of materials in products used in everyday life (insulation, carpets, clothing, and synthetics), especially synthetic plastic and resins containing nitrogen that burn when the fire is hot and in an enclosed space. Common synthetic materials that generate cyanide gas during combustion include nylon, polyurethane, melamine, and acrylonitrile. HCN poisoning has even been indicated in injuries and deaths during prison fires when inmates set fire to mattresses (Fortin *et al*., 2011; Ferrari *et al*., 2001). Increasingly, research is pointing to HCN as a substance that is as much a threat to first responders and victims encountering fire smoke as carbon monoxide (Alarie, 2002; Stamyr *et al*., 2012). The diverse components of the fire (e.g., heat, CO) can have additive and possibly synergistic effects with the HCN present. Such an environment may induce sublethal intoxication and limit the ability to escape the situation or perform rescue operations (Eckstein & Maniscalco, 2006), as may have been the case for several U.S. aircraft incidents involving fires during flight (Chaturvedi & Sanders, 1996).

Table 6.6 Comparison of the HCN levels found in tobacco versus marijuana smoke under two smoking conditions.

	Mainstream Smoke[a]				Sidestream Smoke			
	ISO[b]		Extreme[c]		ISO		Extreme	
	tobacco	*marijuana*	*tobacco*	*marijuana*	*tobacco*	*marijuana*	*tobacco*	*marijuana*
HCN (μg/cig)	208	526	320	1668	84	685	103	678

[a]All data obtained from Moir *et al*. (2008).

[b]International Organization for Standardization standard (ISO 3308), Routine Analytical Cigarette-Smoking Machine, Definitions and Standard Conditions

[c]Extreme conditions: > 700°C

6.8.2 Cigarette smoke

Although cigarette smoking among American high school students is declining, the proportion of students smoking (19.5%) (National Institute on Drug Abuse, 2011) is in fact the same as the proportion of adults over 18 that smoke in the United States (19.3%) (CDC, 2011). Consequently the net level of smoking in the overall population is likely to remain relatively stable for years to come. The proportion of people worldwide exposed to secondhand smoke is estimated to be up to 40% of children, 35% of women, and 33% of men (Öberg *et al*., 2011) – in 2006 this represented 126 million Americans. It has been suggested that cyanide and thiocyanate can cross the placenta, putting fetuses of smoking mothers at risk of exposure (U.S. EPA, 2010). Even though the composition of cigarette smoke and its effects have been studied for many years, new research continues to uncover the various dangers associated with this persistent behavior.

Cigarette smoke is a complex, dynamic aerosol containing approximately 4,000 distinct chemicals (O'Connor & Hurley, 2008). Smoking cigarettes is known to increase levels of HCN in the blood (Chandra *et al*., 1980). In non-smokers, cyanide levels are reported to be between ~ 0.2 μM (Tsuge *et al*., 2000) and ~3 μM (Borowitz *et al*., 2006); whereas in smokers blood cyanide levels are reported to vary between ~0.3 μM (Tsuge *et al*., 2000) and ~7 μM (Borowitz *et al*., 2006). While these absolute estimates vary by an order of magnitude, there seems to be a consensus regarding the relative levels of blood cyanide between the two groups: 1.6–2.3 times higher in smokers than non-smokers (Borowitz *et al*., 2006; Tsuge *et al*., 2000). Due to the complex nature of cigarette smoke and especially the combined effects of its components, disentangling the particular role of cyanide in smoking-related health outcomes continues to be challenging.

Research suggests that the levels of cyanide in mainstream, or inhaled, smoke from cigarettes purchased in the United States range from 10 to 400 μg per cigarette, and the ratio of HCN in sidestream compared to mainstream smoke to be about 0.006–0.27 per cigarette (ATSDR, 2006, Guo *et al*., 2012, Guthery and Taylor, 2011). However, one recent study has reported a higher range of 170–830 μg/cigarette (Bodnar *et al*., 2012). Cigarettes available outside of the United States show relatively higher ranges of HCN in smoke: 280 – 550 μg/cigarette (mainstream) and 53 – 111 μg/cigarette (sidestream), respectfully (ATSDR, 2006). Comparatively, marijuana use among youth in the U.S. is now higher than cigarette smoking according to certain parameters (seemingly due to both decreases in cigarette use and increases in marijuana use) (National Institute on Drug Abuse, 2011). While smoking marijuana may be considered a safer alternative to cigarettes by some (Zimmer & Morgan, 1997), marijuana smoke appears to contain roughly five times more cyanide in both mainstream and sidestream smoke compared to tobacco (Moir *et al*., 2008). See Table 6.6.

6.9 Conclusion

The transport and fate of manufactured cyanide compounds entering soil and water has quite recently been reviewed in considerable detail by Dzombak *et al*. (2006). Within this discourse, Ghosh *et al*. (2006) have presented both anthropogenic and natural cycles describing the recycling and transformation of cyanides. At the risk of being too parsimonious with the

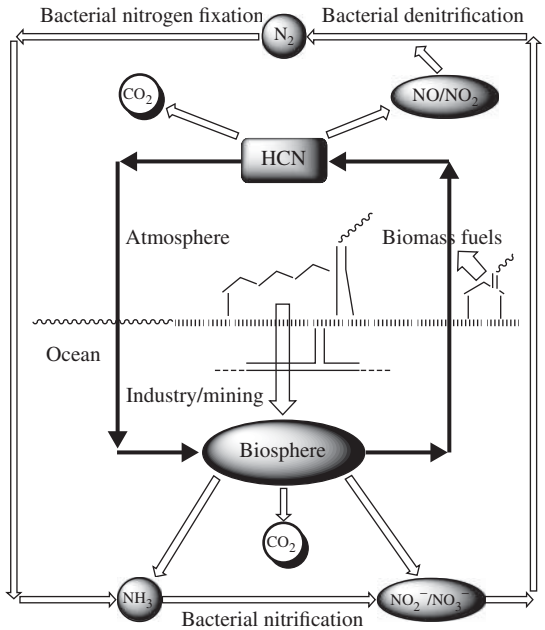

Figure 6.9 A parsimonious global cyanide cycle.

information content, we present here a minimal global cyanide cycle – delineating the major cyanide fluxes between the biosphere and the environment as they currently appear to be understood (see Figure 6.9).

Industrial/mining activity is currently responsible for relatively little release of cyanide into the soil and groundwater. Bacteria in landfills process cyanogenic effluent efficiently, preventing any cyanide migration into the wider biosphere/environment. Providing due diligence continues to be observed with regard to the monitoring and management of industrial/mining practices, this situation need not change. The majority of HCN released into the atmosphere originates in the burning of biomass fuels for both domestic and industrial purposes. Most atmospheric HCN partitions into bodies of water, the oceans being the largest, before it can be transformed in the atmosphere. Bacteria in the hydrosphere initialize the biochemical conversion of HCN to metabolites, some of which will eventually form biomass to be used as fuel, thus beginning the cycle again. The cyanide cycle is connected to and subordinate to both the global nitrogen cycle (as shown) and the global carbon cycle (through CO_2). Provided the carbon and nitrogen cycles remain stable, it would require an enormous increase in the level of anthropogenic HCN release to significantly disturb the global steady-state levels of the cyanide-cycle components. Consequently, cyanide in the environment is of low concern at this time and, given current trends in the development of cleaner energy sources, can probably remain so in the future despite the increasing demands of Earth's growing population.

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CHAPTER 7

Cyanide in the production of long-term adverse health effects in humans

Julie Cliff, Hipolito Nzwalo, and Humberto Muquingue

At a Glance

- More than 2000 plant species contain cyanogenic glycosides.
- Cassava (Manihot esculenta) is one of these plants.
- Cassava is a staple food for millions of people in tropical and sub-tropical areas of the world.
- When properly prepared, Cassava is safe to eat.
- When improperly prepared, the cyanide components (linamarin and lotaustralin) may cause neurological diseases.
- These neurological disease are konzo (a sudden-onset spastic paraparesis) and tropical ataxic neuropathy (a rather typical dying-back peripheral neuropathy).
- Available cyanide antidotes have generally not been efficacious in these neurological conditions.

7.1 Introduction

In this chapter, we will discuss the long-term adverse health effects of cyanide exposure in humans. These long-term effects are distinct from the effects of acute poisoning caused by single and short-term exposure, which are discussed in other chapters.

7.1.1 Sources of cyanide exposure

Various cyanide-generating compounds, such as hydrogen cyanide, cyanide salts and cyanogenic glycosides, may be sources of cyanide exposure. These compounds occur naturally or as a result of human activity; human exposure may result from ingestion and, less commonly, inhalation and skin contact.

Most people are exposed to very low levels of cyanide in the environment, but in some specific groups and circumstances the potential exists for much higher levels of exposure. This exposure over the medium and long term can cause long-term adverse health effects.

Cyanide ingestion

Over 2000 species of plants contain cyanogenic glycosides, which can liberate hydrogen cyanide as a defense against animals and marauding insects (White *et al.*, 1994; Jones, 1998; WHO, 2004). This phenomenon is called cyanogenesis. Cassava (*Manihot esculenta*) is by far the most important human food source that uses cyanide as a defense mechanism (Jones, 1998). Cyanogenic glycosides are also present in lima beans, linseed, sorghum, sweet potato, and bamboo shoots (Sang *et al.*, 2011). Stone fruit kernels such as bitter almonds and apricot kernels contain the cyanogenic glycoside amygdalin, and accidental ingestion may cause acute poisoning (Shragg *et al.*, 1982; WHO, 2004; Akyildiz *et al.*, 2010; Sanchez-Verlaan *et al.*, 2011).

Contamination of the food chain has been linked to accidental contamination of water sources. Crop treatment with cyanide-containing fumigants is also a possible source of contamination. Recently, there has been increased concern regarding possible terrorist acts targeting food and water supplies with cyanide as a plausible adulteration vehicle (Khan *et al.*, 2001).

Toxicology of Cyanides and Cyanogens: Experimental, Applied and Clinical Aspects, First Edition.
Edited by Alan H. Hall, Gary E. Isom and Gary A. Rockwood.

The alternative cancer treatments of amygdalin and laetrile (a semi-synthetic form of amygdalin) may also be a source of cyanide exposure. Although banned by the Food and Drug Administration (FDA) in the United States and by the European Commission, they are available for purchase on the Internet (Milazzo *et al*., 2011). Cases of acute cyanide poisoning continue to be reported (Bromley *et al*., 2005; O'Brien *et al*., 2005), but the recent literature has no reports of the chronic syndromes cited by Wilson (1987). This may be due to the treatment's declining popularity, as awareness of the side effects has increased.

Cassava as a source of cyanide exposure

Cassava is a staple for over 800 million people in approximately 80 countries in the tropics and subtropics, mostly in sub-Saharan Africa, but also in other parts of Africa, Asia, the Pacific, and South America (Burns *et al*., 2010).

Roots and leaves of cassava of all varieties contain varying concentrations of cyanogenic glycosides, mainly as linamarin but also as lotaustralin, in their cellular vacuoles (Mkpong *et al*., 1990; Jones, 1998). Cassava varieties with high concentrations of linamarin are usually classified as bitter, although taste is not always a reliable predictor of cyanogenic glycoside content (King & Bradbury, 1995; Chiwona-Karltun *et al*., 2004; Oluwole *et al*., 2007). The concentration of cyanogenic glycosides depends on both genetic and environmental factors, and agro-ecological differences such as water stress can influence the cyanogenic potential of the same cassava cultivar (Bokanga *et al*., 1994; Nwosu & Onofeghara, 1994; Burns *et al*., 2012).

Cyanogenic glycosides in cassava roots and leaves can be reduced by appropriate processing prior to consumption. Cyanogenesis is initiated when the plant tissue is damaged, and linamarase, a cell wall enzyme, hydrolyzes linamarin to produce acetone cyanohydrin. Processing also improves palatability and increases the shelf life of the root (Mlingi, 1995; Cardoso *et al*., 2005).

High dietary cyanogen exposure mainly occurs when high cyanogenic cassava is insufficiently processed, usually in a context of food shortage. Drought, crop failure, and sometimes commercialization may all result in short cuts in processing (Nzwalo & Cliff, 2011). Water stress may increase the cyanogenic glycoside concentration in cassava to a level where the traditional processing methods can no longer avoid high retention of cyanogens (Cardoso *et al*., 1999, 2005; Dufour, 2011). The sun-drying method used in much of sub-Saharan Africa is also relatively inefficient at removing cyanogens, and high levels may remain, even in normal conditions (Cardoso *et al*., 2005; Nhassico *et al*., 2008).

Inhalation and skin absorption

In the general population, active and passive smokers are a subgroup with high potential for cyanide exposure.

Inhalation and skin absorption are principal routes of occupational exposure. Workers involved in many industries – for example, electroplating and fumigation – may be exposed to higher concentrations of cyanide (WHO, 2004). Cassava processors may also be at risk through inhalation; in Nigeria, a study found that cassava processors had higher concentrations of urinary thiocyanate than cassava consumers, suggesting possible long-term exposure (Okafor *et al*., 2002).

Exposure from microorganisms

Cyanide is commonly found in microorganisms, including fungi and bacteria, where it provides a source of nitrogen and carbon, needed for growth (Kunz *et al*., 1992). It may also provide a defense mechanism (as in the case of non-cyanogenic fungi that detoxify cyanide as they infest cyanogenic plants). Soil microorganisms can hydrolyze exuded cyanogenic glycosides (such as linamarin from cassava tubers) into hydrogen cyanide.

Cyanide-producing bacteria include *Pseudomonas*, *Chromobacterium*, *Anacystis*, *Nostoc*, *Plectonema* and *Rhizobium*, all of which are able to metabolize glycine, the first being the most studied (Castric, 1977; Gallagher & Manoil, 2001; Rudrappa *et al*., 2008). Several hundred fungi also produce cyanide (Wilson, 1987). Paradoxically, the ability of numerous other prokaryotes to degrade cyanide has led to an increased commercial and industrial utilization of cyanide-degrading microorganisms to treat contaminated waste, water, and soils. Frequently used in these bioremediation processes are *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Nocardia*, and *Mycobacterium*.

The role of microorganisms in inducing cyanide intoxication in humans is unknown, as most of the cyanide produced diffuses into the soil, bound to metallic ions, and eventually evaporates into the air. The likelihood of significant food contamination by cyanogenic fungi and bacteria is low.

7.1.2 Detoxification in the body

Most cyanide is converted to thiocyanate in the liver by sulfurtransferases, including the mitochondrial sulfurtransferase enzyme rhodanese; thiocyanate is then excreted in the urine (WHO, 2004). The transformation to thiocyanate requires sulfur donors, provided from dietary sulfur amino acids (SAAs) (Tor-Agbidye, Palmer, Lasarev, *et al.*, 1999). Capture of cyanide with iron in erythrocytic hemoglobin is an important temporary detoxification mechanism. Minor pathways include combination with hydroxocobalamin (vitamin B12a) to yield cyanocobalamin (vitamin B12) and reaction with cystine to produce 2-iminothiazoline-4-carboxylic acid (WHO, 2004).

7.2 Long-term adverse health effects

7.2.1 Introduction

Many diseases have been attributed to dietary cyanide exposure from cassava. They include the neurological disorders of tropical ataxic neuropathy and konzo, iodine deficiency disorders, diabetes, endomyocardial fibrosis, and malnutrition. The possibility of subtle cognitive decline resulting from repeated episodes of acute or subacute cyanide intoxication suggested by Spencer (1994) has still not been investigated.

Serious injury from long-term occupational exposure is rather rare. Reported symptoms include gastrointestinal disturbances, headache, dizziness, confusion, muscular weakness, poor vision and slurred speech, and thyroid disorders (WHO, 2004). Cyanide from tobacco smoke may contribute to tobacco amblyopia, a now rare syndrome (WHO, 2004). Subacute combined degeneration of the spinal cord has also been attributed to cyanide exposure.

Reproductive and developmental toxicity from cyanides has been shown in experimental animals only (WHO, 2004). No evidence is available of toxic effects on fertility or of teratogenicity in humans.

7.2.2 Tropical ataxic neuropathy

Clinical features

The essential neurological components of tropical ataxic neuropathy (TAN) are posterior column myelopathy of insidious onset, bilateral optic atrophy, bilateral nerve deafness and polyneuropathy (Osuntokun, 1981). The clinical diagnosis requires at least two of these features to be present.

The onset of TAN is usually insidious, but occasionally a history of sudden blurring of vision preceded by paresthesia is obtained (Osuntokun, 1981). Myelopathy and optic atrophy are usually the earliest manifestations. Symptoms and severity may fluctuate during pregnancy, lactation, and the rainy season. The illness is initially progressive, but then becomes static. Wilson (1987) describes significant clinical improvement with hospital admission, probably due to a reduction of dietary cyanide intake.

In a large series of 400 patients described by Osuntokun (1981), stomatoglossitis was present in about one-third of patients. Motor neuron disease, Parkinsonism, cerebellar degeneration, psychosis, and dementia were also found in association with the disease.

History and geographical distribution

Neurological disorders characterized by visual failure, paresthesia, spasticity, and impaired posterior column sensation have been reported in the tropics and subtropics since the 19th century (Wilson, 1987). In the 1930s, various authors in Nigeria postulated that cassava could be the cause of ataxic neuropathy (Osuntokun, 1994). These observations were not followed up until 1958, when Money (1958) reported a similar syndrome in southwestern Nigeria. Monekosso and Wilson (1966) also linked ataxic neuropathy to exposure to cyanide from cassava and showed that cases had higher levels of thiocyanate than controls. Between 1966 and 1968, Osuntokun (1981) and colleagues carried out an extensive series of studies to describe TAN.

TAN was also reported from Tanzania in the 1960s and 1970s (Haddock, 1962; Makene and Wilson, 1972), but no cases have been reported from Tanzania in recent decades. Apart from one isolated report of two cases from Liberia in 1990 (Ngoh, 1990), all reports of tropical ataxic neuropathy associated with cassava in Africa in the past 40 years have been from Nigeria. More recent studies show that TAN persists in southwestern Nigeria (Oluwole *et al.*, 2000). Cases of TAN occurring in association with cyanide exposure have also been reported recently from Kerala in India (Madhusudanan *et al.*, 2008).

Epidemiology

TAN is an endemic disease and does not occur in outbreaks. In Nigeria, TAN has always occurred in poor rural populations living in the tropical rainforest who use cassava as a staple (Wilson, 1987). In 1999, Oluwole and colleagues (2000) carried out a community study to estimate the prevalence rate of TAN in Ososa, an endemic area in southwestern Nigeria, previously studied in 1969. They found a rate of 60/1000, compared to the 24/1000 found in 1969. This may have been due to differences in study design or a change in population structure, but they concluded that new cases of TAN had probably occurred over the decades.

Both incidence and prevalence rates of TAN increase with age (Osuntokun, 1994; Oluwole *et al*., 2003), and the disease occurs only rarely in children under 10 years of age (Osuntokun, 1994). Males and females are affected equally, with often more than one family member affected (Osuntokun, 1994).

7.2.3 Konzo

Clinical features

Konzo is a distinct neurological entity, characterized by an abrupt onset of a permanent, non-progressive, and symmetrical spastic para/tetraparesis, due to an upper motor neuron lesion (Howlett *et al*., 1990).

The disease varies in severity, but always affects upper motor neurons with longer axons to a greater extent (Howlett *et al*., 1990). Hence paraparesis is the dominant feature. The upper limbs are usually involved, with increased reflexes and sometimes functional impairment. Additional neurological findings such as optic neuropathy, rotatory nystagmus, dysarthria, and deafness are seen in some cases (Ministry of Health, 1984; Rosling & Tylleskar, 1995; Howlett, 1994; Howlett *et al*., 1990). Contractures are a common complication in more severe cases without physiotherapy, and painful calf muscle spasm may be a major chronic symptom (Cliff and Nicala, 1997; Tshala-Katumbay *et al*., 2001).

Subclinical signs of upper motor damage, in the form of spasticity resulting in exaggerated reflexes and ankle clonus, are found in apparently healthy people in konzo-affected populations. Studies have shown that the rate of ankle clonus is high in apparently healthy schoolchildren in konzo-affected areas two years after the epidemics (Cliff *et al*., 1999).

History and geographical distribution

Konzo was first reported from the Belgian Congo (now the Democratic Republic of Congo – DRC) in the 1930s (Trolli, 1938). Over 70 years later, outbreaks and persistent disease continue in the DRC (Banea-Mayambu *et al*., 2009). In recent decades, konzo has been reported from Angola (Bettencourt Mateus *et al*., 2011), Cameroon (Ciglenecki *et al*., 2011), the Central African Republic (Tylleskar *et al*., 1994; Mbelesso *et al*., 2009), Mozambique (Ministry of Health, 1984; Cliff *et al*., 1997; Cliff *et al*., 2011), and Tanzania (Howlett *et al*., 1990; Mlingi *et al*., 2011).

Konzo has always been associated with prolonged high dietary cyanogen consumption from insufficiently processed roots of bitter cassava combined with a protein-deficient diet, low in SAAs (Nzwalo & Cliff, 2011).

Epidemiology

Konzo is both epidemic and persistent (Ernesto *et al*., 2002). Epidemics occur during agricultural crises, often associated with war or drought. Those affected in both epidemic and persistent disease belong to the poorest segments of the most remote rural areas of Africa (Nzwalo & Cliff, 2011).

The burden of konzo in communities varies, but can be high. All studies covering large areas give a wide range of incidence and prevalence. For example, in a study of the 1981 epidemic in Mozambique, the incidence rate varied from 0.1 to 29/1000 (Ministry of Health, 1984). Similar variations have been reported in the DRC, with prevalence rates up to 40/1000 (Banea *et al*., 1992; Bonmarin *et al*., 2002). Second attacks of konzo have been reported in around 10% of patients.

In all reported studies, konzo has predominantly affected children and women of reproductive age. The disease spares breastfed infants, who eat the least cassava and have the highest SAA intake in the affected communities. Children begin to acquire the disease from three years on. Familial clustering has been reported in most series (Tylleskar *et al*., 1991).

7.2.4 Pathogenesis and etiological role of cassava in TAN and konzo

As mentioned above, cassava has been suggested as a causative agent in both TAN and konzo.

Between 1966 and 1968, Osuntokun (1981) and colleagues carried out an extensive series of studies to elucidate the cause of TAN and the role of cyanide in cassava. In 1994, Osuntokun (1994) reviewed the evidence from these studies and concluded that a cassava diet was the major cause. Evidence in favor was, first, clinical, including higher plasma concentrations of thiocyanate in patients than controls. Second, epidemiological evidence showed that endemic foci of TAN coincided with the areas where cassava was intensely cultivated and consumed. In endemic areas, evidence existed of increased exposure to cyanide in members of families with multiple or conjugal cases, compared to normal families. Other evidence was lacking, as experimental animal studies had failed to reproduce the lesions of TAN on any scale. Therapeutic trials had also essentially failed.

More recently, Oluwole and his colleagues (Oluwole *et al*., 2002, 2003; Oluwole & Onabolu, 2003) have thrown doubt on the causative role of cassava and cyanide in TAN. An ecological study showed that two communities in non-endemic areas had higher exposure to cyanide from cassava foods than TAN endemic communities. A study of one of these communities with high cyanide exposure found a lower prevalence of ataxic polyneuropathy (1.7/1000) than in an endemic community (49/1000). Plasma thiocyanate was above the reference level in 65% of subjects in the non-endemic area and 40% in the endemic area (Oluwole *et al*., 2002). In a nested case control study, they showed a comparable intake of cassava foods, levels of thiocyanate in the plasma, and levels of thiols in the plasma of ataxic polyneuropathy cases and controls (Oluwole *et al*., 2003).

Osuntokun (1968) had also hypothesized that a dietary deficiency in SAAs causing impairment of cyanide detoxification could be the cause of the neurological lesion in TAN. However, more recent research found no difference in the concentration of SAAs in cases of TAN and controls (Oluwole *et al*., 2003). Thus, different or additional factors may be causing TAN. A role for vitamin and other micronutrient deficiencies has been suggested, but with the exception of one study, which found low levels of riboflavin in patients with TAN (Osuntokun, 1968), no nutritional contribution to TAN pathogenesis has been demonstrated. Furthermore, supplementation with vitamins and protein was not associated with any clinical improvement in patients with TAN (Osuntokun *et al*., 1970, 1974, 1985). The exact pathogenesis of TAN remains uncertain.

For konzo, in the first report of the disease, written in 1938, exclusive consumption of insufficiently processed cassava was suggested as a cause (Trolli, 1938). More recently, the combination of a high intake of cyanogenic glycosides from cassava and a low intake of SAAs has been considered as the cause of neurotoxicity in konzo (Cliff *et al*., 1985; Howlett *et al*., 1990; Tylleskar *et al*., 1991; Tor-Agbidye, Palmer, Lasarev, *et al*., 1999). This combination has been reported in all major epidemics of konzo (Nzwalo & Cliff, 2011).

Arguments in favor and against the cyanide hypothesis have been summarized by Tylleskar (Tylleskar, 1994). Arguments for the cyanide hypothesis include the consistent association of konzo with a cassava-dominated diet, insufficient cassava processing, very high urinary thiocyanate concentrations, shortage of other foods, and a low sulfur intake. An association at individual level between high blood cyanide and konzo has also been demonstrated on one occasion (Tylleskar *et al*., 1992). The recent prevention of new cases of konzo by more efficient processing of cassava and reduction of total cyanogen intake (Banea *et al*., 2012) also provides evidence in favor of the cyanide hypothesis.

Different compounds, all originating from cyanide exposure, have been proposed as candidates for the neurotoxicity in konzo (Nzwalo & Cliff, 2011). The closest human model for konzo, with similar clinical and pathological findings, comes from primate experiments with exposure to cyanate (Shaw, 1974). But other experiments with cyanogens have shown neurological damage, as shown in Table 7.1.

The demonstration in animal experiments of neuronal damage induced by linamarin and the presence of high levels of unmetabolized linamarin in individuals from konzo-affected communities compared with controls suggests that this cyanogen may also be implicated directly in the pathogenesis of konzo (Banea-Mayambu *et al*., 1997; Sreeja *et al*., 2003; Kassa *et al*., 2011). Acetone cyanohydrin, a metabolite of linamarin, is also capable of causing neuronal damage in rats on a low SAA diet (Soler-Martin *et al*., 2010). A labile metabolite, its concentration should decrease with time during storage. But in an agricultural crisis, cassava may be consumed without storage, thus increasing exposure and the risk of toxicity (Nzwalo & Cliff, 2011).

Table 7.1 Experiments showing cyanogen neurotoxicity.

Subjects/ animals	Exposure	Relevant neurotoxic findings	Motor changes and similarities to konzo
Neural pheochromocytoma cell culture (Sreeja *et al*., 2003)	Linamarin (acute)	Direct linamarin-induced lesion neural culture.	None
Rats on low SAA diet (Kassa *et al*., 2011)	Linamarin (chronic)	Structural and functional proteomic modifications in the spinal cord.	Non-motor symptoms; hind limb tremors can occur transiently at onset of konzo.
Rats on low SAA diet (Kassa *et al*., 2011)	Cyanate (chronic)	Structural and functional proteomic modifications in the spinal cord.	Motor weakness, gait abnormalities resembling findings in konzo.
Rats (Tor-Agbidye, Palmer, Spencer, *et al*., 1999)	Cyanate (acute)	Glutathione depletion by inhibition of glutathione reductase activity in the brain.	None
Rats on low SAA diet (Soler-Martin *et al*., 2010)	Acetone cyanohydrin (chronic)	Structural brain lesions in non-motor areas.	None
Goats (Soto-Blanco *et al*., 2002)	Cyanate (chronic)	Structural lesions at different levels of the nervous system (including ventral horn of the spinal cord and brainstem).	None
Rhesus monkeys (Shaw, 1974)	Cyanate (chronic)	Structural lesions at different levels of the nervous system (Betz cells in the motor cortex, basal ganglia, and anterior horn cells).	Sudden onset of irreversible spastic quadriparesis resembling konzo, in association with general signs (wasting, anorexia).

Source: Nzwalo and Cliff, 2011.

Excitotoxic damage related to glutathione deficiency is also a possible mechanism (Nunn *et al*., 2011). Methionine, an SAA, is essential for the synthesis of brain glutathione, the most abundant intracellular antioxidant and an important agent for detoxification of xenobiotics (Dringen, 2000). Exercise induces a transient decrease in the levels of glutathione (Evelo *et al*., 1992), and this may explain the frequent onset of konzo during or after exercise. The exacerbation of a chronic state of neuron glutathione deficiency could cause abrupt neurological damage (Nzwalo & Cliff, 2011).

The occurrence of TAN and konzo, two distinct neurological diseases, is probably explained by the difference in the level and duration of exposure to cyanide. Blood cyanide and serum thiocyanate levels found in TAN patients are similar to those found in smokers, but are much lower than those found in konzo-affected patients (Rosling, 1989; Tylleskar, 1994). This may be explained by different dose rates causing different patterns of neuron damage. Duration of exposure may also be important. Perhaps high concentrations during a shorter period cause acute upper motor neuron damage, while lower levels over a longer period cause a more complex myelopathy (Rosling, 1989). The associated poverty and monotony of the diet is more severe in communities affected by konzo.

The nitriles may provide a unifying hypothesis for the pathogenesis of both diseases. These compounds, which are intermediate metabolites of cyanide, have been implicated in the genesis of TAN, konzo and lathyrism (Llorens *et al*., 2011). The authors propose that different nitriles, probably generated by different cassava processing methods, could be responsible for selective neuronal toxicity. This hypothesis is supported by the demonstration in animal models of selective organ or structure neurotoxicity; for example, to the eyes, vestibulocochlear apparatus, large neurons, and the brain caused by specific nitriles (Llorens *et al*., 2011).

Besides acetone cyanohydrin, other nitriles are capable of inducing neuron damage, thus raising the possibility of a common mechanism of neurotoxicity caused by different nitriles, produced under different environmental conditions and processing methods (Llorens *et al*., 2011). This could explain the different combination of clinical manifestations found in TAN and konzo. If different nitriles are generated by regional differences in cassava processing methods, such differences could be indirectly responsible for causing different neurological disorders (Llorens *et al*., 2011).

7.2.5 Prevention of TAN and konzo

The continuing occurrence of TAN and konzo reflects an unacceptable level of poverty. Communities affected by the diseases should be targets for rural development and agricultural extension to improve food intake and diversity. Replacing bitter cassava is usually not an option because of its high yield, and disease and pest-resistant properties (Chiwona-Karltun *et al*., 1998).

Improved processing methods may also be promoted. A new "wetting method" is feasible and simple, and substantially decreases the cyanide content (Bradbury, 2006; Cumbana *et al*., 2007; Bradbury & Denton, 2010; Bradbury *et al*., 2011). A recent trial in a konzo-affected community in the DRC resulted in lowering of the cyanide content of cassava flour and urinary thiocyanate in schoolchildren. No new cases of konzo occurred during the trial period (Banea *et al*., 2012).

7.2.6 Treatment of TAN and konzo

No proven treatment exists for either TAN or konzo. WHO recommends dietary diversification and immediate treatment with high doses of multivitamins, particularly vitamin B, in order to avoid increased neuron damage due to concurrent vitamin deficiency (WHO, 1996). Thiamine deficiency has been suggested as a cause of konzo, with possible therapeutic implications (Adamolekun, 2010). However, the role of thiamine deficiency has been disputed because of the absence of other typical manifestations such as Wernicke encephalopathy, Korsakoff syndrome, or wet beriberi (Nzwalo, 2011).

A trial of hydroxocobalamin for TAN in Nigeria was unsuccessful (Wilson, 1987), and the recommended antidotes for cyanide poisoning have not been tried at scale for either disease.

Exposure to high doses of cyanide or cyanide-generating compounds can be treated with two categories of drugs, according to their mechanism of action: (i) those that trap cyanide and reduce the concentration of free cyanide, such as hydroxocobalamin (Frankenberg & Sörbo, 1975) and (ii) those that increase the catabolism and excretion of cyanide, such as thiosulfates.

Useful elements in judging benefit versus risk in compounds with potential anti-cyanide activity are toxicity, tolerability and safety at antidotal concentrations, speed of antidotal action, interference with cellular oxygen use, and ease of administration (Hall *et al*., 2009).

Arguably, hydroxocobalamin is the best antidote available for acute cyanide poisoning, in terms of risk–benefit, thus offering potential for its use in prolonged poisoning (Borron *et al*., 2007; Hall *et al*., 2009). Hydroxocobalamin was approved by the FDA in 2006. Availability and cost may constrain utilization in the low income countries where TAN and konzo occur. In addition, system issues such as the distance to health service providers may impede appropriate and timely attention to victims of cyanide exposure, regardless of its source.

New experimental antidotes with similar properties are under development. Cobinamide is a hydroxocobalamin precursor, but binds two cyanide ions per molecule rather than the one cyanide by hydroxocobalamin, and can thus be given in smaller quantities and perhaps intramuscularly. Sulfagen is an alternate sulfane sulfur donor and has properties that would perhaps make it more easy to use clinically than sodium thiosulfate. Colleagues in India continue to work on alpha-ketoglutarate, which can be given orally as prophylaxis (Alan Hall, personal communication).

7.2.7 Tobacco amblyopia

Tobacco amblyopia is a rare disorder that characteristically presents as a painless, progressive, bilateral, symmetrical visual failure. Affected patients typically have smoked heavily for several years before the onset of visual complaints (Wokes, 1958; Victor & Dreyfus, 1965; Krumsiek *et al*., 1985). Because almost two-thirds of patients also drink heavily, the condition is frequently designated tobacco–alcohol amblyopia (Carroll, 1944; Victor, 1963; Victor & Dreyfus, 1965; Krumsiek *et al*., 1985). Central vision is particularly impaired, with dyschromatopsia and relative sparing of peripheral and night vision. Examination shows impaired visual acuity with cecocentral scotoma. The ocular fundus is usually normal, although temporal pallor of the optic discs may occur in chronic stages (Krumsiek *et al*., 1985; So & Simon, 2007).

The pathogenesis of tobacco amblyopia has yet to be elucidated. Freeman (1988) has proposed that the cyanide from tobacco smoke causes toxic damage to the optic nerve in genetically susceptible patients. The occurrence of cecocentral scotoma, a common finding in toxic amblyopias, provides indirect evidence of an underlying toxic etiology (Wokes, 1958; Kerrison, 2004). Bilateral and symmetrical lesions of the optic

nerves have been documented in animals after chronic exposure to sodium cyanide (Lessell, 1971).

There is also evidence of a possible contribution of nutritional deficiencies (Carroll, 1944; Victor, 1963; Grzybowski, 2007; Grzybowski & Holder, 2011). Studies have shown an improvement in visual acuity in tobacco amblyopia following dietary supplements (Carroll, 1944; Victor, 1963). Injections of vitamin B12 in the form of hydroxocobalamin may be an effective treatment of tobacco amblyopia, even without smoking cessation (Rizzo & Lessell, 1993). Its protective effect is believed to be due to enhanced conversion of free cyanide to cyanocobalamin by hydroxocobalamin. In patients with tobacco amblyopia, the serum concentration of thiocyanate is relatively low, considering the levels predicted from their tobacco consumption (Chisholm & Pettigrew, 1970), and serum and urinary thiocyanate levels increase with hydroxocobalamin supplementation (Foulds *et al*., 1968). These findings suggest a possible genetic deficiency in cyanide detoxification preventing the conversion of cyanide to thiocyanate and predisposing tobacco smokers to cyanide toxicity (Foulds *et al*., 1968; Jestico *et al*., 1984). Furthermore, in tobacco–alcohol amblyopia, vitamin B12 absorption can be impaired, and cyanide insufficiently metabolized because of alcohol-related hepatic dysfunction (Watson-Williams *et al*., 1969; Wilson, 1983). Jestico *et al.* (1984) found high levels of cyanide in three alcohol–tobacco amblyopia patients. They proposed that the toxicity from tobacco in these patients could be facilitated by concomitant impairment of liver rhodanese activity in association with pre-existent hepatic failure. In addition to stopping tobacco use, vitamin supplementation including B12 is indicated in the treatment of tobacco amblyopia (Carroll, 1944; Krumsiek *et al*., 1985).

The 1991–1994 Cuban epidemic of amblyopia and peripheral neuropathy gave rise to a discussion on the possibility of a common mechanism between tobacco amblyopia and Leber's hereditary optic neuropathy (LHON). The clinical and histopathological findings described in this epidemic optic neuropathy were similar to those described in LHON (Ordunez-Garcia *et al*., 1996; Sadun, 1998), a disorder associated with an impairment of the conversion of cyanide to thiocyanate caused by deficiency of the rhodanese enzyme (Wilson, 1983). Vitamin deficiencies, exposure to alcohol and cyanide from tobacco were among the risk factors in the Cuban epidemic of amblyopia (Ordunez-Garcia *et al*., 1996; Sadun, 1998). Because these identified risk factors can interfere with the process of mitochondrial oxidative phosphorylation, Sadun (1998) proposed that both the toxic and nutritional deficiency optic neuropathies could be caused by mitochondrial dysfunction, as in LHON. More recent studies have shown that tobacco smoke is associated with an increased rate of visual loss with a dose relationship in LHON (Kerrison, 2004; Kirkman *et al*., 2009). The availability of genetic testing has proven that patients who were labeled in the past as having tobacco–alcohol amblyopia in fact have the primary mtDNA mutation of LHON (Cullom *et al*., 1993; Purohit & Tomsak, 1997). Cyanide from tobacco could therefore be causing optic nerve toxicity in susceptible patients with an underlying genetic deficiency of cyanide metabolism.

7.2.8 Subacute combined degeneration of the spinal cord

Subacute combined degeneration of the spinal cord (SACD) is a rare neurological complication of vitamin B12 deficiency with extensive lesions of the dorsal and lateral columns of the spinal cord. The disease is characterized by spastic paraparesis in association with signs and symptoms of profound sensitivity impairment (Russell *et al*., 1900; Scalabrino, 2001). Magnetic resonance imaging of the spinal cord shows the typical abnormal hyper-intense signal changes on T2-weighted imaging of the posterior and lateral columns (Hemmer *et al*., 1998).

The mechanisms of SACD, and why a few individuals with vitamin B12 deficiency develop the disorder, are not completely understood (Scalabrino, 2005). Based on the similarities between vitamin B12 optic nerve neuropathy and tobacco amblyopia, and on the common response to B12 supplementation, Wilson and Langman (1966) proposed that in a chronic stage of vitamin B12 deficiency, the occurrence of neurological complications of vitamin B12 could be precipitated by exposure to exogenous sources of cyanide, including tobacco. But since then, only one study has addressed the association between cyanide from tobacco and SACD (Wells *et al*., 1972). In this study, the levels of plasma thiocyanate were surprisingly found to be significantly lower in patients with SACD than in control smokers. The authors suggested that the lower thiocyanate levels in tobacco smokers with SACD could

be attributed to a blockage in the conversion of cyanide to thiocyanate as a result of a low level of vitamin B12. Different acquired etiologies such as hypocupremia in individuals with normal vitamin B12 levels and nitrous oxide in patients with vitamin B12 deficiency are also associated with SACD (Scalabrino, 2005). These data suggest that SACD may be an endpoint of different insults to the spinal cord in susceptible individuals. As in other disorders associated with a disturbance of conversion of cyanide to thiocyanate such as LHON, smoking could possibly facilitate the development of the SACD in predisposed individuals with vitamin B12 deficiency (Wilson, 1983).

7.2.9 Iodine deficiency disorders

Iodine Deficiency Disorders (IDD) result from insufficient production of the iodine-containing thyroid hormones, elicited by low dietary intake of iodine.

The health consequences of iodine deficiency can occur at all ages. Besides goiter and endemic cretinism, hypothyroidism resulting from moderate to severe deficiency can cause delayed physical development, decreased educability, apathy, impaired mental function, and reduced work productivity. In the fetus and neonate, stillbirth, congenital anomalies and increased perinatal and neonatal mortality may result (Zimmermann *et al*., 2008).

Thiocyanate, the metabolic product of cyanide detoxification in the body, inhibits the iodide concentration mechanism. Its effects are identical to those of iodine deficiency, and they can be corrected entirely by increasing the intake of iodine. Even a very high thiocyanate load from cassava will not induce goiter if the iodine intake is within the daily requirement (Cliff *et al*., 1986; Delange *et al*., 1994). Thus, distinguishing the role of cassava and iodine deficiency in IDD is difficult.

Historically, cassava was an important goitrogen and the potential effect of cassava on the thyroid gland was extensively reviewed in three monographs in the early 1980s (Ermans *et al*., 1980; Delange *et al*., 1982; Ahluwalia & Delange, 1983). In Africa, many countries suffered from endemic goiter before the iodization of salt was widely adopted, and the goitrogenic action of thiocyanate in cassava was implicated in some areas. African countries where goiter has been reported in association with cassava consumption include Cameroon (Aquaron, 1977), the Central African Republic (Biassoni *et al*., 1990; Peterson, 1995), DRC (Delange *et al*., 1994), Ethiopia (Abuye *et al*., 1998, 2008), Guinea (Konde *et al*., 1994), Nigeria (Ekpechi *et al*., 1966; Nwokolo *et al*., 1966; Oluwasanmi & Alli, 1968; Adewusi *et al*., 1992, 1993), and Togo (Jaffiol *et al*., 1992).

A review article published in 2008 found that, since 1990, worldwide the number of households using iodized salt had risen from less than 20% to more than 70% (Zimmermann *et al*., 2008). Many countries with previous areas of endemic goiter are no longer considered to have a public health problem from IDD. But as the areas where cassava-related diseases are reported are often remote and underserved by basic services, and sometimes war-torn, pockets with IDD associated with cassava consumption may still exist.

For example, in 2009, Chabwine (2009) reported on the current situation on Idjwi Island in Lake Kivu in the DRC, one of the sites with a known high prevalence of goiter in the colonial period. Iodine supplementation had greatly improved the situation, at times even leading to thyrotoxicosis due to excessive iodine. But with ongoing war, since the 1990s, some health workers thought that the resurgence of endemic goiter was due to disruption in the supply of iodized salt. No report was available on the current situation.

In Ethiopia, Abuye *et al*. (2008) reported in 2008 that cassava consumption had been increasing in the past 30 years, with a concomitant increase in goiter rate.

In Tanzania, a study in Kigoma region where goiter and high cassava consumption coincided, concluded that the goitrogenic effect of cassava consumption was negligible (Mlingi *et al*., 1996). More recently, Kulwa and colleagues (Kulwa *et al*., 2007) suggested that cassava might have a role as a goitrogen in the Arusha region. An area of southern Tanzania that suffered a konzo outbreak in 2000–2002 was an endemic goiter region, and large goiters were commonly seen in adults (Assey & Mtunda, 2002).

In Asia, despite high cassava consumption, cassava no longer plays a goitrogenic role in most countries, probably because iodine intake is adequate. Previously, in Malaysia in the 1970s and 1980s, goiter was widespread in Sarawak and cassava was implicated (Khor, 2002). In a study of endemic goiter in Vietnam in 1983, an association with cassava consumption was found in Dich Giao, a highland commune (Hershman *et al*., 1983).

In India, Chandra (Chandra and Ray, 2001; Chandra, Mukhopadhyay, *et al*., 2004; Chandra, Tripathy,

et al., 2004; Chandra *et al.*, 2006, 2009; Chandra, Bhattacharjee, *et al.*, 2008; Chandra, Singh, *et al.*, 2008) postulated a possible role for goitrogens, from a variety of vegetables and cassava, as one reason for the persistence of goiter in the post-salt iodination phase.

Pathogenesis

Underlying the pathogenesis of IDD is an insufficient supply of iodine to the thyroid gland, usually due to insufficient dietary iodine intake. More rarely, cells are unable to appropriately take up and process iodine due to substances generically called goitrogens, for they cause goiter, the most common form of IDD. Many substances can act as goitrogens, including thiocyanates derived from the metabolism of cyanide-generating compounds, which are well-established goitrogens.

Goitrogenic action can be direct, by interference with the uptake and/or metabolic processes in the thyrocyte, or indirect, by altering reutilization processes such as iodine salvage pathways; for example, enterohepatic cycles (Gaitan, 1980). The simultaneous occurrence of thiocyanate exposure and iodine and selenium deficiencies have been shown to contribute to altered thyroid function (Contempre *et al.*, 2004).

Regardless of its cause, the metabolic imbalance resulting from iodine deficiency has two typical features: an increased susceptibility to goitrogens when the iodine supply is chronically low and exposure to goitrogens is prolonged (Eastman & Zimmermann, 2009), and an increased pituitary output of thyroid stimulating hormone (TSH), which may lead to hypertrophy and hyperplasia of the thyroid gland.

7.2.10 Diabetes

Cyanide exposure from cassava has been postulated as a cause of malnutrition-related diabetes, based on a link between cassava consumption and diabetes prevalence (McMillan & Geevarghese, 1979). However, a more recent study in Tanzania cast doubt on this hypothesis, by showing that prevalence of diabetes was not increased in a village with high dietary cyanide exposure compared to a control village (Swai *et al.*, 1992).

7.2.11 Endomyocardial fibrosis

An etiological role for cassava consumption in this chronic disease of the heart in tropical areas has been suggested (Sezi, 1996; Rutakingirwa *et al.*, 1999). Many other causes have also been suggested, including worm infestation, malaria, genetic susceptibility and cerium or thorium in monazite deposits in the soil. No single cause explains endomyocardial fibrosis (EMF) in all the areas where it has been reported (Mocumbi *et al.*, 2008). A recent review article concluded that the cause of EMF is still a mystery (Bukhman *et al.*, 2008). Although cassava has never been excluded as a possible cause, there are many other stronger candidates.

7.2.12 Malnutrition

Based on an ecological study in DRC, Banea hypothesized that cyanide exposure from cyanogenic glycosides in insufficiently processed cassava could contribute to growth retardation because of the preferential use of SAAs for cyanide detoxification (Banea-Mayambu *et al.*, 2000). These observations have not been followed up.

7.3 Conclusions

The most important long-term health effects of exposure to cyanide come from the ingestion of cassava with high cyanogenic glycoside content in poor rural communities in Africa. The resultant neurological diseases of TAN and konzo cause severe long-term disability in affected communities. The pathogenesis of these diseases is yet to be elucidated and an effective treatment for cyanide poisoning is neither available nor deliverable in most communities where the diseases occur. Prevention of high levels of cyanide exposure from cassava should be a priority for rural development and agricultural extension services.

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CHAPTER 8
Pediatric cyanide poisoning

Robert J. Geller

At a Glance

- Confirmed cases of pediatric cyanide poisoning are rare.
- Children may be more vulnerable to cyanide poisoning than adults.
- Available cyanide antidotes may have some unique risks for children.
- Especially, the methemoglobin-forming antidotes may have increased risk in children.
- Fire smoke inhalation is a particular risk in children. In a fire scenario, *adults flee* and *children hide*.

8.1 Introduction

Confirmed cases of childhood exposure to cyanide are rare, despite multiple potential sources including inhalation of fire smoke, ingestion of toxic household and workplace substances, and ingestion of cyanogenic foods. Because of its infrequent occurrence, medical professionals may have difficulty recognizing and treating cyanide poisoning. The sources and manifestations of acute cyanide poisoning appear to be qualitatively similar between children and adults, but children may be more vulnerable than adults to poisoning from some sources. Antidotes available in the United States to treat cyanide poisoning include hydroxocobalamin (Cyanokit®); a kit containing amyl nitrite (administered by inhalation), sodium nitrite, and sodium thiosulfate; and Nithiodote® (a kit containing sodium nitrite and sodium thiosulfate). The nitrite antidotes have been successfully used in children, but have particular risks associated with their use in pediatric patients. Because of age-related idiosyncrasies in hemoglobin kinetics, methemoglobinemia associated with nitrite-based antidotes may be excessive at standard dosing in children. This chapter reviews the available data on the sources, manifestations, and treatment of acute cyanide poisoning in children.

Cyanide is among the most potent and deadly poisons, and sources of potential human exposure to it are numerous (World Health Organization, 2004). Existing in gaseous, solid, and liquid forms, cyanide is used in many industries, found in certain household substances, and produced by the combustion of common materials such as fabrics containing nylon, silk, or wool and many plastics such as melamine, polyurethane, and polyacrylonitrile (Alarie, 2002; Betol *et al*., 1983). The release of cyanide and cyanogenic compounds from combustion of such products is the most common source of human exposure to cyanide, and may be second only in importance to carbon monoxide as a toxicant in these circumstances (Alarie, 2002; Barillo *et al*., 1994; Baud *et al*., 1991; Betol *et al*., 1983; Lundquist *et al*., 1989; Riordan *et al*., 2002; Walsh & Eckstein, 2004; Yeoh & Braitberg, 2004).

Humans can also be exposed to cyanide by eating foods, such as the tropical root cassava, apricot seeds, and bitter almonds that contain cyanogenic glycosides that liberate cyanide when metabolized in the body (Akyildiz *et al*., 2010; Nader *et al*., 2010). Additional sources of cyanide exposure include chemicals (used in various occupations) (Garlich *et al*., 2012) and malicious acts such as murder attempts or terrorist attacks (Eckstein, 2004). Cyanide is a potential chemical weapon for use by terrorists because it can be easily obtained and dispersed, and may be rapidly incapacitating or even lethal.

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Little is known about the benefits and risks of antidotes and other aspects of intervention in pediatric patients. Only limited additional information has been published since the last comprehensive review was published on pediatric cyanide poisoning (Geller *et al*., 2006). Most information still comes from case reports of pediatric cyanide poisoning by ingestion. This chapter reviews the available data on the sources, manifestations, and treatment of acute cyanide poisoning in children.

8.2 Sources of acute cyanide poisoning in children

Fire smoke is a common source of cyanide poisoning in children (Barillo *et al*., 1994; Riordan *et al*., 2002). Additional sources described in case reports include household or workplace substances containing cyanide or cyanogenic compounds, cyanogenic foods, laetrile, and nitroprusside, and exposure to these have been reported more frequently in children than in adults (Table 8.1) (Akyildiz *et al*., 2010; Caravati & Litovitz, 1988; Cheok, 1978; Davies *et al*., 1975; Dawood, 1969; Garlich *et al*., 2012; Humbert *et al*., 1977; Krieg & Saxena, 1987; Nader *et al*., 2010; Ruangkanchanasetr *et al*., 1999). The sources of acute cyanide toxicity are similar between children and adults, although their relative frequency of poisoning varies with age.

8.2.1 Inhalation of fire smoke

About one-fourth of the approximately 4000 fire- and burn-related deaths each year in the United States occur in children younger than 15 years (AAP Committee on Injury and Poisoning Prevention, 2000). In children, as in adults, the majority of fire-related deaths are attributed to smoke inhalation rather than burns. Cyanide is an important contributor to death by smoke inhalation. The presence of cyanide in the blood of fire victims (regardless of age) appears to be the norm rather than the exception (Alarie, 2002; Barillo *et al*., 1994; Yeoh & Braitberg, 2004). In a meta-analysis of smoke inhalation-associated deaths occurring in seven major fire incidents from 1971 to 1990, cyanide was found in victims' blood in each study in which it was measured (Alarie, 2002). The percentage of fatalities having toxic blood concentrations (i.e., in adults, >/ = 1 mg/l or >/ = 100 μmol/l) of cyanide ranged from 33% to 87% in the meta-analysis. Toxic blood concentrations of cyanide in the absence of lethal carbon monoxide concentrations were documented in some fire victims, a finding suggesting a cause of death other than carbon monoxide in these children.

Consistent with the results of this meta-analysis, other studies found cyanide in the blood of 62–77% of deaths (Barillo *et al*., 1994; Fortin *et al*., 2010; Yeoh & Braitberg, 2004). In one study of the role of cyanide in smoke-inhalation injury and death, 30 of the 109 victims of smoke inhalation in residential fires in Paris were younger than 14 years (Baud *et al*., 1991). Among those 30 children, 13 died and 17 survived. Cyanide was present in both children who survived (mean concentration 27.4 μmol/l) and those who died (mean concentration 87.0 μmol/l). Blood carbon monoxide concentrations were below the lethal level in some children who survived and some who died, a result suggesting that carbon monoxide poisoning was not the sole cause of death in some children.

8.2.2 Ingestion of household or workplace substances containing cyanogenic compounds

Accidental ingestion of household substances containing poisons often involves young children, who place substances in their mouths and/or ingest them as a means of exploration (Michael & Sztajnkrycer, 2004). Although the U.S. Consumer Product Safety Commission prohibits the sale of consumer products containing cyanide salts (CPSC, 2002), cyanide may be accessible from industrial sources (Garlich *et al*., 2012), as are some cyanogenic compounds that may also be contained in products marketed for consumer use.

Acetonitrile is used as a solvent in industrial and laboratory settings and is sometimes present in cosmetics. Its toxicity is attributed to its metabolism to inorganic cyanide (Table 8.1) (Caravati & Litovitz, 1988; Geller *et al*., 1991; Losek *et al*., 1991).

As would be expected from a cyanogenic compound requiring metabolic activation to be converted to cyanide, the onset of acetonitrile-associated cyanide poisoning typically occurs after a delay of 6 to 14 hours, during which it is slowly metabolized to inorganic cyanide via hepatic microsomal enzymes (Caravati & Litovitz, 1988) (Table 8.1). A similar delay between exposure and onset of toxicity has been observed in adults (Turchen *et al*., 1991). Absence of toxicity in the

Table 8.1 Summary of case reports of pediatric cyanide poisoning from causes other than smoke inhalation

Reference	Age/Sex	Source/Cause of cyanide poisoning	Signs and symptoms	Blood cyanide concentration*	Intervention	Outcome
			Ingestion of substances containing acetonitrile			
Caravati and Litovitz, 1988	16 months/male	Acetonitrile-containing nail remover	• Vomiting, respiratory distress • Found dead in bed the morning after ingesting the product	3.1 µg/ml 12 hours after ingestion	• None	Died
Caravati and Litovitz, 1988	2 years/male	Acetonitrile-containing nail remover	• Vomiting, coma, respiratory distress, shock 8 hours after ingestion	6.0 µg/ml 12 hours after ingestion	• Oxygen, intravenous fluids	Survived with no sequelae
Geller *et al.*, 1991	3 years/male	Acetonitrile-containing nail remover	• No noticeable symptoms upon presentation to the emergency department 30 minutes after ingestion • 13 hours after ingestion (and after gastric lavage and administration of activated charcoal upon emergency department admission), the patient vomited • 16 hours after ingestion, confusion, vomiting, abnormal venous blood hemoglobin desaturation	124 µg/dl 3 hours and 45 minutes after ingestion	• Gastric lavage and activated charcoal 30 minutes after ingestion (while patient asymptomatic) • Sodium thiosulfate 16 hours after ingestion	Survived with no sequelae
Kurt *et al.*, 1991	2 years/female	Acetonitrile-containing nail remover	• Vomiting, seizures, coma 14 hours after ingesting the product • Marked hypoxia and acidosis • No odor of bitter almonds	70.1 µmol/l 14 hours after ingestion	• Oxygen • Cyanide antidote kit (inhaled amyl nitrite followed by sodium nitrite and sodium thiosulfate) • Activated charcoal	Survived with no sequelae

(*continued overleaf*)

Table 8.1 (*continued*)

Reference	Age/Sex	Source/Cause of cyanide poisoning	Signs and symptoms	Blood cyanide concentration*	Intervention	Outcome
Losek *et al.*, 1991	23 months/male	Acetonitrile-containing nail remover	• Vomiting, 6 hours after ingestion; otherwise normal • Beginning 24 hours after ingestion, altered responsiveness (staring episodes, not responding to mother), low oxygen saturation • No odor of bitter almonds	2.1 µg/ml 12 hours after ingestion; 3.8 µg/ml 25 hours after ingestion	• Amyl nitrite • Sodium thiosulfate	Survived with no sequelae
			Ingestion of other household and workplace substances			
Berlin, 1970	17 months/male	Ingestion of Drabkin's solution containing 50 mg potassium cyanide, 200 mg potassium ferrocyanide, and 1 g sodium bicarbonate in 1 liter	• Asymptomatic upon arrival at the emergency department 30 minutes after ingestion • After administration of antidote, vomiting, apneic spells, generalized seizure • Cardiac arrest	Postmortem blood cyanide concentration < 10 mcg%	• Amyl nitrite, sodium nitrite, sodium thiosulfate • Oxygen • Diazepam for seizure • Sodium bicarbonate • Methylene blue	Died. Authors attributed the death to sodium nitrite-induced methemoglobinemia.
Krieg *et al.*, 1987	2.5 years/female	Ingestion of metal cleaning solution containing cyanide salt	• Unresponsive, unconscious, hypotension, tachycardia • Odor of bitter almonds present	Not reported	• Cyanide antidote kit (amyl nitrite, sodium nitrite, sodium thiosulfate)	Survived with no sequelae
Garlich *et al.*, 2012	16 years/female	Ingestion of metal button cleaning powder containing sodium cyanide	• Vomiting, followed by apnea	Not reported	• None	Found deceased
Garlich *et al.*, 2012	15 years/female, 25 weeks pregnant	Ingestion of jewelry cleaner	• Unresponsive, GCS 3 • Bradycardia, HR 47, BP 102/68 torr	Blood thiocyanate 38.6 mcg/ml (normal 1–4 mcg/ml in nonsmokers)	• Sodium nitrite, sodium thiosulfate	Died 72 hours after presentation

Garlich *et al.*, 2012	72 years/female	Ingestion of white powder used to "wash silver"	• Unresponsive • Hypotensive • Bradycardia, HR 50 • Hypothermic • Apneic • Fixed and dilated pupils	Blood glucose 584 mg/dl Cyanide level 5.6 mcg/ml 20 hr after presentation		Died 56 hours after presentation
Garlich *et al.*, 2012	56 years/male	Ingestion of white and gray rock sold as herbal remedy	• Cardiac arrest	Postmortem analysis of product confirmed inorganic cyanide	• None	Died
Garlich *et al.*, 2012	41 years/female	Ingestion of jewelry cleaner	• Cardiac arrest	Blood cyanide 289.5 mcg/ml	• None	Died
Garlich *et al.*, 2012	35 years/female	Ingestion of coin cleaner	• Unresponsive • Bradycardia, HR 30 • Hypotensive 40/20 torr	Blood cyanide 7.3 mcg/ml	• None	Died
Garlich *et al.*, 2012	43 years/female	Ingestion of jewelry cleaner	• Unresponsive • HR 120 • BP 120 systolic • RR 30-40	Level not obtained	• Amyl nitrite • Sodium nitrite • Sodium thiosulfate	Recovered, discharged in good condition 2 days later
Garlich *et al.*, 2012	24 years/male	Ingestion of coin cleaning tablets	• BP 163/83 • HR 138 • RR 20	Qualitative cyanide level "high"	• Amyl nitrite • Sodium nitrite • Sodium thiosulfate	Recovered, discharged in good condition 4 days later
			Ingestion of cyanogen-containing food			
Dawood, 1969	3.5 years/female	Ingestion of cooked wild tapioca	• Vomiting 5 hours after ingestion, frothing around the mouth • Upon admission to the hospital, the girl was in shock, acidotic, drowsy, hypotensive, irritable, breathless, pale. Pupils were dilated and nonreactive.	Not reported. Analysis of the specimen of uncooked tapioca tuber showed 0.0094% w/w hydrogen cyanide. The authors characterized this amount as being moderately to severely poisonous.	• Sodium bicarbonate	Survival with no sequelae
Cheok, 1978	2.5 years/male	Ingestion of tapioca cake	• Vomiting, drowsiness, weakness approximately 9 hours after ingestion	Not reported	• Sodium bicarbonate • Sodium thiosulfate	Survived with no sequelae

(*continued overleaf*)

Table 8.1 (*continued*)

Reference	Age/Sex	Source/Cause of cyanide poisoning	Signs and symptoms	Blood cyanide concentration*	Intervention	Outcome
Cheok, 1978	1.5 years/female	Ingestion of tapioca cake	• Vomiting, drowsiness, weakness, dyspnea, cyanosis a few hours (time not specified) after ingestion	Not reported. Analysis of the cooked tapioca, the upper tuber of uncooked tapioca, and the lower tuber of uncooked tapioca revealed 3, 15, and 28 ppm cyanide, respectively.	• Gastric lavage • Oxygen • Sodium bicarbonate • Sodium thiosulfate	Survived with no sequelae
Akintonwa and Tunwashe, 1992	8 years/male	Ingestion of a cassava-based meal	• Coma	Blood and urine concentrations 0.85 mg l^{-1} and 0.56 mg l^{-1}, respectively	• Supportive therapy (not specified)	Died of cardiorespiratory arrest
Akintonwa and Tunwashe, 1992	17 years/female	Ingestion of a cassava-based meal	• Dizziness, headache, vomiting progressing to shock with acute renal failure	Blood and urine concentrations 1.35 mg l^{-1} and 0.40 mg l^{-1}, respectively	• Not specified	Died of cardiorespiratory arrest
Ariffin *et al.*, 1992	3 sisters: 6 years	Ingestion of tapioca blocks	• Vomiting and diarrhea approximately 10 hours after ingestion. Otherwise normal	• 4 µg/ml. Authors suggested that this value was erroneously high	• Gastric lavage, oxygen, intravenous dextrose-saline	Survival with no sequelae
	1.5 years		• Abdominal cramps, nausea, diarrhea, vomiting approximately 6.5 hours after ingestion	• Not reported	• Not treated	Died en route to the hospital
	8 years		• Vomiting	• Not reported	• Not treated	Survived with no sequelae

Espinoza *et al*., 1992	8 children 8-11 years/males	Ingestion of rhizomes of bitter cassava	• Vomiting, excessive weakness, respiratory failure, bradycardia, hypotension, cardiovascular collapse • Generalized seizures in 2 children • Bright cherry-red blood when samples for blood gases obtained	Not reported	• 100% oxygen to all 8 children • Sodium nitrite followed by sodium thiosulfate to 4 children • Hydroxocobalamin to 4 children	All 8 survived with no sequelae
Ruangkanchanasetr *et al*., 1999	4 years/female	Ingestion of boiled cassava	• Vomited and went unconscious 9 hours after ingestion. Upon arrival at the hospital, the girl was stuporous but responsive to pain stimuli. • 19 hours after ingestion, hypocapnea and lactic academia were present.	0.56 μg/ml 19 hours after ingestion	• Intubation with ventilatory support • Gastric lavage and activated charcoal • Sodium nitrate and sodium thiosulfate and supportive treatment 19 hours after ingestion	Survived with no sequelae
Ruangkanchanasetr *et al*., 1999	1.5 years/male	Ingestion of boiled cassava	• Vomited and went unconscious 9 hours after ingestion. Upon arrival at the hospital, stupor, spasticity, and hypoventilation with cyanosis were noted. • 23 hours after ingestion, had bitter almond breath, respiratory alkalosis, mild lactic acidemia	0.32 μg/ml 23 hours after ingestion	• Mechanical ventilation with hyperventilation and circulatory support by intravenous fluid loading, dopamine, and dobutamine • Gastric lavage	Survived with no sequelae
Chang *et al*., 2004	14 years/male 7 years/female 10 years/female	Ingestion of Cycas seeds	• Asymptomatic • Vomiting, headache, dizziness, weakness • Vomiting, abdominal pain, diarrhea	Not reported	• Not reported	Survived with no sequelae (all cases)

(*continued overleaf*)

Table 8.1 (*continued*)

Reference	Age/Sex	Source/Cause of cyanide poisoning	Signs and symptoms	Blood cyanide concentration*	Intervention	Outcome
Nader *et al.*, 2010	30 months/ female	Ingestion of 5 bitter almonds freshly shelled	• Hypotonia 15 min after ingestion, followed by generalized seizures • Tachycardia • BP 105/55 torr	Blood cyanide 2.33 mcg/ml	• Diazepam, clonazepam • Hydroxocobalamin	Recovered, discharged 2 days later without sequelae
Akyildiz *et al.*, 2010	Case series of 13 children (4 male, 9 female; mean age 5.7 years, range 3-9 years)	Ingestion of apricot seeds	• Hypotension (2 cases) • Coma (2 cases) • Seizures (1 case) • Lactic acidosis (9 cases)	Hyperglycemia in 9 patients	• Hydroxocobalamin as only antidote (4 patients) • Hydroxocobalamin plus nitrites plus thiosulfate (2 patients)	All recovered without sequelae (mean 3 days after admission, range 2–6 days)
			Nitroprusside for surgical hypotension			
Davies *et al.*, 1975	14 years	Nitroprusside 400 mg for surgical hypotension	• Tachyphylaxis and acidosis 80 minutes after administration of nitroprusside	140 µmol/l (authors suggested the presence of an abnormality of cyanide metabolism)	Supportive care	Died
Pershau *et al.*, 1977	14 years	Nitroprusside 130 mg for surgical hypotension	• Acidosis and tachyphylaxis 5 hours after administration of nitroprusside	Not reported	• Supportive care	Survived with no sequelae
Quinlan *et al.*, 2008	14 years	Nitroprusside as treatment for refractory postoperative malignant hypertension after renal transplant, total 200 mg (4.8 mg/kg) over 56 hours	• Elevated level on routine monitoring	Peak cyanide level 3.1 mcg/ml	• Sodium nitrite plus sodium thiosulfate	Survived with no sequelae

Moffett and Price, 2008	Case series of 63 patients	Nitroprusside postoperative pediatric cardiac surgery		Elevated cyanide level in 11% of patients; dose of 1.8 mcg/kg/min predicted elevated cyanide levels	• Supportive care	No cyanide-related sequelae
			Laetrile			
Humbert *et al*., 1977	11 months/female	Laetrile tablets	• Coma	Not reported	• Hospital treatment	Died
Ortega *et al*., 1978	2 years and 10 months/male	Laetrile enema for cancer treatment	• Vomiting and diarrhea after second daily enema • After the third daily enema, lethargy, unresponsiveness, tachypnea, cyanosis	214 µg/dl 5 hours after admission to the hospital after the third daily enema	• Oxygen therapy and intravenous hydration	Survived with no sequelae
Hall *et al.*, 1986	4 years/male	Laetrile tablets ingested shortly after a meal of fresh fruits, vegetables, and peanuts	• Over the 1.5 hours after ingestion, progressively increasing lack of responsiveness, seizures • Upon arrival at hospital, boy was hypotensive, pupils widely dilated but responsive, acidotic • No bitter almond smell noted	16.2 µg/ml 5 hours after ingestion	• Initially, diazepam for seizures, 100% oxygen, gastric lavage, amyl nitrite • 6 hours after ingestion, sodium nitrite and sodium thiosulfate	Survived with no sequelae

*Reported in the units used in the original publication. In adults, thresholds for toxic and potentially lethal blood cyanide concentrations are 39 µmol/l (1 mg/l) and 100 µmol/l (2.7 mg/l), respectively.

initial minutes and hours after acetonitrile exposure should not be interpreted as the absence of toxicity.

This potential confusion between acetone poisoning and acetonitrile is compounded by the initial similarity of their early features, including vomiting, lethargy, slurred speech, ataxia, stupor, coma, and respiratory depression (Losek *et al*., 1991). Delayed onset of vomiting is a frequent presenting symptom of acetonitrile toxicity, even though it is not typically a clinical indicator of other forms of cyanide poisoning (Table 8.1).

8.2.3 Ingestion of cyanogenic foods

Cyanogenic compounds are found in foods including almonds, the pits of stone fruits, lima beans, and cassava (Akyildiz *et al*., 2010; Akintonwa & Tunwashe, 1992; Ariffin *et al*., 1992; Chang *et al*., 2004; Espinoza *et al*., 1992; Humbert *et al*., 1977; Ruangkanchanasetr *et al*., 1999). When these foods are ingested in large quantities or without adequate preparation, they can cause cyanide toxicity. Cyanide poisoning by ingestion of cyanogenic foods appears to occur very rarely in the United States where they are not major components of the diet, but is more frequently reported in children in countries where such foods are more important parts of the diet.

Roots and/or leaves of the cyanogenic plant cassava (or tapioca) are a staple food for millions of people in the tropics. Cassava contains glycosides, which are hydrolyzed to glucose, hydrogen cyanide, and acetone by intestinal β-glucosidase or β-glucosidase liberated from the cassava plant itself (Speijers, n.d.). Numerous cases of acute cyanide poisoning after ingestion of cassava have been reported in children in tropical countries (Akintonwa & Tunwashe, 1992; Ariffin *et al*., 1992; Chang *et al*., 2004; Cheok, 1978; Dawood, 1969; Espinoza *et al*., 1992; Ruangkanchanasetr *et al*., 1999). Cassava poisoning in adults has also been reported, but pediatric poisoning may be more severe (as discussed further below in Manifestations of Acute Cyanide Poisoning). Frequent ingestion of cassava over the long term, particularly in the presence of protein-calorie malnutrition, is also associated with chronic poisoning syndromes such as tropical ataxic neuropathy (lesions of skin, mucous membranes, optic and auditory nerves, spinal cord, peripheral nerves) and konzo, a relatively sudden onset upper motor neuron spastic paraparesis (Akintonwa & Tunwashe, 1992; Mayambu, 1993; Tylleskär *et al*., 1991).

Cyanogenic compounds are also present in the pits of stone fruits such as peaches and apricots (Speijers, n.d.). Multiple cases of pediatric cyanide poisoning from eating large quantities of cooked and/or ground apricot pits have been reported (Lasch & El Shawa, 1981; Sayre & Kaymakcalan, 1964). Apricot pits contain amygdalin, which is hydrolyzed to hydrogen cyanide, glucose, and benzaldehyde, as well as the β-glucosidase, emulsin, which catalyzes amygdalin hydrolysis (Akyildiz *et al*., 2010; Suchard *et al*., 1998). Chewing apricot pits releases emulsin and increases the toxicity of cyanide. Swallowing one or two whole stone fruit pits usually does not result in cyanide poisoning, as the amygdalin and the β-glucosidase enzyme are located in different parts of the pit and do not interact to release cyanide.

8.2.4 Other causes of cyanide poisoning in children

Cases of acute cyanide poisoning secondary to use of nitroprusside have also been reported in children (Davies *et al*., 1975; Pershau *et al*., 1977; Quinlan *et al*., 2008; Thomas *et al*., 2009). Sodium nitroprusside is indicated for reduction of blood pressure in patients in hypertensive crises and for production of controlled hypotension to reduce bleeding during surgery. Within minutes of intravenous infusion, sodium nitroprusside is converted to free cyanide at approximately 44% of the amount infused on a weight basis (w/w) (Schulz, 1984).

Acute poisoning in 127 children ages 2 months to 17 years was attributed to cyanide poisoning after an ecological accident resulting in spilling of acetone cyanohydrin and ammonia into the Siret River in Romania in January 2001 (Iordache *et al*., 2002). Nursing infants whose mothers ingested contaminated fish also developed symptoms. Although the poisoning was attributed to ingestion of contaminated fish from the river, cyanide levels and other confirmatory data are not available.

8.3 Manifestations of acute cyanide poisoning

Cyanide prevents cellular use of oxygen by inactivating mitochondrial cytochrome oxidase and thereby causing cells to switch from aerobic to anaerobic metabolism (Mégarbane *et al*., 2003). Anaerobic metabolism favors

Table 8.2 Signs and symptoms of cyanide poisoning (adapted from Dart and Bogdan, 2004; Mégarbane *et al*., 2003; Ruangkanchanasetr *et al*., 1999).

System	Sign or symptom
Dermatologic	Cherry red color of skin
Neurologic	Headache, agitation, disorientation, confusion, weakness, malaise, dizziness, lethargy, seizures, coma, cerebral death
Cardiovascular	Hypotension, tachycardia or bradycardia, ST-T wave changes, dysrhythmias, atrioventricular block, cardiovascular collapse
Respiratory and Metabolic	Tachypnea or apnea, venous hyperoxemia, red venous blood, increased mixed venous oxygen content and decreased oxygen consumption resulting in narrow arteriovenous oxygen differential
	Acidosis, elevated blood lactate, elevated lactate: pyruvate ratio
Gastrointestinal	Nausea, vomiting, abdominal pain
Other	Bitter almond breath in some patients

production of toxic byproducts, such as lactic acid, over production of cellular energy in the form of adenosine triphosphate (ATP). Accordingly, clinical manifestations of acute cyanide poisoning mainly reflect energy deprivation of the heart and brain due to this inability to fully utilize oxygen (Table 8.2) (Dart & Bogdan, 2004; Mégarbane *et al*., 2003; Ruangkanchanasetr *et al*., 1999).

Following intense exposure, rapid death may ensue. Following less severe exposure, early manifestations may include weakness, malaise, confusion, headache, dizziness, and shortness of breath. Later manifestations include nausea and vomiting, hypotension, generalized seizures, coma, apnea, cardiac arrhythmias, and death attributed to cardiac arrest. Additional physical findings sometimes include cherry red discolorations of the skin and retinal veins and arteries arising from the inability of cells to extract oxygen from blood. Because of elevated venous oxygen levels, cyanosis is typically not present in spontaneously breathing or artificially ventilated patients. Breath may or may not have a bitter almonds-like odor attributed to exhalation of unmetabolized cyanide (Gonzalez, 1982; Kirk & Stenhouse, 1953; Schragg *et al*., 1982).

Elevated oxygen content of venous blood is often present in cyanide poisoning (Mutlu *et al*., 2002). Lactic acidosis was shown in a sample of 11 patients to be highly sensitive and moderately specific for cyanide poisoning. A plasma lactate >/ = 72 mg/dl (8 mmol/l) was 94% sensitive and 70% specific for a blood cyanide level >/ = 1.0 mg/l (Baud *et al*., 2002). Although cyanide concentrations in whole blood are also elevated in acute poisonings, the long time usually required for the result of this test to return limits its clinical utility. Nevertheless, in cases of suspected cyanide toxicity, blood levels should be considered for confirmation of the poisoning.

The time between exposure to cyanide and the onset of toxicity depends on the form of cyanide and the route and concentration of exposure (WHO, 2004; Dart & Bogdan, 2004; Mégarbane *et al*., 2003). Exposure to cyanide gas at high concentrations can result in death within seconds to minutes, but toxicity develops over minutes to hours after ingestion or dermal exposure. Cyanide salts and cyanogenic compounds also typically cause delayed onset of effects.

Whether or not children or adults are differentially sensitive to cyanide poisoning has not been systematically studied. Manifestations of cyanide poisoning appear to be qualitatively similar between children and adults (ATSDR, 2006). In children as in adults, cyanide poisoning has been characterized by varying degrees of neurologic impairment, respiratory distress, and cardiovascular compromise; the occasional presence of bitter-almond breath and bright red venous blood; and metabolic acidosis (Table 8.1).

Factors that could render children more vulnerable than adults to cyanide poisoning include higher respiratory rates, which might contribute to greater systemic toxicity from inhalation exposure, and lower body mass and immature metabolic mechanisms, which might make children more susceptible than adults to toxicity from small amounts of poison (Lynch & Thomas, 2004). Young organs can be particularly sensitive to toxicants during critical periods of structural and functional development, the timing of which depend on the organ system (ATSDR, 2006).

Data from the Paris study described above (Baud *et al*., 1991) are consistent with the possibility of greater vulnerability of children than adults to cyanide poisoning from inhalation of fire smoke. In smoke-inhalation victims, the fatality rate was slightly higher among patients less than 14 years old than among older patients (43% versus 38%). In another study of Paris fire victims found in cardiac arrest, all 8 involved children died,

while 5 of 53 adults survived to discharge (Fortin *et al.*, 2010). In the first study, mean blood cyanide concentrations of victims who died were lower among patients less than 14 years old than they were among older patients (87.0 ± 76.1 µmol/l versus 129.0 ± 93.1 µmol/l, respectively; 2.62 ± 0.16 mg/l versus 3.35 ± 2.42 mg/l, respectively) (differences not statistically tested).

Several authors have suggested that children are more susceptible than adults to poisoning by ingestion of cyanogenic foods including cassava and apricot pits (ATSDR, 2006; Cheok, 1978; Dawood, 1969; Ariffin *et al.*, 1992). Consistent with this possibility is the observation that adults concurrently ingesting cassava sometimes did not develop cyanide toxicity or developed less severe toxicity than children.

8.4 Cyanide antidotes

Management of acute cyanide poisoning in both children and adults entails removal of the victim from the source of cyanide in inhalation exposure, or gastric decontamination with aspiration of gastric contents and administration of activated charcoal in the event of poisoning by ingestion (if care for the victim begins soon after ingestion). Ensuing supportive care includes 100% oxygen, cardiopulmonary resuscitation if necessary, and an appropriate antidote (Dart & Bogdan, 2004; Lambert *et al.*, 1998; Mégarbane *et al.*, 2003). Because cyanide toxicity can culminate quickly in death, rapid intervention is crucial and is usually undertaken on the basis of a presumptive diagnosis before confirmatory blood cyanide concentrations are available. However, in many real-life situations, cyanide antidotes have not been administered even to critically ill patients (Bebarta *et al.*, 2011).

Currently available antidotes in the United States include hydroxocobalamin (Cyanokit®), a kit containing amyl nitrite plus sodium nitrite and sodium thiosulfate, and a kit containing sodium nitrite and sodium thiosulfate (without the amyl nitrite) (Dart and Bogdan, 2004). Amyl nitrite, contained in ampoules intended to be crushed and the contents inhaled, can be administered to stabilize the victim before intravenous administration of sodium nitrite and sodium thiosulfate. The nitrite moieties from amyl nitrite and sodium nitrite oxidize hemoglobin to create methemoglobin, which competes with cytochrome oxidase for the cyanide ion. Binding of cyanide to methemoglobin frees the cytochrome oxidase necessary for aerobic cellular respiration. Sodium thiosulfate serves as a sulfur donor that increases the rate of rhodanase-catalyzed transformation of cyanide to much less toxic thiocyanate.

Incongruously, the putative mechanism of the nitrites in the Cyanide Antidote Kit – induction of methemoglobinemia – can be dangerous and even lethal (Berlin, 1970; Dart & Bogdan, 2004; Hall *et al.*, 1989; Van Heijst *et al.*, 1987; Walsh & Eckstein, 2004). Methemoglobinemia reduces the amount of hemoglobin available to transport oxygen to the cells. In some cases, nitrite-induced methemoglobinemia can be excessive, even to the extent of likely fatal reduction in the oxygen-carrying capacity of the blood (Berlin, 1970). However, a study of four critically ill adult smoke-inhalation patients treated with the Cyanide Antidote Kit demonstrated methemoglobin levels of 7.9% to 13.4%, and their total reduction in oxygen-carrying capacity caused by carbon monoxide, cyanide, and methemoglobinemia never exceeded 21% (Kirk *et al.*, 1993). These values were not considered dangerous.

Nitrite-induced methemoglobinemia may pose a particular danger for victims of cyanide poisoning from smoke inhalation – probably the most common cause of cyanide poisoning in children – because of the likely presence of carboxyhemoglobinemia secondary to concomitant carbon monoxide poisoning (Becker, 1985; Hall *et al.*, 1989; Van Heijst *et al.*, 1987; Walsh & Eckstein, 2004). Like methemoglobinemia, carboxyhemoglobinemia reduces the amount of hemoglobin available to transport oxygen to the cells. The additive effects of nitrite-induced methemoglobinemia and carboxyhemoglobinemia can exacerbate the patient's condition. This possibility raises concern about the use of the Cyanide Antidote Kit in the management of smoke inhalation associated cyanide poisoning, particularly in the pre-hospital setting (Gracia & Shepherd, 2004; Walsh & Eckstein, 2004).

To avoid the complications of methemoglobinemia associated with nitrites, hydroxocobalamin has been used extensively in France and other settings. Sodium thiosulfate has also been recommended for use as a sole agent (without nitrites) for cyanide toxicity to enhance rhodanase activity (Chen *et al.*, 1933; Sylvester *et al.*, 1983). Advocates of this therapeutic regimen contend

that the enhanced safety of this approach outweighs the potentially slower reduction of the cyanide level in the body.

Nitrite-induced methemoglobinemia can pose a particular safety hazard to young children because of age-related idiosyncrasies in hemoglobin kinetics. A proportion of hemoglobin in infants and young children is available in the form of fetal hemoglobin, which is more easily oxidized by nitrites to form methemoglobin than is adult hemoglobin (ATSDR, 2007). In addition, infants and very young children have significantly reduced activity of methemoglobin reductase compared with normal adults – the enzyme responsible for converting methemoglobin back to normal hemoglobin (ATSDR, 2007; Nilsson *et al.*, 1990). These factors render young children especially susceptible to excessive nitrite-induced methemoglobinemia.

The dangers of excessive nitrite-induced methemoglobinemia are illustrated by the case of a 17-month-old child who died after administration of the Cyanide Antidote Kit for ingestion of potassium cyanide (Berlin, 1970). The Cyanide Antidote Kit, which was given according to the published adult dosing schedule, appears to have been a more important contributor to this death than cyanide. At 10 µg per dl (0.01 mg/l), the patient's blood cyanide concentration based on samples taken shortly after ingestion was estimated at between 1/50 and 1/140 of the published lethal dose. On the other hand, the cumulative amount of hemoglobin estimated to have been oxidized to methemoglobin by nitrites administered during antidotal treatment was estimated at up to 92% – well above the 70% that is considered potentially lethal. The author suggested that the adult dosing schedule for treatment of cyanide poisoning with the Cyanide Antidote Kit is potentially lethal for children weighing less than 25 kg because of their weight and the lower hemoglobin concentrations often observed.

The vitamin B_{12} precursor hydroxocobalamin is the most recent cyanide antidote introduced into the U.S. market. Hydroxocobalamin detoxifies cyanide by binding with it to form cyanocobalamin (vitamin B_{12}), a nontoxic compound excreted in the urine (Mégarbane *et al.*, 2003; Sauer & Keim, 2001). With human fibroblasts *in vitro* incubated in a cyanide solution, addition of hydroxocobalamin decreased intracellular concentrations by 75% and resulted in formation of intracellular cyanocobalamin, a finding suggesting that hydroxocobalamin penetrates cells and can act intracellularly (Astier & Baud, 1996).

In experimental animals, hydroxocobalamin crosses the blood-brain barrier and enters the cerebrospinal fluid from the blood circulation (Evans, 1964; Van den Berg *et al.*, 2003). It has been shown to be an efficacious cyanide antidote in mice, rabbits, guinea pigs, dogs, and baboons (Faure *et al.*, 1977; Mizoule, 1966; Mushett *et al.*, 1952; Paulet & Olivier, 1963; Pill *et al.*, 1980; Posner *et al.*, 1976; Rose *et al.*, 1965). Preclinical studies in normal human volunteers have shown safety and efficacy in clearing the blood of the small amounts of cyanide detectable in heavy smokers (Forsyth *et al.*, 1993).

Licensed as a cyanide antidote in France many years earlier than U.S. licensure, hydroxocobalamin has been used to treat known or suspected cyanide poisoning associated with smoke inhalation, industrial exposure to cyanide gas, and ingestion of cyanide salts (Borron *et al.*, 2004; Mégarbane *et al.*, 2003). It has been administered to pediatric patients as well as adults. The recommended dose in the United States follows the recommended pediatric dose in France of 70 mg/kg.

In a recent study of 41 French children (median age: 5 years) with fire smoke inhalation, the total mortality was 44% (14/41), with a pre-hospital mortality of 27% (11/41) and an in-hospital mortality of 23% (7 of the 30 hospitalized children) (Haouach *et al.*, 2005). Pre-hospital administration of hydroxocobalamin was associated with only a 4% mortality in children not found in cardiac arrest. Of 23 children not found in cardiac arrest at the fire scene, 70% (16/23) had loss of consciousness, 74% (17/23) were intubated at the scene, 23 (100%) were hospitalized, and there was one fatality. Children found in cardiac arrest had a mortality of 94% (only 1 of 18 survived); 11 died at the scene, and 6 died in the intensive care unit despite supportive care and administration of hydroxocobalamin.

Espinoza and colleagues reported 8 pediatric cases (aged 8–11 years) of suspected acute cyanide poisoning from improperly prepared bitter cassava (*Manihot esculenta*) (Espinoza *et al.*, 1992). These children had vomiting, weakness, respiratory failure, bradycardia, hypotension, and cardiovascular collapse. Two had generalized seizures. The four most acutely ill children were each treated with limited supplies of sodium nitrite/sodium thiosulfate. The four other children were treated with 500 mg of hydroxocobalamin in a dextrose

solution. All children improved within a few minutes of antidote administration, remained asymptomatic, and were discharged from the hospital the following day with normal cardiovascular and neurological assessments.

Pediatric pharmacokinetic and safety data on hydroxocobalamin are lacking. Although safety and tolerability of hydroxocobalamin in children have not been systematically studied, its use without adverse effects has been reported in pediatric patients (Breton *et al*., 1993; Haouach *et al*., 2005). The most common side effects (in patients regardless of age) – transient interference with colorimetric clinical laboratory tests and transient reddish-brown discoloration of the urine and mucous membranes – appear not to be clinically significant and are attributed to the red color of the hydroxocobalamin molecule (Curry *et al*., 1994; Sauer & Keim, 2001). Elevations in blood pressure and rash have been observed in ongoing clinical trials of hydroxocobalamin. Other allergic reactions to hydroxocobalamin – primarily with long-term low-dose for indications other than cyanide poisoning – have been occasionally observed (Branco-Ferreira *et al*., 1997; Heyworth-Smith & Hogan, 2002) but have not been reported in the relatively small number of children treated to date. On the basis of available data, hydroxocobalamin appears to constitute a useful alternative to the Cyanide Antidote Kit for acute cyanide poisoning in pediatric patients. However, additional data about the risks and benefits of hydroxocobalamin and other potential cyanide antidotes are needed, particularly in children.

8.5 Conclusion

Acute exposure to cyanide from inhalation of fire smoke, toxic household and workplace substances ingestion of cyanogenic foods, and other sources has caused morbidity and mortality in children. Children may be more vulnerable than adults to some sources of cyanide poisoning. Children also seem to be more susceptible to the dangers of nitrite-induced methemoglobinemia caused by administration of the nitrites in two antidote kits. Hydroxocobalamin is a useful antidote, but additional information about its dosing, pharmacokinetics, and risks and benefits in children is still needed.

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CHAPTER 9

Sodium nitroprusside in intensive care medicine and issues of cyanide poisoning, cyanide poisoning prophylaxis, and thiocyanate poisoning

Prasad Abraham, Alissa Lockwood, John Patka, Marina Rabinovich, Jennifer Sutherland, and Katleen Chester

At a Glance

- Sodium nitroprusside (Na2Fe (CN)5NO.2H2O) (SNP) is comprised of a ferrous iron molecule complexed with 5 CN moieties and a nitrosyl group.
- SNP has been utilized to manage blood pressure in a variety of conditions such as hypertensive emergencies, intraoperative hypotension, aortic surgery, pheochromocytoma, etc.
- SNP generates Nitric Oxide (NO) spontaneously.
- Concerns over cyanide toxicity led the FDA to issue a black box warning in 1991, suggesting an upper dosing limit of 2 mcg/kg/min.
- However, cyanide toxicity during SNP infusion seems to be a rare occurrence and is dose-rate and duration dependent.
- Co-infusion of sodium thiosulfate usually prevents development of cyanide toxicity during SNP infusion.

9.1 Introduction

Discovered in the early 1800s, sodium nitroprusside (SNP) is a blood red crystalline compound that has applications in both the laboratory and clinical setting. Its ability to react with multiple substances to generate uniquely colored compounds lead to its utility as a chemical reagent for almost two centuries (Swinehart, 1967). The hypotensive effects of SNP were realized in the late 1800s but the clinical application of SNP for its blood pressure reducing potential did not occur until the 1950s. SNP has been utilized in the management of hypertension in a myriad of medical conditions as well as the induction of hypotensive states for the reduction of intra-operative blood loss (Tinker and Michenfelder, 1976; Friederich and Butterworth, 1995). Over the years growing concerns over the risk of cyanide (CN^-) toxicity related to its use has led to various publications either recommending it as a last line therapy or condemning its use (Marik and Varon, 2007; J. Broderick *et al.*, 2007; Adams *et al.*, 2003; Rhoney and Peacock, 2009). Despite the negative press, SNP has important clinical applications in the management of malignant hypertension and is thus worth reviewing.

9.2 History

Discovery of SNP occurred in the 19th century when Gmelin observed that the addition of nitric acid to ferri- or ferro-cyanides ("prussides") produced a vividly purple solution, particularly when sulfides were added to the mixture at an alkaline pH. Playfair would later name

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the compounds formed by this reaction the "nitroprussides" (Playfair, 1849). In 1886, Hermann observed that SNP produced lethal effects similar to sodium cyanide when injected into animals. Later, Davidsohn along with Gibbs and Reichert recognized that at sublethal doses, SNP produced significant reductions in blood pressure (Davidsohn, 1887; Gibbs and Reichert, 1891).

Johnson later rediscovered its hypotensive effects (in humans) while evaluating the compound as a respiratory stimulant. His research further elucidated that SNP's effects were due to a direct action on the vascular smooth muscle similar to sodium nitrite, but significantly more potent (Johnson, 1929). Despite its potent effects, it would take over 30 years for SNP to enter clinical practice, when Page published his experiences with both oral and IV administration of SNP (Page *et al*., 1955). The rapid reductions in blood pressure with the IV formulation made SNP an ideal agent for in hospital use such that the American Medical Association (AMA Committee on Hypertension, 1974) and later the World Health Organization (1979) both recommended it as an essential agent for the management of hypertensive emergencies. Over the years, SNP has been utilized to manage blood pressure in a variety of conditions such as hypertensive emergencies, intraoperative hypotension, aortic surgery, pheochromocytoma, and so on (Friederich and Butterworth, 1995). Despite its almost ubiquitous use, the concerns over cyanide toxicity led the FDA to issue a black box warning in 1991, suggesting an upper dosing limit of 2 mcg/kg/min (FDA, 1994). In the last decade, fears of toxicity, along with the availability of alternative agents, have allowed clinicians to push SNP back to the shelf. Nonetheless, SNP's potency still allows it to play a role in malignant hypertension when other agents fail.

9.3 Mechanism of action

Sodium nitroprusside ($Na_2Fe\ (CN)_5NO.2H_2O$) (SNP) is comprised of a ferrous iron molecule complexed with 5 CN moieties and a nitrosyl group (Tinker and Michenfelder, 1976). SNP interacts with oxyhemoglobin in the blood to produce methemoglobin while releasing CN^- and nitric oxide (NO) (Ivankovich *et al*., 1978). In contrast to nitroglycerin which requires specific thiol-containing compounds to generate NO, SNP generates this product spontaneously (Moncada *et al*., 1991). NO activates guanylate cyclase, which is located in the vascular smooth muscles, to produce cyclic guanosine monophosphate (cGMP). The increased intracellular concentration of cGMP leads to inhibition of calcium entry into the cell, as well as an increased uptake of calcium by the smooth endoplasmic reticulum resulting in vasodilation (Moncada *et al*., 1991; Palmer *et al*., 1987).

9.4 Metabolism

Once SNP is broken down, the 5 molecules of CN^- that are generated have two generally accepted fates. CN^- can react with thiosulfate ions to produce thiocyanate, which is catalyzed by the mitochondrial enzyme rhodanese and subsequently renally eliminated (Friederich and Butterworth, 1995; Hall and Guest, 1992; Himwich and Saunders, 1948; Schulz, 1984; Schulz *et al*., 1979). CN^- may also react with physiologically available methemoglobin to generate cyanomethemoglobin, which is in dynamic equilibrium with CN^- and nontoxic (Ivankovich *et al*., 1978; Smith and Kruszyna, 1974; Kulig, 1991). CN^- accumulation is of concern as it halts aerobic metabolism by inhibiting the final step of oxidative phosphorylation. CN^- binds to ferric iron in the cytochrome oxidase enzyme and renders it inactive. As a result, there is a shift from aerobic to anaerobic metabolism leading to cellular ATP depletion, reduction of pyruvate to lactate, and rapid accumulation of lactic acid (Vogel and Sultan, 1981; Salkowski and Penney, 1995; Baud *et al*., 2002). Although inhibition of mitochondrial oxidative phosphorylation is not the only consequence of CN^- accumulation, significant CN^- intoxication has been associated with the development of lactic acidosis, both in animals and humans (Vogel and Sultan, 1981; Salkowski and Penney, 1995; Baud *et al*., 1991, 2002; Graham *et al*., 1977). Depending on the whole blood concentration of CN^-, patients may present with mild clinical symptoms such as tachycardia and flushing at 0.5–1 mcg/ml (20–38 micromol/l), depressed levels of consciousness at 1–2.5 mcg/ml (48–65 micromol/l), coma at 2.5–3 mcg/ml (95–114 micromol/l) and death at levels greater than 3 mcg/ml (> 114 micromol/l) (Hall and Rumack, 1986).

Historically, rhodanese, also known as thiosulfate sulfurtranferase, has been the primary enzyme implicated in the detoxification of SNP via adding a

sulfur atom to the CN^- molecule to form the much less toxic thiocyanate ion. Rhodanese is distributed widely throughout the body, with the highest concentrations located in the liver (Vincent *et al.*, 1981). The rate-limiting step for this reaction appears to be the availability of thiosulfate (sulfur donor), which is generated endogenously from cysteine and methionine (Tinker and Michenfelder, 1976; Vincent *et al.*, 1981; Curry and Arnold-Capell, 1991; Kun, 1961).

Studies suggest that the body has a limited amount of thiosulfate and can detoxify a maximum of approximately 50 mg of SNP (Curry and Arnold-Capell, 1991; Ivankovich *et al.*, 1983). In a study by Ivankovich *et al.*, mean plasma thiosulfate concentrations in healthy individuals age 24 to 45 years were determined to be 1.13 ± 0.11 mg/dl (range 0.43 to 2.54 mg/dl), and healthy males can produce 3 mg/day, regardless of dietary intake. Elevated thiosulfate concentrations were found in starving patients and children, possibly due to increased protein utilization (Ivankovich *et al.*, 1983). Based on these data, the authors reported that adults should be able to detoxify 66 mg of SNP with endogenous thiosulfate stores. However, if thiosulfate concentrations need to be 3 times higher to act as a preferred substrate for rhodanese, this amount drops to less than 50 mg. With these considerations in mind, the authors recommended a maximum safe dose of 1–1.5 mg/kg of SNP based on clinical experience. A limitation of this data is that it was a short-term study and does not reflect the true amount of SNP that the body can handle. They also recommended that sodium thiosulfate should be administered as a continuous infusion. A 10 : 1 ratio of thiosulfate to nitroprusside as a mixed infusion provides enough sulfur to promote metabolism for excretion (Hall and Guest, 1992).

More recent data suggests that other enzymes also have the capability of metabolizing CN^-, and these include mercaptopyruvate sulfurtransferase and thiosulfate reductase in the mitochondria, and cystathionine gamma-lyase in the cytoplasm (Nagahara *et al.*, 2003). It is now evident that rhodanese and mercaptopyruvate sulfurtransferase are the primary enzymes responsible for CN^- metabolism (Westley, 1973; Westley *et al.*, 1983). Rhodanese is a non-specific enzyme that can interact with many sulfur donors. Besides thiosulfates, other sulfane-sulfur (divalent sulfur bound to another sulfur ion) sources in the body include polythionates, thiosulfonates, persulfides, and elemental sulfur (Schneider and Westley, 1969). Thus, the "sulfane pool" is larger than initially proposed resulting in the body's enhanced ability to detoxify CN^-, contrary to our prior understanding.

Mercaptopyruvate sulfurtransferase also aids in the detoxification process by generating sulfane-sulfur from cysteine, hence adding to the sulfane pool (Way, 1984; Way *et al.*, 1984). Patients who are receiving oral or enteral nutrition will have additional sources of cysteine, which may add to the sulfane pool although there are no published data to support this hypothesis at this time. In addition, mercaptopyruvate sulfurtransferase plays a key role in the incorporating of exogenous thiosulfate into the sulfane pool, as thiosulfate by itself has poor intracellular penetration (Beasley and Glass, 1998).

While rhodanese is found in high concentrations in the liver, it is very scant in the brain (Isom and Way, 1976). The primary toxic effects of CN^- are elucidated in the brain as it has a high affinity for cytochrome a,a_3, the cytochrome oxidase found specifically in brain mitochondria (Piantadosi and Sylvia, 1984). Albumin also appears to play a role in CN^- detoxification as it has been reported to act as a sulfur carrier as well as have the capacity to directly react with CN^- to form thiocyanate (Sörbo, 1955; Vennesland *et al.*, 1981). This mechanism might be another way thiosulfate is able to relieve the toxic effects of CN^- within the brain.

Besides enzymatic reactions, the body has other chemical pathways to inactivate/remove CN^-, and they include reactions with formic acid, 2-aminothiazoline-4 carboxylic acid, hydroxycobalamin, hemoglobin, exhalation of hydrogen cyanide (HCN), and incorporation into choline and methionine (Hall and Rumack, 1986; Silverman *et al.*, 1988). In conclusion, the body possesses a much larger capacity for the detoxification of CN^- than once realized. While there are a couple of primary pathways that account for the majority of the detoxification, it is unknown how the other systems interact *in vivo* (i.e., in the presence of a large CN^- load can other systems up regulate to handle the burden). Nonetheless, the data suggest that larger doses of SNP may be used in patients, contrary to what has been proposed previously (FDA, 1994; Curry and Arnold-Capell, 1991; Ivankovich *et al.*, 1983; Vesey *et al.*, 1976; Hall and Guest, 1992; Zerbe and Wagner, 1993).

9.5 Evidence for CN^- toxicity associated with SNP

Table 9.1 and Table 9.2 are a summary of all published cases that have been reported in the literature regarding either mortality or morbidity associated with CN^- toxicity related to SNP administration. Several things can be gleaned from these reports. In the four of the cases related to intra-operative use, gross over dosing (> 10 mcg/kg/min) of SNP lead to CN^- toxicity and death in two cases. In most of the other case reports, SNP was used for a prolonged period of time before CN^- toxicity was manifested. CN^- toxicity was typically presumed, for lack of measured CN^- levels, especially in the patients that had morbidity related to CN^-. Finally, in the patients that died, other complications may have contributed to their demise as well.

In addition to these reports, Patel *et al.* (1986) published a case series of 7 patients, out of a total of 292 patients undergoing CABG surgery, who developed

Table 9.1 Summary of case reports of mortality associated with SNP use.

Cit	No. pts	Age	Wt	NO_2 use	Dose	Duration	CN level	pH	Treatment	Comments
48	1	14	40	Y	120 mcg/kg/min Total 400mg	80 min	0.5 mg/dl	6.92	Supportive	Used for intraop hypotension. Pt developed tachyphylaxis. Post op complications
49	1	20	–	Y	750 mg (25 mcg/kg/min if 100 kg)	5 hrs	–	6.8	Supportive	Used for intraop hypotension. Pt developed tachyphylaxis. Arrested 20 min post op and died 32 hours later. K^+ was 8.5 during arrest. Post op complications
50	1	40–50	–	Y	–	–	–	7.12	Supportive	Used for intraop hypotension. Pt developed tachyphylaxis. Post op complications Base excess = –22
51	1	43	–	N	2878 mg (0.5–8 mcg/kg/min)	14 days	–	–	Supportive	Hepatitis, ARF, HTN encephalopathy, obtunded on presentation. Cardiac arrest on day 12. Died day 47. Autopsy path suggestive of CN toxicity. Globus Pallidus lessions
52	1	66	74	N	238 mg (max 2.3 mcg/kg/min)	38hr	0.29 mg/dl	–	Supportive	ICH. ARF by day 3. Had some acidosis on admission Base excess = –11
53	1	42	50	N	13.5 mg (max 3 mcg/kg/min)	90 min	–	7.1	Supportive	Scleroderma, HTN emergency, ARF, CHF. Developed profound hypotension unresponsive to intervention. Autopsy did not show obvious cause so assumed it was CN
54	1	78	75	N	1300 mg (max 4.4 mcg/kg/min)	5 days	0.2 mg/dl		Na nitrite and thiosulfate	Aortic pseudoaneurysm. Patient developed tachyphylaxis. Death from CNS damage from trauma. Lactate increased from 2.4 to 4.3
55	1	1 day	4.4	N	Max 5 mcg/kg/min	30 hours	RBC CN 400 nmol/ml	7.19	Thiosulfate	HTN. Tachyphylaxis. Gave SNP with thiosulfate for 3 days. CN level normal. Child died of other causes
56	1	59	–	N	2029 mg (max 8 mcg/kg/min)	7 days	–	–	Supportive	Patient had CHF. No electrolyte or pH values were reported but author stated they were "acceptable." Patient had several days of confusion, agitation. Sz on day of death
57	3	?	–	N	–	> 48 hours	–	–	Supportive	All pts suffered from severe pre-eclampsia. Mortality only reported for the fetus

Abbreviations: Cit – citation, Y – yes, N – no, CN – cyanide, RBC – red blood cell, ICH – intracranial hemorrhage, ARF – acute renal failure, HTN – hypertension, CHF – congestive heart failure, CNS – central nervous system, SNP – sodium nitroprusside, SZ – seizure.

Table 9.2 Summary of case reports of morbidity associated with SNP use.

Cit	No. pts	Age	Wt	NO_2 use	Dose	Duration	CN level	pH	Treatment	Comments
58	1	42		Y	250 mg	90 min	–	7.19	$NaHCO_3$	Intraop hypotension. Developed tachyphylaxis. Altered mental status post op. Use in more than 600 pts. Rare occurrence
59	1	41	82	Y	15 mcg/kg/min	75 min	–	7.35	Supportive	Intraop hypotension. Patient developed tachyphylaxis.
60	1	14	44	Y	10 mcg/kg/min	300 min	–	7.34	Thiosulfate	Intraop hypotension, Developed tachyphylaxis. Cardiac arrest for 45 min. Developed pulm edema with PaO_2 of 44. Base excess −6
61	1	58	80	N	1000 mg (max 3 mcg/kg/min)	7 days	0.5 mg/ml	–	HD	CHF and Aortic valve insufficiency. Patient developed tachyphylaxis
62	1	52	66	N	1093 mg (max 8 mcg/kg/min)	34 hours	–	7.08	$NaHCO_3$	HTN and AMI. Smoker. Patient developed tachyphylaxis. Pt developed lactic acidosis.
63	1	21 days		N	Max 8 mcg/kg/min	4 days	–	–	Supportive	
64	1	53		N	2.5 mcg/kg/min	7 days	–	7.32	Dimethylaminophenol and thiosulfate	ICH from HTN. Base excess −7.4
65	1		70	N	215 mg (max 4.9 mcg/kg/min)	33 hours	–	–	Supportive	
66	1	27	68	N	1120 mg (max 12.5 mcg/kg/min)	48 hours	–	7.2	Supportive	Renal transplant pt with history of resistant HTN. Serum creatinine −1.6. Had gall bladder rupture. Base excess = −16, Cardiac arrest, no complications. No tachyphylaxis
67	1	66		N	max 5 mcg/kg/min	29 days	–	–	Supportive	HTN, aneurysmal bleed. Patient developed tachyphylaxis developed by 48 hours. No signs of metabolic derangements
68	1	66	21	N	490 mg(max 7 mcg/kg/min)	28 hours	–	7.21	$NaHCO_3$	HTN emergency. ARF. Became agitated.
69	1	6		N	Max 5.5 mcg/kg/min	22 days	–	–	Supportive	HTN. ARF with intermittent HD. No CN Toxicity. Had elevated SCN levels that corrected with HD
70	1	3 days	3.8 kg	N	Max of 2.5 mcg/kg/min	3 days	–	7.26	Supportive	Pt had a lactate of 19 mmol/L. Patient was receiving concomitant thiosulfate infusion
71	1	14		N	Max of 2.2 mcg/kg/min	3 days	3.1 mcg/ml	7.39	Supportive	

Abbreviations: Cit – citation, Y – yes, N – no, CN – cyanide, ICH – intracranial hemorrhage, ARF – acute renal failure, HTN – hypertension, CHF – congestive heart failure, HD – hemodialysis, SCN – thiocyanate.

CN^- toxicity related to SNP use for the management of post-operative hypertension (Patel *et al*., 1986). Three of these patients died. Patients were administered SNP for 26–160 hours with doses ranging from 1.8 to 12 mg/kg body weight. All seven patients had blood CN^- levels > 500 mcg/l, which was considered toxic by their laboratory (although this level is clinically associated with mild symptoms; see previous section) (Hall and Rumack, 1986). According to the authors, serum lactate, pH, and base excess did not correlate with elevated CN^- levels, although the timing of these measurements is not mentioned in the paper. Finally, Robin and McCauley published a paper which reviewed 52 cases that were reported to the FDA between 1974

and 1992. Twenty-nine of the 52 cases resulted in death (Robin and McCauley, 1992). Very few patients had CN^- levels measured and from the paucity of data provided, it appears that many of the patients had complications which could have significantly contributed to the morbidity and mortality. Nonetheless, the authors concluded that CN^- toxicity related to SNP is a frequent event, resulting in negative outcomes and thus routine use of SNP should be avoided. The authors also stated that lactic acidosis presented as a terminal event only and was not a useful marker for CN^- toxicity evaluation; although no supporting data were provided.

While there are several cases of cyanide associated toxicity published in the literature, it is important to note that CN^- levels were not drawn for all patients. It is difficult to confirm the diagnosis of death related to CN^- toxicity (Table 9.1, Table 9.2). The data from the FDA is scant, which makes it difficult to make any true inferences about a causal relationship (Robin and McCauley, 1992). Furthermore, in several of the FDA reports CN^- toxicity was assumed because there was no other explanation.

Though rare, SNP has been reported to cause methemoglobinemia at high doses (> 10 mg/kg), as well as carboxyhemoglobinemia (McEvoy, 1994; Lopez-Herce *et al.*, 2005). Nitrous oxide, which was used in the OR frequently for anesthesia, also has the potential to cause methemoglobinemia (Chiodi *et al.,* 1983). The combination of these agents could, theoretically, increase the risk of a patient developing methemoglobinemia. This highlights the concern that there are potentially other causes for negative outcomes, especially in the intra-operative setting. Nonetheless, the emphasis here is that while there are confounders related to the data, indiscriminate use of SNP *does* cause CN^- toxicity, as evident from several of the cases.

9.6 Incidence of CN^- toxicity

One of the common misgivings is that CN^- toxicity related to SNP is a very frequent phenomenon. Robin and McCauley's paper postulated greater than 1% annual excess in mortality or 1000 deaths related to CN^- toxicity alone (Robin and McCauley, 1992). Sarvotham also estimated about 1000 deaths per year and 3000 cases per year attributed to CN^- toxicity in patients undergoing CABG surgery (Sarvotham, 1987). Estimates from Patel *et al*. suggest an incidence of 2.4% (7/292) for CN^- related toxicity and a 1% mortality rate, which is significant considering the number of patients who undergo CABG surgeries annually in the United States, not to mention the other multitude of patients that receive it for other indications (Patel *et al*., 1986). However, in a letter to the editor, McRae reported only one incidence of metabolic acidosis related to SNP use and no deaths in over 1000 patients that his center had treated (McRae, 1976). A review of the published literature, searching MEDLINE using the MeSH term "sodium nitroprusside" and limiting the search to "clinical trials, all" was recently conducted. The results were further reviewed by one of the authors (PA) and refined to ensure the citations met above criteria. These studies were then evaluated for reports of CN^- toxicity, lactic acidosis, and death from all causes. The final result included 59 trials that are summarized in Table 9.3. Approximately 4300 patients were included in these trials of which 2999 were exposed to SNP. Over 80% of the patients received SNP outside of the OR setting. CN^- toxicity or lactic acidosis was reported in 66 patients and 16 patients died from any cause. This gives us an incidence of SNP-related adverse events of 2.73% (82/2999) and a mortality rate of 0.5%. Interestingly, in reviewing several of the studies that specifically evaluated SNP and its potential for CN^- toxicity, a majority reported no evidence of clinical symptoms of CN^- toxicity despite detection of elevated CN^- levels in several of the patients (Moore *et al*., 1985; Lundquist *et al*., 1989; Benitz *et al*., 1985; Kunathai *et al*., 1989; Linakis *et al*., 1991; Moffett and Price, 2008). Further investigation of mortality associated with SNP reveals that the 16 deaths came from two specific trials where 11 patients died in the SNP group compared to 9 in the placebo group in the first study and 5 patients died in the SNP group compared to 18 in the placebo group in the second study (Cohn *et al.,* 1982; Durrer *et al.,* 1982). Assuming that the 2 additional deaths in the first study compared to the placebo group were due to CN^- toxicity, the mortality rate is less than 0.1% (2/2,999). It should be noted that this information is extracted from controlled trials and is thus a conservative estimate. The overall use of SNP has waned over the years leading to a low number of reports of CN^- toxicity. It is important to understand the even in its peak use, the true incidence is very small, suggesting that prudent use of SNP is still a relatively safe therapeutic option.

Table 9.3 Review of adverse events related to SNP use in clinical trials.

Study	Patient population	N	Treatment and control group regimens	Mean duration of study	Mean SNP dose	Outcomes		
						CN toxicity (#)	Deaths (#)	Lactic acidosis (#)
Vesey 1976	Orthopedic surgery	26	Prospective observational study in intraoperative hypotension	NR (range 90–168 min)	NR Total SNP dose (range 4–95.4 mg)	7	0	0
Aitken *et al.,* 1977	Adult and pediatric pts undergoing craniotomies	13	Prospective study evaluating CN toxicity following SNP induced hypotension	35.5 min	4.4 mcg/kg/min	5	0	4
Thompson *et al.,* 1978	Total hip arthroplasty	30	Prospective, randomized, controlled trial comparing effects of isoflurane (n = 10), SNP (n = 10) and placebo (n = 10) for intra op hypotension on organ function and blood loss	NR (range 65–115 min)	0.5 mg/kg	0	0	0
Pasch 1983	Surgical patients	55	Prospective observational study in intraoperative hypotension. Divided into 5 groups based on SNP dose and administration of thiosulfate	NR (range 103–152 min)	NR	8	0	0
Fahmy 1985	Major orthopedic procedures	20	Prospective, randomized trial comparing SNP (n = 10) to SNP + trimethaphan (n = 10) for intra op hypotension	SNP alone – 258 min SNP + trimethaphan – 266 min	SNP alone – 5.82 mcg/kg/min SNP + trimethaphan – 1.39 mcg/kg/min	4	0	0
Moore 1985	CABG pts	6	Prospective study of hypothermia effects on SNP metabolism	20 min	7.3 mcg/kg/min	1	0	0
Corr *et al.*, 1986	CABG surgery on bypass	12	SNP (N = 6) or trimetaphan (N = 6) to maintain MAP 70–85 before and after bypass and ≤ 70 during bypass	63.5 min on bypass with SNP	25.4 mg over 63.5 min (400 mcg/min)	0	0	0
Bernard *et al.,* 1987	Total hip arthroplasty	16	Prospective, randomized trial comparing hemodynamic and metabolic effects isoflurane (n = 8) to SNP (n = 8) for intra op hypotension	90 min	NR (range 1–6 mcg/kg/min)	0	0	0
Cole 1987	Major elective orthopedic procedures	30	Prospective, randomized trial comparing blood CN concentrations in pts who received TS with SNP (n = 15) and those who did not (n = 15) for intra op hypotension	SNP alone = 98 min SNP + TS = 89 min	SNP alone = 0.36 mg/kg SNP + TS = 0.45 mg/kg	0	0	0
Simpson *et al.,* 1987	Major middle ear surgery	30	Prospective, randomized, study comparing metoprolol 25, 50 mg and oxprenolol 20 mg for intraop hypotension in addition to SNP and trimetaphan	NR	NR (range 0.25–1.75 mcg/kg/min)	0	0	0

Table 9.3 (*Continued*)

Study	Patient population	N	Treatment and control group regimens	Mean duration of study	Mean SNP dose	Outcomes		
						CN toxicity (#)	Deaths (#)	Lactic acidosis (#)
Van Wezel *et al.,* 1987	CABG pts	20	Prospective, randomized trial comparing nifedipine (n = 9) to SNP (n = 11) effects on myocardial metabolism and coronary sinus blood flow	32 min	3.4 mcg/kg/min	0	0	0
Van Wenzel 1987	CABG pts	37	Placebo (n = 12) controlled trial comparing effects of nifedipine (n = 12) vs SNP (n = 13) on myocardial metabolism and catecholamine balance	31 min	1.9 mcg/kg/min	0	0	0
Porter *et al.,* 1988	Spinal fusion	21	Prospective randomized study comparing nitroglycerin (n = 8), SNP + captopril (n = 6), and SNP (n = 8) for intra op hypotension	SNP – 194 min SNP + captopril – 256 min	NR (max 2 mcg/kg/min)	0	0	0
Hodsman *et al.,* 1989	CABG pts	41	Randomized, placebo controlled comparing 3 different doses of ketanserin. Patients also received closed loop SNP	7.2 hours in placebo group vs 2.1–3 hours in ketanserin groups	0.57 mg/kg in placebo group vs 0.056 to 0.28 mg/kg in ketanserin groups	0	0	0
Lundquist 1989	CABG pts	18	Prospective study evaluating CN release from SNP during CPB. Nine pts received TS concomitantly	62.8 minutes (during CPB)	0.13 mcg/kg/min (during CPB)	2	0	0
Geniton 1990	Undergoing elective carotid endarterectomy	19	Prospective, randomized trial comparing labetalol (n = 9) to SNP (n = 10) for intra op HTN	12 hours	1.2 mcg/kg/min	0	0	1
Godet *et al.,* 1990	CABG pts	20	Prospective, randomized study comparing isoflurane (n = 10) to SNP (n = 10)	26 min	7 mcg/kg/min (total dose of 11 mg)?	0	0	0
Ornstein *et al.,* 1991	Deliberate hypotension (MAP 60–65) during intracranial AVM resection	29	Isoflurane (n = 9) SNP (n = 10), or esmolol (n = 10).	NR	2.3 ± 1.3 mcg/kg/min	0	0	0
Owall and Sollevi 1991	Abdominal aortic aneurysm	6	Prospective crossover study comparing myocardial effects of adenosine and SNP	20 min	0.7 mcg/kg/min	0	0	0
Blau *et al.,* 1992	Orthognathic surgery	30	Randomized study comparing esmolol (n = 15) to SNP (n = 15) for intra op blood loss (intra op hypotension)	95 min	Median dose 1.75 mcg/kg/min Total dose – 10.5 mg	0	0	0
Chaudhri *et al.*, 1992	Resection of intraoccular melanoma	20	Patients randomized to receive closed loop control or manual control of arterial pressure with a mixture of SNP and trimetaphan	204 min for closed loop and 158 min for manual	0.038 mg/kg in closed loop and 0.026 mg/kg in the manual	0	0	0

Table 9.3 (*Continued*)

Study	Patient population	N	Treatment and control group regimens	Mean duration of study	Mean SNP dose	Outcomes		
						CN toxicity (#)	Deaths (#)	Lactic acidosis (#)
Bernard *et al.*, 1993	Induced-hypotension in healthy patients undergoing spinal fusion for scoliosis	20	Diltiazem plus SNP (n = 10) or SNP (n = 10)	Diltiazem plus SNP (186 ± 17 min). ySNP (214 ± 26 min)	Diltiazem plus SNP (0.24 ± 0.05 mg/kg). SNP (0.47 ± 0.07 mg/kg)	0	0	0
Dentz *et al.*, 1995	Abdominal aortic aneurysms	20	Prospective randomized study comparing amrinone (n = 10) to SNP (n = 10) for hemodynamic control	NR	NR (range 1–8 mcg/kg/min)	0	0	0
Newton *et al.*, 1996	Induced-hypotension (MAP = 55) for middle ear surgery	30	Isoflurane (n = 9) SNP (n = 10), or trimetaphan (n = 9).	Isoflurane (105 ± 19 min) SNP (87 ± 6 min), or trimetaphan (85 ± 10 min).	0.18 ± 0.06 mg/min	0	0	0
Przybylo *et al.*, 1995	Pediatric patients undergoing CPB	10	Prospective study	75 min	6 mcg/kg/min	6	0	0
Van der Stroom *et al.*, 1996	CABG	60	Open label, randomized study comparing effects urapidil (n = 31) and SNP (n = 29) on myocardial metabolism, hemodynamic state	59 min	1 mcg/kg/min (range 0.1–2.6)	0	0	0
Tugrul *et al.*, 1997	Cardiac surgery	66	Prospective, randomized study comparing effects of isoflurane (n = NR) and SNP (n = NR) on rewarming after cardiopulmonary bypass	32.6 min	1.55 mcg/kg/min	0	0	0
Deakin *et al.*, 1998	CABG	120	Unknown study design Comparing effects of SNP (n = 59) to placebo (n = 61) in rewarming after cardiopulmonary bypass	NR (max 490 min)	1.4 mcg/kg/min	0	0	0
Suttner *et al.*, 1999	Induced-hypotension in radical prostatectomy	30	SNP (n = 15) Control (n = 15)	97 ± 13 min	Total dose = 23.4 + 7.8 min	0	0	0
Armstrong *et al.*, 1975	Patients with MI and MAP ≥105 and/or PCP >15	26	All (N = 26) received SNP gtt, 18 received NTG gtt	First 24 hrs post-MI	76 mcg/min	0	0	0
Kotter *et al.*, 1977	Acute MI	29	Unknown study design SNP + phentolamine vs SNP + glyceril trinitrate	88 mcg/min (range 33–273 mcg/min)	NR	0	0	0
Cohn 1982	Acute MI	812	Prospective, randomized double blinded study comparing SNP (n = 407) to placebo (n = 405)	48 hours	NR (range 72.8–94.4 mcg/min)	0	11 (9 in PC at 48 h)	0
Durrer 1982	Acute MI	328	Prospective, randomized trial comparing effects of SNP (n = 163) to placebo (n = 165) on mortality	24 hrs	181.3 mg	0	5 (18 died in PC)	0

Table 9.3 (*Continued*)

Study	Patient population	N	Treatment and control group regimens	Mean duration of study	Mean SNP dose	Outcomes		
						CN toxicity (#)	Deaths (#)	Lactic acidosis (#)
Schulz and Roth1982	Multiple indications	70	Prospective observation trial of SNP use for intra-operative hypotension in 51 pt and hypertensive crisis or aortic aneurysm (medical) in 19	Intra op – 80 min Medical – NR (up to 2 weeks)	Intra op – NR Medical – NR	9	0	0
Flaherty 1983	Patients with MI	17	SNP and NTG in a randomized crossover protocol	At least 60 min	NR	0	0	0
Gray *et al.,* 1985	Post cardiac surgery	12	Cross-over study comparing esmolol vs SNP	1.6 mcg/kg/min (range 0.5–2.75 mcg/kg/min	NR	0	0	0
Vesey 1985	NR	30	Prospective, observation study of long term SNP infusion and CN levels	NR (range 12–314 hr)	NR	5*	0	0
Benitz 1985	Neonates with various pulmonary and vascular conditions	58	Retrospective study evaluating safety and efficacy of SNP	8 hrs (median)	2 mcg/kg/min (median)	0	0	1
Gray *et al.,* 1987	Post cardiac surgery	20	Prospective, randomized, open label, crossover study comparing esmolol to SNP	NR	1.8 mcg/kg/min	0	0	0
Installé *et al.,* 1987	Patients with NYHA class III or IV HF	10	All patients received SNP, then dobutamine, then enoximone. (30–40 min washout after SNP)	SNP titrated to goal then washout < 8 hours?	1.8 ± 0.75 mcg/kg/min	0	0	0
Breisblatt *et al.,* 1988	Unstable angina	40	SNP (N = 18) NTG (N = 22)	SNP titrated to goal < 8 hrs?	77 ± 25 mcg/min	0	0	0
Mullen *et al.,* 1988	CABG pts	62	Prospective, randomized, study comparing diltiazem (n = 22), nifedipine (n = 20) and SNP (n = 20) for post op HTN	NR	NR	0	0	0
Kunathai 1989	Pediatric pts undergoing CPB	22	Prospective study	34.4 hours	4.62 mcg/kg/min	0	0	0
Chitwood *et al.*, 1992	Post cardiac surgery	1089	Prospective study of closed loop vs manual management of post op HTN with SNP	15 hours in loop group vs 19 hours in manual group	NR	0	0	0
Underwood *et al.,* 1989	CABG pts	20	Prospective, randomized, pilot dose finding study comparing isradipine (n = 10) to SNP (n = 10) for post op HTN	2 hours	1.6 mcg/kg/min	0	0	0
David *et al.,* 1991	CABG pts	74	Open label, randomized trail comparing nicardipine (n = 38) to SNP (n = 36) for post op hypertension	NR (Max. range 18–24 hrs)	1.43 mcg/kg/min	0	0	0
Linakis 1991	Various	52	Retrospective analysis of pediatric patients on SNP that have a CN or SCN level ordered	NR (range 51–228 hrs)	NR (range 13.6–159.3 mg)	5	0	0

Table 9.3 (*Continued*)

Study	Patient population	N	Treatment and control group regimens	Mean duration of study	Mean SNP dose	Outcomes		
						CN toxicity (#)	Deaths (#)	Lactic acidosis (#)
Underwood *et al.,* 1991	CABG pts	20	Prospective randomized trial comparing isradipine (n = 10) to SNP (n = 10) for post op hypertension	2 hours	1.6 mcg/kg/min	0	0	0
Combes & Durand, 1992	CABG pts	20	Prospective, randomized, open label trial comparing nicardipine (n = 10) to SNP (n = 10) for post op HTN	Up to 24 hours	4.5 mcg/kg/min	0	0	0
Gretler *et al.,* 1992	Hypertensive emergencies	21	Prospective, randomized trial comparing ECG changes in patients treated with fenoldopam (n = 10) and SNP (n = 11)	NR	NR	0	0	0
Panacek *et al.,* 1995	Treatment of severe acute HTN (DBP ≥120 mmHg)	183	Fenoldopam (n = 90) SNP (n = 93)	6 to 24 hours	1.67 mcg/kg/min	0	0	0
Nathan *et al.,* 1992	Post-CABG hypertension	60	SNP (n = 28) or nifedipine (n = 32)	2 hour infusion, and 2 hour follow-up monitoring	Not stated	0	0	0
Ruegg *et al.,* 1992	CABG pts	198	Prospective, randomized trial comparing isradipine (n = 98) to SNP (n = 100) for post op HTN	6 hours	0.45 mg/kg	0	0	1
Hill *et al.,* 1993	CABG	20	Unknown study design Comparison of fenoldopam (n = 10) to SNP (n = 10) for post op hypertension	NR	1.36 mcg/kg/min	0	0	0
Lestrate 1993	CABG	27	Open label, randomized study comparing isradipine (n = 13) to SNP (n = 14) for post op hypertension	2 hours	NR (max dose 8 mcg/kg/min)	0	0	0
Pilmer *et al.,* 1993	Hypertensive urgency	33	Unknown study design Comparison of fenoldopam (n = 15) to SNP (n = 18) for severe systemic HTN	NR (range 6–24 hours)	1.2 mcg/kg/min	0	0	0
Hirschl *et al.,* 1997	Treatment of hypertensive emergency	81	Urapidil (n = 46) SNP (n = 35)	5.5 hours	0.5 to 3 mcg/kg/min	0	0	0
Khot *et al.,* 2003	Pts with aortic stenosis and LVD	25	Prospective study	24 hours	128 mcg/min	0	0	0
Moffett, 2008	Pediatric cardiac sugery pts	63	Retrospective study of pts receiving SNP after surgery	42.7 hours	1.24 mcg/kg/min	7	0	0

Abbreviations: NR – not reported, SNP – sodium nitroprusside, CABG – coronary artery bypass graft, MAP – mean arterial pressure, TS – thiosulfate, HTN – hypertension, MI – myocardial infarction, PCP – pulmonary capillary pressure, NTG – nitroglycerin, CN – cyanide, HF – heart failure, ECG – electrocardiogram, DBP – diastolic blood pressure, CPB – cardiopulmonary bypass, LVD – Left ventricular dysfunction.

9.7 Challenges associated with CN^- monitoring

CN^- has the potential to distribute into three general compartments – RBCs, plasma, and tissues (Schulz, 1984; Curry and Arnold-Capell, 1991; Cole and Vesey, 1987). Most investigators believe that the conversion of SNP to CN^- takes place in extracellular space or plasma (Smith and Kruszyna, 1974; Schulz, 1984; Curry and Arnold-Capell, 1991; Pasch *et al.*, 1983; Vesey *et al.*, 1980). CN^- becomes concentrated within erythrocytes; however, it is suggested that the plasma CN^- concentration remains in equilibrium with tissue and correlates to CN^- toxicity (Hall and Rumack, 1986; Curry and Arnold-Capell, 1991; Pasch *et al.*, 1983). The relationship between plasma and erythrocyte CN^- concentrations is proportional; thus, a rise in erythrocytic CN^- correlates to a rise in plasma and tissue CN^- concentrations (Vincent *et al.*, 1981). Because of ease of measurement, most investigators evaluate erythrocytic CN^- concentrations as opposed to plasma CN^- concentrations (Vincent *et al.*, 1981). However, most laboratories only report whole blood CN concentrations, which can take days to weeks to result and can be misinterpreted due to the significant distribution into erythrocytes. Whole blood CN^- levels may be elevated without manifestation of toxicity, or, in some cases, may be falsely low due to a delay between sample collection and analysis (Friederich and Butterworth, 1995; Hall and Rumack, 1986; Cole and Vesey, 1987). Therefore, most clinicians must rely on symptomatology rather than whole blood CN^- concentrations.

In 1976, Vesey and colleagues studied the CN^- and thiocyanate concentrations following SNP infusion for intra-operative hypotension in 26 patients undergoing major orthopedic surgery (Vesey *et al.*, 1976). The results indicated that for infusions lasting approximately 2 hours plasma and erythrocyte CN^- concentrations were more closely related to the total dose of SNP than to the rate of infusion ($r = 0.924$, $P < 0.001$; $r = 0.849$, $P < 0.001$, respectively), Vesey and colleagues found that 98.4% of blood CN^- was located in the red cells as previously described. Based on these results and previously published data, Vesey and colleagues hypothesized that the lethal plasma CN^- concentration is between 10 and 20 μmol/l, and the lethal intravenous dose of SNP would be less than 250 mg. The authors recommended that the total dose of SNP be limited to 1.5 mg/kg of the duration of an average surgical operation lasting 1 to 3 hours.

Pasch and colleagues studied the CN^- concentrations in the blood following intraoperative SNP administration with and without thiosulfate (Pasch *et al.*, 1983). SNP was administered to produce controlled hypotension during surgery in 55 patients. Patients were divided into 5 groups. Groups I through III were retrospectively divided based on the rate of SNP infusion (< 2 mcg/kg/min, 2–4 mcg/kg/min, and > 4 mcg/kg/min, respectively), group IV received an intravenous bolus of sodium thiosulfate at set times in addition to SNP doses $\geq$ 2.4 mcg/kg/min and group V received SNP at doses $\geq$ 3.5 mcg/kg/min in addition to sodium thiosulfate continuous infusion. The authors determined that the mean values of erythrocyte CN^- concentrations increased with the quantity of SNP administered in groups I through III. Most patients in group I had a CN^- level less than 10 μmol/l. In group II, the mean CN^- concentration was 14.4 μmol/l, and in group III the mean CN^- concentration was 48.2 μmol/l. In patients not receiving thiosulfate, there was a clinically significant correlation between the SNP infusion rate and the maximum CN^- concentration ($r = 0.862$; $p < 0.01$). In groups I through III, the concentration of CN^- increased with time, with the most notable increase in group III which received the greatest SNP infusion rate. Groups IV and V had low CN^- concentrations due to the administration of thiosulfate.

According to previously published data and cases, Pasch and colleagues assumed that metabolic changes (increase in base deficit and a decrease in mixed venous oxygen saturation) were detectable at erythrocyte CN^- concentrations of 40 μmol/l, severe clinical symptoms occur at concentrations from 200 to 250 μmol/l and upward, and concentrations from 400 to 500 μmol/l are considered lethal. Based on the calculated CN^- detoxification rate of 0.1–0.2 mg/kg/h, the authors computed time to the development of significant CN^- levels for given SNP infusion rates (Pasch *et al.*, 1983). The authors concluded that infusion rates of up to 2 mcg/kg/min led to a slight increase in red cell CN^- concentrations of less than 20 μmol/l and presented no danger to the patient, while doses greater than 2.5 mcg/kg/min led to significant elevations in CN^- levels in a matter of hours to minutes. Admittedly, the authors note considerable inter-individual fluctuation in the rate of SNP infusion and the red cell CN^- concentration.

In the 1970s, various authors published safe upper dosing limits for short SNP infusions during deliberate hypotension, but little was known about the toxicity of long-term SNP infusions (Vesey *et al.*, 1976; Vesey and Cole, 1985; Michenfelder, 1977). In 1985, Vesey and Cole studied plasma and red cell CN^- concentrations and thiocyanate concentrations in 30 patients receiving long-term therapy (12–314 hours) with SNP (Vesey and Cole, 1985). Blood samples were obtained during and/or near the end of the SNP infusion. The authors found a significant correlation between plasma and red cell CN^- concentrations and the rate of SNP infusion but not to total dose ($r = 0.64$, $P < 0.001$; $r = 0.71$, $P < 0.001$). In addition, erythrocyte CN^- concentrations correlated closely with plasma CN^- concentrations ($r = 0.92$, $P < 0.001$). The authors showed that plasma CN^- concentrations may increase above normal values when SNP is infused at rates greater than 1 mcg/kg/min, but concerning CN^- levels did not occur until doses exceeding 4 mcg/kg/min. They hypothesized that when SNP is infused at higher rates, hydrogen cyanide may be released faster than it can be detoxified by endogenous thiosulfate, which results in elevated CN^- concentrations.

A limitation with this study is that there were only a handful number of levels measured at doses greater than 4 mcg/kg/min; therefore, it is not certain that there is a linear relationship with plasma CN^- levels at the higher doses. The study did demonstrate that there is a difference in toxicity when SNP is administered at lower doses over a longer period of time versus rapid boluses in the intra-operative setting and that there is safety with more chronic use. Admittedly, the authors did state that the safe CN^- level in long term SNP infusions is unknown, however, they derived a lower and upper limit for SNP based on a couple of assumptions as follows: data from patients suffering from tropical ataxic neuropathies, which is believed to be due to CN^-, reveals that these patients have a mean plasma CN^- concentration of 1.1 micromol/l (Osuntokun, 1973).

Vesey *et al.* correlated this to a SNP dose of approximately 4 mcg/kg/min, which the authors suggested as the upper limit of safety for SNP. The average smoker inhales 25 mcg of HCN a day, which is equivalent to about 1 mcg/kg/min of SNP and they correlated this to the lower tolerable limit, as the average smoker has a negligible CN^- level.

While these assumptions provide us with useful guidance as to the safe administration of SNP, they cannot be taken at face value. The limitation with the above studies is that estimations of toxicity were based on either RBC or plasma CN^- levels without correlations to clinical symptoms. The best data we have correlating levels to clinical symptoms is by Rumack and Hall (1986) which utilizes whole blood levels. Whole blood levels are generally what routine laboratories report as well. As such, it is hard to suggest that the proposed dose limits by the previously mentioned studies are absolute. In routine clinical practice, as it relates to use in the post-operative or ICU setting, the proposed dose ranges are routinely exceeded without clinical symptomatology.

It is also important to note that all CN^- assays are not created equal. A common procedure that had been utilized up until at least the early 1980s is the colorimetric procedure, which in comparison to alternate methods like the potentiometric or spectrometry, has the potential to introduce light to the biological assay. The significance of this lies in the fact that light converts SNP to aquapentacyanoferrate ion, which is still colorless. This ion readily decomposes to release CN^-, which is the reason SNP must be protected from light during administration (Wolfe and Swineheart, 1973).

When CN^- levels were evaluated in a study by Bisset *et al.* utilizing an ion-sensitive electrode, they were unable to measure CN^- in whole blood, plasma and red blood cells (Bisset *et al.*, 1981). Therefore, some of the data regarding CN^- levels pre-1980 may have been spuriously overestimated because of the assay technique. Also of note, sodium thiosulfate has the potential to interfere with several assays and cause falsely elevated CN^- levels (Way, 1984). Assays that require acidification as part of their process, or samples that are frozen and thawed, can have falsely elevated CN^- levels due to the generation of CN^- from thiocyanate (Hasuike *et al.*, 2004; Vesey *et al.*, 1979). Finally, there are discrepancies in arterial versus venous samples (Curry, 1963; Trunkey, 1978). This calls into question the accuracy of estimations of CN^- levels from much of the data, making it difficult to correlate CN^- levels to outcomes.

9.8 Safe use of SNP – clinical monitoring

Even with the limitations of the current data, one is still left with the question; how do I safely use this drug? This drug still has great utility and can be monitored

for safety without the use of CN^- levels, which are impractical today. The utilization of lactate levels does provide clinical utility. As reviewed previously, CN^- accumulation results in halting of aerobic metabolism by inhibiting the final step of oxidative phosphorylation, leading to the generation of lactic acid. Although inhibition of mitochondrial oxidative phosphorylation is not the only consequence of CN^- accumulation, significant CN^- intoxication has been associated with various degree of lactic acidosis both in animals and humans (Vogel and Sultan, 1981; Salkowski and Penney, 1995; Baud *et al.*, 1991, 2002; Graham *et al.*, 1977).

Baud *et al.* conducted a prospective study of 39 smoke inhalation victims without severe burns and found a serum lactate level of > 10 mmol/l (> 90 mg/dl) to significantly correlate ($r = 0.47$, $p < 0.001$) with toxic CN^- levels of > 0 μmol/l or > 0.0 mg/l (blood CN^- concentrations < 40 μmol/l were defined as nontoxic, ≥ 40 to 100 μmol/l as potentially toxic, and $\geq$ 100 μmol/l as potentially lethal) (Baud *et al.*, 1991). Based on their results, the authors reported that the sensitivity of plasma lactate concentration > 10 mmol/l for CN poisoning (blood level > 40 μmol/l) was 87%, with 94% specificity and the positive predictive value of 95%. Baud and colleagues therefore concluded that high plasma lactate concentrations were largely indicative of CN^- intoxication in smoke inhalation victims with no or minor burns ($< 15\%$ of body surface area) (Baud *et al.*, 1991). While this information was useful for patients involved in fires, little was known about the correlation between acute pure CN^- poisoning and plasma lactate concentration.

Baud *et al.* hypothesized that serial plasma lactate concentrations could be used as a marker for the evolution of CN^- toxicity, and have confirmatory and therapeutic value. To test their hypothesis, Baud *et al.* conducted a retrospective study of 11 patients in the toxicological intensive care unit who had confirmed exposure to "pure" CN^- (Baud *et al.*, 2002). Victims of smoke inhalation were excluded from the study. The median age was 38 years with the majority of patients having ingested potassium cyanide (7/11). At baseline, there was a significant correlation ($r = 0.74$, $p = 0.017$) between the median blood CN^- concentration of 4.2 mg/l ($n = 10$), $0.34 - 6.9$ mg/l) and median plasma lactate concentration of 168 mg/dl ($n = 10$, $43 - 477$ mg/dl). It was determined that plasma lactate concentration of 72 mg/dl (8 mmol/l) had the best sensitivity and specificity (94% and 70%, respectively) in predicting CN^- toxicity (blood CN^- concentration of $\geq$ 1.0 mg/l) with positive and negative predictive values of 64% and 98%, respectively.

In patients that did not receive catecholamine infusion, the specificity and the positive predictive values increased to 85% and 86%, respectively. Although limited by a small sample size, the authors concluded that immediate and serial measurement of plasma lactate concentrations would be useful in assessing severity of CN^- poisoning, especially in a situation where immediate laboratory confirmation of CN^- intoxication is not possible (Baud *et al.*, 2002). Finally, Fahmy demonstrated a correlation between base excess and whole blood CN^- levels in patients who received SNP for intraoperative hypotension (Fahmy, 1985). The available literature supports a positive correlation between plasma lactate and CN^- concentrations, although most of the data are in acute CN^- poisonings. While it is not certain that plasma lactate level is a sensitive or a diagnostic marker in cases of gradual CN^- accumulation (i.e., administration of SNP) due to lack of randomized controlled trials, the current data suggest that it is a reasonable monitoring tool.

9.9 Prevention and treatment of CN^- toxicity

The wide variability in SNP dosing, metabolism by the patient and concurrent clinical factors can still permit the occurrence of CN^- poisoning and can have disastrous consequences if not recognized and treated early and aggressively. A laboratory diagnosis can take hours to days to obtain, thus, empiric treatment is primarily initiated on clinical symptoms alone. An understanding of the antidotes to combat this complication is prudent. Three general classes of agents exist for treatment of cyanide toxicity: methemoglobin generators (sodium nitrite, amyl nitrite), sulfur donors (sodium thiosulfate), and direct binding agents (hydroxocobalamin, dicobalt EDTA). From these three classes, there are four antidotes or antidote kits in various countries that are routinely used for the treatment of cyanide poisoning. Two cyanide antidote kits are available and are preferred for empiric treatment of cyanide poisoning. The Cyanide Antidote Kit consists of amyl nitrite, sodium nitrite, and sodium thiosulfate (Lilly, 1990).

The Cyanokit®, a newer cyanide antidote kit, consists of hydroxocobalamin (Meridian Medical Technologies, 2011). Sodium thiosulfate and hydroxocobalamin have both been empirically used for cyanide toxicity and have specifically been evaluated for cyanide toxicity associated with sodium nitroprusside. Cobinamide is an investigational agent currently being evaluated for efficacy and safety in the treatment of cyanide toxicity. A detailed review of these antidotes follows below.

9.9.1 Sodium thiosulfate/nitrite therapy

Amyl nitrite, sodium nitrite, and sodium thiosulfate are available in a three-drug cyanide antidote kit, and have been evaluated in the treatment of sodium-nitroprusside induced cyanide toxicity. Amyl nitrite and sodium nitrite exert their antidotal by effectively converting hemoglobin to methemoglobin. This conversion is beneficial because methemoglobin has a higher affinity for cyanide than cytochrome oxidase. Cyanide clearance may be enhanced by the addition of sodium thiosulfate. Sodium thiosulfate expedites the conversion of cyanide to thiocyanate, allowing it to be excreted in the urine (Hall *et al.*, 2007).

Numerous studies have proven that synergism exists with nitrites and thiosulfate. In 1952, Chen and Rose published a study in which they administered thiosulfate, amyl nitrite, and/or sodium nitrite in various doses and combinations to cyanide poisoned dogs and reported the ratio of observed 50% lethal doses (LD_{50}) between treated and untreated animals. Results demonstrated that the combination of thiosulfate with either nitrite was superior to thiosulfate or any nitrite alone. The combination allowed survival at LD_{50} at 11–18 times higher rates than the control group (Chen and Rose, 1952).

In 1982, Schulz and colleagues published a study of 19 patients receiving mixed infusions of thiosulfate and nitroprusside for hypertensive emergency. None of the patients evaluated reached toxicologically high levels of cyanide for up to two weeks. Additionally, there was no reduction in the efficacy of nitroprusside when mixed with thiosulfate (Schulz *et al.*, 1982).

In 1987, Cole and Vesey evaluated concentrations of cyanide and thiocyanate in 30 patients undergoing intraoperative nitroprusside-induced hypotension. Half of the patients received a bolus of thiosulfate upon discontinuation of nitroprusside with a dosing range of 10.6–38.5 mg/kg. The average infusion rate and duration of sodium nitroprusside was 3.89 mcg/kg/min for 98.7 minutes in the control group and 5.43 mcg/kg/min for 88.9 minutes in the thiosulfate group. Cyanide and thiocyanate levels were measured 10, 30, and 60 minutes post infusion. The results showed that patients treated with thiosulfate had significantly lower cyanide levels ($p < 0.05$) and a significantly shorter time to decrease in cyanide levels ($p < 0.001$) (Cole and Vesey, 1987). To prevent nitroprusside-induced cyanide toxicity, sodium thiosulfate should be administered at 5–10 times the rate of sodium nitroprusside. When given acutely for cyanide toxicity, antidotal therapy is initiated by breaking an amyl nitrite inhalant, holding it in front of the patient's mouth, and allowing the patient to inhale for 15 seconds. The inhalant should then be taken away for 15 seconds. Amyl nitrite has a time to onset of approximately 30 seconds, and last 3–5 minutes. This procedure may be repeated until an intravenous (IV) line is established and sodium nitrite is prepared. Once IV access is established, sodium nitrite is administered at a dose of 300 mg for adults and 180 to 240 mg per square meter of body surface area for children, at a rate of 75 to 150 mg/min. The time to peak effect of sodium nitrite is 30–70 minutes. Sodium thiosulfate should be administered intravenously, immediately following the infusion of sodium nitrite. If signs of cyanide toxicity are still present two hours following administration of sodium nitrite and sodium thiosulfate, administration of both may be repeated at one-half the original dose. The cyanide antidote kit includes 300 mg sodium nitrite, 0.3 ml amyl nitrite inhalants, and 12.5 g of sodium thiosulfate (Lilly, 1990).

Short term therapy with sodium thiosulfate has very few adverse reactions, and these are primarily infusion-related reactions. A rare side effect associated with thiosulfate therapy is thiocyanate accumulation and degradation which can occur in the setting of renal failure. Nitrite therapy can cause significant adverse events and patients should be closely monitored. Vasodilation and hypotension are commonly seen with nitrites, which may preclude their use in patients already in shock. Nitrites should be avoided in pregnancy to avoid oxidative stress to fetal hemoglobin. Nitrites should also be avoided in patients with impaired oxygen carrying capacity, such as patients with certain cardiovascular conditions or carbon monoxide poisoning (Shepherd and Velez, 2008). Blood pressure and methemoglobin concentrations should be monitored

closely, and the administration of sodium nitrite discontinued if the systolic blood pressure goes below 80 mm Hg. The methemoglobin concentration should not exceed 40% in adults or 30% in children (Lilly, 1990).

Co-infusions of sodium nitroprusside and sodium thiosulfate may result in higher levels of thiocyanate. The half-life of sodium thiosulfate is very short, at 15–20 minutes. The half-life of thiocyanate is approximately 2.7 days in patients with normal renal functions; however, it can be up to 9 days in patients with renal dysfunction. Signs of thiocyanate toxicity are primarily neurologic, and can include confusion, hallucinations, convulsions, and coma. Toxicity can be seen with thiocyanate in approximately nine days in patients with normal renal function, and three days in patients with renal dysfunction. Thiocyanate is considered toxic at serum levels exceeding 100 mg/l. The most effective treatment is hemodialysis, and there is generally rapid improvement in symptoms (Schulz; 1984; Hall *et al.*, 2007).

9.9.2 Hydroxocobalamin

Hydroxocobalamin has been proven to be safe and effective in treating cyanide toxicity associated with sodium nitroprusside based on both animal and human data. Hydroxocobalamin traps the cyanide ion, preventing transfer of cyanide from red blood cells and plasma to the tissues. Following intravenous administration of hydroxocobalamin, significant binding to plasma proteins and low molecular weight physiological compounds occurs, forming various cobalamin-(III) complexes by replacing the hydroxo ligand. The final product of this process is cyanocobalamin (vitamin B12). Once converted to cyanocobalamin, cyanide is safely excreted unchanged in the urine (Meridian Medical Technologies, 2011; Zerbe and Wagner, 1993).

Dose-proportional pharmacokinetics was observed in a study following single dose intravenous administration of 2.5 to 10 g of hydroxocobalamin in healthy volunteers. Mean free and total cobalamin-(III) complex values were 113 and 579 mg eq/ml, respectively, following a dose of 5 g of hydroxocobalamin. Similarly, mean free and total cobalamins-(III) complex values were 197 and 995 mg eq/ml, respectively, following the dose of 10 g of hydroxocobalamin. The predominant mean half-life of free and total cobalamins-(III) was found to be approximately 26 to 31 hours at both the 5 g and 10 g dose level. Overall, the total urinary excretion was calculated to be at least 60% to 70% of the administered dose. The majority of the urinary excretion occurred during the first 24 hours, but red-colored urine was observed for up to 35 days following the intravenous infusion. When normalized for body weight, male and female subjects revealed no major differences in pharmacokinetic parameters of free and total cobalamins-(III) following the administration of 5 and 10 g of hydroxocobalamin (Meridian Medical Technologies, 2011).

Simultaneous administration of hydroxocobalamin with nitroprusside significantly decreases cyanide concentrations. Cottrell *et al.* published a study in 1978 evaluating the use of hydroxocobalamin in 14 patients receiving nitroprusside infusions. Patients received either nitroprusside alone or in combination with hydroxocobalamin at 25 mg/hr. Doses of nitroprusside were not significantly different between groups. The study demonstrated that the small amounts of cyanide produced by nitroprusside were neutralized by high doses of hydroxocobalamin. Concomitant administration of hydroxocobalamin and nitroprusside significantly decreased (60% reduction, $p < 0.025$) the red blood cell concentration of cyanide when compared to the placebo group. Acidosis did not occur in patients who received a continuous infusion of hydroxocobalamin, showing that cyanide transfer from red cells via plasma to tissue had been prevented. The inhibition of the transfer could possibly be due to hydroxocobalamin's greater affinity for cyanide than cytochrome oxidase, as well as the rapid urinary elimination of cyanocobalamin. Another explanation could be that hydroxocobalamin decreases the diffusion gradient of blood cyanide to tissue, keeping the concentration of cyanide low in the plasma. When the hydroxocobalamin infusion was stopped prior to cessation of nitroprusside, acidosis and other signs of cyanide toxicity were similar to the patients who had not received hydroxocobalamin. Adverse events only included transient reddening of the skin, mucus membranes, and urine. The authors concluded that hydroxocobalamin should be continued in patients for 10 hours after cessation of nitroprusside based on the half-life of cyanide on the red blood cell (Cottrell *et al.*, 1978).

The recommended starting dose of hydroxocobalamin is 5 g given IV over 15 minutes (approximately 15 ml/hr or 7.5 minutes per vial). Depending on the severity of the poisoning and the clinical response, a repeat dose of

5 g dose may be administered for a maximum total dose of 10 g. Infusion rates for the second infusion can vary from 15 minutes (for patients in extremis) to 2 hours. The rate of infusion should be adjusted in cases where infusion reactions occur. Hydroxocobalamin should be administered through a dedicated IV line since it is incompatible with many medications, including other cyanide antidotes. Hydroxocobalamin is a lyophilized powder which turns red upon reconstitution and should be protected from light. The Cyanokit® consists of 2.5g vials, each requiring reconstitution with 100 ml of diluent, or a single-dose 5 g vial to be reconstituted in the same manner (Meridian Medical Technologies, 2011; Zerbe and Wagner, 1993). Hydroxocobalamin can interfere with certain laboratory tests, including liver enzymes, bilirubin, creatinine, creatine kinase, phosphorous, glucose, magnesium, and iron. Results can be falsely high or low (Hall *et al.*, 2007).

The safety of hydroxocobalamin was evaluated in healthy volunteers in a randomized, placebo-controlled, ascending dose study. A total of 136 patients were randomized to 2.5 g, 5 g, 7.5 g, and 10 g of hydroxocobalamin or placebo by intravenous infusion during 7.5–30 minutes. The most common adverse events noted were reddening of the skin and chromaturia. Other common adverse events included pustular or papular rash, erythema at the injection site, headache, decreased lymphocyte percentage, and chest pressure. There were moderate increases in systolic blood pressure, ranging from 22.6 to 27 mm Hg in the hydroxocobalamin group and tended to be greatest near the end of the infusion. Changes tended to be larger and persist longer in the patients receiving 7.5 g and 10 g of hydroxocobalamin. All blood pressure changes associated with hydroxocobalamin were self-limiting. The authors theorized that the increases of blood pressure may be beneficial in cyanide toxicity to counteract the hypotension associated with nitrite containing compounds. Two patients had allergic reactions, one in the 5 g hydroxocobalamin group and the other in the 10 g group. The reactions were developed within minutes and the patients were successfully controlled with corticosteroids (dexamethasone) and antihistamines (dimethindene maleate) (Uhl *et al.*, 2006).

9.9.3 Cobinamide

Cobinamide is a new compound currently undergoing evaluation for its use as a cyanide antidote. Cobinamide is the resultant compound of cobalamin synthesis. It binds two cyanide ions, each with about 100 times higher affinity than hydroxocobalamin. Cobinamide differs from cobalamin in that it lacks a dimethylbenzimidazole ribonucleotide group. This lack of a dimethylbenzimidazole ribonucleotide group leads to several key differences between cobinamide and hydroxocobalamin. First, cobinamide provides two ligand binding sites instead of only one on hydroxocobalamin, leading to a higher binding affinity for cyanide. Second, it increases the binding affinity of the upper ligand binding site. This is because the dimethylbenzimidazole ribonucleotide group exerts a negative trans- effect on the binding site, decreasing the binding affinity of cobalamin. And lastly, the lack of the dimethylbenzimidazole ribonucleotide group increases the solubility of cobinamide. This could potentially make it a superior pharmacologic agent since it can be administered in smaller volumes or by various routes. Cobinamide also binds nitric oxide, but much less tightly than it binds cyanide. Once cobinamide binds cyanide, it can no longer bind to nitric oxide. Decreased nitric oxide binding could help limit the harmful hypertensive effects of cobinamide when administered with sodium nitroprusside (K. Broderick *et al.,* 2007; Chan *et al.*, 2010).

To date, cobinamide has only been evaluated in animal studies and has been compared to hydroxocobalamin. Research with *Drosophila melanogaster* and cultured cells has shown cobinamide to be a superior agent to hydroxocobalamin in cyanide detoxification. Studies analyzing the effect of lethal doses of cyanide in mice have found that inhaled or intramuscular formulations may be effective for neutralization of cyanide. These formulations may be beneficial in out of hospital cyanide overdoses or in situations where intravenous access cannot be made. Studies specifically evaluating the intramuscular formulation in sodium nitroprusside-induced cyanide toxicity found that cobinamide sulfite does not bind nitric oxide *in vivo*, mitigating any hypertensive effects of neutralizing the action of sodium nitroprusside. In addition, no clinical toxicity was observed even at concentrations exceeding 2000 mg/kg. However, postmortem examination of injected animals found significant quantities of residual cobinamide, indicating there may be some localized vasoconstriction at the injection site (Chan *et al.*, 2010).

In 2010, Brenner and colleagues completed a study evaluating cyanide concentrations in rabbits who had received either cobinamide or hydroxocobalamin after a 10 mg infusion of sodium cyanide. Doses were equimolar and consisted of 0.0816 moles of each agent in 5 milliliters of normal saline, and were administered over 30 seconds. The authors found that intravenous infusions of cobinamide yielded lower cyanide concentrations than did hydroxocobalamin infusions. In addition, it saw a faster time to complete recovery of oxy- and deoxyhemoglobin concentrations (13.8 minutes in the cobinamide group compared to 75.4 minutes in the hydroxocobalamin group, $p < 0.001$). This study indicated that cobinamide more rapidly and completely reverses the physiologic effects of cyanide compared with equivalent doses of cobalamin at the doses evaluated in the study (Brenner and Mahon, 2010).

Safety data is lacking on cobinamide. However, evidence from animal studies indicates a mild adverse event profile. One study in mice showed no effect on growth of leukemic cells, monocytes, or lymphocytes. When administered continuously for 14 days, cobinamide had no apparent toxic effects and did not inhibit methionine synthase or methymalonyl-CoA mutase, two cobalamin-dependent enzymes (K. Broderick *et al*., 2007).

Preliminary evidence of cobinamide shows that it may be a new avenue for treatment in cyanide toxicity. Advantages of cobinamide treatment include its superior pharmacologic profile, allowing it to be administered quickly and through multiple routes. Studies also show cobinamide rapidly decreases cyanide concentrations, likely due to its multiple binding sites and increased affinity for cyanide ions. While early animal study data indicate an efficacious and safe option for treatment, future studies are needed to evaluate the safety and efficacy of cobinamide in human patients.

9.10 Conclusions

SNP is a potent vasodilator with excellent blood pressure lowering effects, however, health-care providers must be aware of its limitations. With close monitoring of arterial blood pressure, serum lactate levels, and clinical symptoms of CN^- toxicity SNP can be a safe first-line agent in hypertensive emergencies or as an adjunct in difficult to control patients.

9.11 Disclosure

The author reports no conflicts of interest in this work.

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CHAPTER 10
Smoke inhalation

Alan H. Hall and Stephen W. Borron

At a Glance

- Inhalation of smoke in enclosed-space fires is the most common etiology of acute cyanide poisoning.
- Cyanide inhalation from fire smoke impairs the ability to self-rescue.
- Carbon Monoxide and Cyanide in fire smoke are at least additive and are possibly synergistic toxicants.
- Plasma lactate levels are a surrogate measure for significant cyanide poisoning, both in smoke inhalation victims (>/ = 10 mmol/l) and in "pure" cyanide poisoning (>/ = 8 mmol/l).
- Of all cyanide antidotes in current clinical use, Hydroxocobalamin has the most favorable safety/efficacy profile for empirical administration to smoke inhalation victims.

10.1 Introduction

Eight major factors can cause death in fires (Kimmerle, 1974):

1 Direct consumption by the fire (flame contact)
2 Very high temperatures
3 Oxygen deficiency
4 Presence of carbon monoxide
5 Presence of other toxic gases
6 Presence of smoke
7 Development of fear, shock, and panic
8 Secondary fire effects due to mechanical reasons (trauma, bone fractures, etc.).

This chapter focuses on numbers 5 and 6 of the above: presence of other toxic gases (i.e., cyanide) and presence of smoke. Adults attempt to escape a fire scene; children hide. Certainly it is possible that cyanide in fire smoke impairs the ability to self-rescue/escape.

Fire smoke is a quite complex mixture of liquid and solid aerosols, fumes, gases, and vapors produced by pyrolysis or thermal decomposition (Landrock, 1983; Gad & Symington *et al.*, 1978; Anderson & Harland, 1982; Linden, 2007; Stefanidou *et al.*, 2008). Bauer and Gimbel (2004) divided the effects of the potentially large number of chemical substances in fire smoke and the low oxygen environment produced by fires into three categories: (i) *thermal injury* caused by intense heat, heated air, or steam; (ii) *chemical injury* caused by any number of chemical substances in fire smoke that can cause upper and/or lower airway irritation; and (iii) *hypoxia and asphyxia* from the decrease in oxygen in the breathing atmosphere due to depletion by combustion and systemic asphyxiants, particularly carbon monoxide and cyanide (Bauer & Gimbel, 2004).

Since antiquity, death from smoke inhalation has been appreciated. In certain ancient conflicts, captured enemy soldiers were executed by being placed in cages over green wood-fueled fires. Carbon monoxide poisoning as a cause of serious poisoning or death in smoke inhalation victims has long been recognized, but it was only in the 1960s to 1980s when the potential for a significant cyanide poisoning component contributing to or in some cases being the major cause of serious poisoning or fatality began to be appreciated (Wetherell, 1966; Birky & Clarke, 1981; Clark *et al.*, 1981; Jones *et al.*, 1987). Additional cases were reported in the 1990s (Shusterman *et al.*, 1996; Kirk *et al.*, 1993; Jones & Krohmer, 1990; Yoshida *et al.*, 1991; Baud

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et al., 1991; Fornes *et al.*, 1994) and later (Gill *et al.*, 2003; Yeoh & Braitberg, 2004; Borron *et al.*, 2007; Fortin *et al.*, 2010). However, in some smoke inhalation cases, there may be no cyanide involved (Gill *et al.*, 2003).

Wetherell (1966) reported 53 fire victims who died and found that both carbon monoxide and cyanide were present in 39/53. Some of these victims were badly burned and others were hardly burned at all. Cyanide was found in all but 14/53. Wetherell noted: "It would be particularly embarrassing to overlook such a potent cause of death [cyanide]." Despite this admonition, currently, some more than 40 years later, victims of fatal smoke inhalation and firefighters with possible smoke inhalation during line-of-duty deaths may not have autopsy analytical samples obtained for cyanide.

At present with the proliferation of plastics and synthetic polymer building materials, the risk of a significant cyanide poisoning component in victims of enclosed-space fire smoke inhalation has increased (Alarie, 2002; Stefanidou & Athanaselis, 2004). There are approximately 4000 smoke inhalation deaths each year in the United States, and in 50–80% of these the cyanide poisoning component may play a critical role. Firefighters are at especial risk, as are children, the elderly, and those with pre-existing cardiac or respiratory disease. An ideal cyanide antidote for pre-hospital administration on the fireground would have the following characteristics (Hall *et al.*, 2009):

- rapid onset of action;
- neutralize cyanide without interfering with oxygen transport;
- safety and tolerability profile conducive to use in pre-hospital settings as well as in the ED/ICU;
- safe for use in smoke inhalation victims;
- not harmful if administered to non-poisoned patients;
- easy to administer.

Hydroxocobalamin, of all the cyanide antidotes in clinical use worldwide, best meets these criteria. Hydroxocobalamin (as Cyanokit® Hydroxocobalamin 5 grams) is currently one of the two *FDA-approved antidotes in the United States* for *known or suspected cyanide poisoning*, including smoke inhalation victims. The second available FDA-approved antidote kit, Nithiodote®, consists of sodium nitrite and sodium thiosulfate. The sodium nitrite portion which induces methemoglobin is relatively contraindicated for the empirical treatment of smoke inhalation victims where a significant cyanide poisoning component may or may not be present.

A number of years of experience of the Paris Fire Brigade (Borron *et al.*, 2007) have shown that using the following criteria, early and efficacious pre-hospital administration of hydroxocobalamin can be safely done:

- extricated from an enclosed-space fire scene with smoke;
- soot in the nose, mouth, or throat or sooty expectorations;
- *any* alteration of consciousness; and
- *especially hypotension* (although hypotension need not be present for the decision to administer hydroxocobalamin).

Hydroxocobalamin is most efficacious when administered as soon as possible following extrication from the fireground, and thus should be available for pre-hospital use.

10.2 Cyanide in smoke inhalation

The diagnosis of cyanide poisoning as a significant part of the toxicity of fire smoke inhalation is often missed because it is not considered, although it has been recognized since the 1960s (Wetherell, 1966) and was further recognized in the 1970s and 1980s (Mayes, 1985; Bowes, 1974; Birky *et al.*, 1979, 1980; National Fire Protection Agency, 1983; Von Meter *et al.*, 1979). The non-availability of a readily available analytical method for emergent measurement of blood cyanide concentrations and the non-specific and non-pathognomonic nature of cyanide poisoning signs and symptoms continue to render making the diagnosis difficult (Baud, 2007).

In 2007, NIOSH published "Preventing Fire Fighter Fatalities Due to Heart Attacks and Other Sudden Cardiovascular Events" (NIOSH, 2007), wherein it is noted that hydrogen cyanide is formed by incomplete combustion of any substance that contains carbon and nitrogen (both naturally occurring and synthetic) and that airborne concentrations exceeding those of established occupational exposure limits occur in structural fires (Jankovic *et al.*, 1991; Brandt-Rauf *et al.*, 1988; Gold *et al.*, 1978). Cyanide impairs cellular utilization of oxygen, which can result in cellular hypoxia and a variety of cardiac manifestations (Purser *et al.*, 1984). A study in dogs exposed to both carbon monoxide by inhalation and cyanide by intravenous infusion to mimic inhalation exposure showed that carbon

monoxide had little or no effect on cardiovascular parameters, while cyanide severely depressed such functions (Breen *et al.*, 1995).

An air monitoring study at more than 200 fires in Boston did not find significant amounts of hydrogen cyanide present (Treitman *et al.*, 1980), while another such study found cyanide present in about 50% of fires (Gold *et al.*, 1978). Perhaps different materials were burning in these two studies, which might account for the differing results.

When whole blood cyanide levels are not assayed at autopsy, cyanide poisoning as a significant component of fatal smoke inhalation may go unrecognized. For firefighters, this is particularly disturbing in line-of-duty deaths. The U.S. Fire Administration's Firefighter Autopsy Protocol (U.S. Fire Administration, 2008) specifically lists hydrocyanic acid in its section of Toxicological Examination. Perhaps this protocol is not followed as often as would be advisable?

Ambient air monitoring for CO and CN^- can now be done on the fireground in real time which can alert firefighters to the potential for poisoning. A significant cyanide poisoning component should be suspected in smoke inhalation victims with otherwise unexplained respiratory failure or a persistent anion-gap metabolic acidosis (Lee-Chiong, 1999).

Barillo *et al.*'s (1994) study is sometimes quoted by those who question the frequency and importance of a significant cyanide poisoning component in smoke inhalation (Barillo *et al.*, 1994). These authors state in their abstract that: "Cyanide poisoning is infrequent in fire fatalities" and "Specific assay and treatment for cyanide poisoning is rarely necessary in the treatment of smoke and fire." This study examined the New Jersey State Medical Examiner Office's records for a two-year period from 1985 to 1987 and was of fatalities only, not specifically of victims who were still alive when extricated from a fire scene and who therefore might have benefited from specific cyanide antidote treatment.

Victims had deaths attributed to fires, burns, and smoke inhalation. Thus, unlike the study from Paris by Baud *et al.* (1991) where only living victims with < 20% total body surface area (TBSA) burns were studied (Baud *et al.*, 1991), thermal burns were present in 82% of cases, the average TBSA was 71%, and 61 victims were charred or incinerated (had non-survivable burn/thermal injuries) (Barillo *et al.*, 1994). Also, the blood cyanide samples were obtained at autopsy, not shortly after extrication from the fire scene as in the Paris study. Only 56 of these the total of 433 fire fatalities (13%) received pre-hospital, emergency department, or inpatient medical treatment, and most died within 1–2 hours (Barillo *et al.*, 1994). Whole blood cyanide levels were obtained in 364 cases. Of these, 85 (23%) had no detectable blood cyanide, and 31 (9%) had blood cyanide levels > 3 mg/l (generally considered to be lethal). These authors do not report whether the victims with the potentially lethal blood cyanide levels received pre-hospital or hospital treatment.

While the fact that only 9% of the fatalities in the Barillo *et al.* study (1994) had potentially fatal blood cyanide concentrations does indeed represent a relatively small fraction (Barillo *et al.*, 1994), patients with such blood cyanide levels administered hydroxocobalamin in the pre-hospital setting have survived with similar blood cyanide levels (Borron *et al.*, 2007; Fortin *et al.*, 2006).

In a prospective series of 69 smoke inhalation patients from Paris, France, treated with between 5 and 15 grams of hydroxocobalamin in the pre-hospital setting or in the intensive care unit, 50 (72%) survived (Borron *et al.*, 2007). Of the 67% of patients confirmed to have cyanide poisoning (whole blood cyanide level >/ = 39 μmol/l (1 mg/l)), 67% administered hydroxocobalamin survived. No serious adverse events were attributed to administration of the antidote (Borron *et al.*, 2007).

The issue is sometime raised regarding substituting sodium thiosulfate alone for hydroxocobalamin because of the lesser cost. This issue was reviewed by Hall *et al.* (2007). In general, there are much fewer published data on the use of sodium thiosulfate alone than there are for hydroxocobalamin for the antidotal treatment of cyanide poisoning. There are contradictory conclusions regarding the efficacy of sodium thiosulfate in animal studies, and the onset of sodium thiosulfate antidotal action may be too slow for it to be recommended as the only cyanide antidote for emergent administration (Hall *et al.*, 2007).

Because a significant cyanide poisoning component in smoke inhalation victims, both firefighters and civilians, continues to be under-diagnosed and untreated, anything that can be done to increase awareness of the possibility of this potentially fatal and treatable condition should be undertaken. For those in positions of

influence in the fire service, this could include opening a dialog with the local Medical Examiner's Office and strongly urging that all firefighter line-of-duty deaths and civilian smoke inhalation fatalities that come to autopsy have blood cyanide levels determined on a routine basis. The results of such assays should be published and should also be discussed with pre-hospital care providers, EMS medical directors, and emergency physicians locally at mortality and morbidity conferences and medical grand rounds. Medical examiners and forensic toxicologists should be encouraged to report their findings at regional and national medical meetings.

10.3 Plasma lactate levels as a screening assay

The diagnosis of acute cyanide poisoning, even in "pure" cyanide poisonings has always been difficult in the absence of a credible exposure history because cyanide poisoning has no pathognomonic signs and symptoms (Baud, 2007). This is particularly difficult in cases of smoke inhalation where there may or may not be a significant cyanide poisoning component. In smoke inhalation victims, a plasma lactate level of >/ = 10 mmol/l was a sensitive and specific indicator of the presence of a blood cyanide concentration >/ = 39 μmol/l (1 mg/l) which is generally agreed to be a toxic blood level (Baud *et al.*, 1991, 2002; Baud, 2007).

Obtaining a plasma lactate level is, however, restricted to the emergency department/hospital environment. A promising pre-hospital test that could be obtained in the field to aid in the suspicion of a significant cyanide poisoning component in smoke inhalation victims is the use of portable lactate meters which can be used with a finger-stick blood sample (similar to finger-stick blood glucose meters now in common pre-hospital use). Originally developed as training tools for high-performance athletes, such meters have been used to monitor type I glycogen storage disease and were found to have good correlation with the standard laboratory plasma lactate assay (Saunders *et al.*, 2005). They have also been used in obstetrics as a replacement for pH measurements in fetal monitoring during labor (Ridenour *et al.*, 2008) and for hyperlactatemia (elevated plasma lactate) screening during antiretroviral therapy in Africa (Pérez *et al.*, 2008). Such point-of-care devices have not yet been studied in smoke inhalation victims, but hold out the potential for rapid field screening that could increase clinical suspicion of a significant cyanide poisoning component and support pre-hospital administration of a specific cyanide antidote.

10.4 Exhaled breath cyanide meters

A meter is being developed that has the potential to measure both carbon monoxide and cyanide in exhaled breath. The developer anticipates that it would be adaptable to either collecting samples exhaled by voluntary effort, or that it could be placed in line between an endotracheal tube and a ventilation bag. Should this method prove to be accurate, reproducible, and well-correlated with blood cyanide concentrations, it would provide a very rapid means for the pre-hospital diagnosis of a significant cyanide poisoning component in smoke inhalation victims.

10.5 Cobinamide colorimetric quantitative/qualitative blood cyanide measurements

Cobinamide is a hydroxocobalamin precursor under development as a cyanide antidote. It has the properties of binding two molecules of cyanide ion per cobinamide molecule, and also undergoes a color change and a change in its ultraviolet/visible light spectrophotometric spectrum. Both a quantitative method using this color change for rapid laboratory measurement of blood cyanide concentrations in the emergency department/hospital setting and a qualitative finger-stick method with cobinamide-impregnated paper have recently been described (Blackledge *et al.*, 2010). Further development of this method has the potential for a rapid finger-stick test to determine blood cyanide concentrations in the pre-hospital setting, which would provide further information to support pre-hospital administration of a specific cyanide antidote.

10.6 Additional information

In experimental animals, inhalation of polymer pyrolysis products including hydrogen cyanide in smoke has

been shown to result in cardiotoxicity with elevated creatine phosphokinase (CPK) activity and an increased number of ectopic heartbeats (O'Flaherty & Thomas, 1982).

The production of hydrogen cyanide during combustion and pyrolysis is both material- and temperature-dependent (Hartzell, 1996). Cyanide is released from nitrogen containing material and relatively high temperatures are required (Hartzell, 1996). In one study of ambient atmospheric concentrations of cyanide and other gases at structural fires, hydrogen cyanide was found in only 12% of studied fires and the maximum measured concentration was 40 ppm (Lowry *et al*., 1985).

Hydrogen cyanide and carbon monoxide in fire smoke are at least additive toxicants (Hartzell, 1996; Levin *et al*., 1987; Pitt *et al*., 1979), and may indeed be synergistic (having greater toxicity than predicted from the concentrations of either toxicant alone) (Pryor *et al*., 1975; Moore *et al*., 1985, 1991; Becker, 1985; Norris *et al*., 1986). Clinically, this was observed in smoke inhalation victims in the classic Paris, France, study, where some fatalities were associated with blood carbon monoxide and cyanide concentrations, neither of which were predicted to cause death (Baud *et al*., 1991).

Firefighting involves strenuous physical activity. One animal study showed that the time to lethality from breathing pyrolysis products of polyacrylonitrile was decreased with increased physical activity (Moore *et al*., 1987).

Amongst a group of 479 Baltimore firefighters, exposure to hydrogen cyanide in fire atmospheres was sufficient to increase their serum thiocyanate (a metabolite of cyanide) concentrations above those of a control population (Levin & Radford, 1978).

Victims of smoke inhalation in enclosed-space house fires have been noted to have soot in the nose or throat and carbonaceous sputum, and to have alterations of consciousness, including coma (Hart *et al*., 1985). As compared to a control group of critically ill patients without smoke inhalation exposure, 66 smoke inhalation survivors had significantly lower mean blood cyanide levels than 43 such victims who died (Baud *et al*., 1991). Potentially fatal blood cyanide levels have been documented in smoke inhalation victims who have survived with supportive treatment and administrations of specific cyanide antidotes (Hart *et al*., 1985). It is also relevant that inhaled hydrogen cyanide has a rather short half-life, such that blood samples must be obtained close to the time of extrication from the fire scene to accurately reflect the degree of impairment due to cyanide (Purser, 1992).

Two Boston fire companies participated in personal toxic gas monitoring in the ambient fireground atmosphere and it was found that low levels of hydrogen cyanide were detected in half the collected samples (Gold *et al*., 1978).

Higher yields of hydrogen cyanide occur in small oxygen vitiated flaming fires in closed compartments and in fully developed, post-flashover fires in open compartments at high temperatures (Purser, 1992). In such fires, the systemic asphyxiant effects of carbon monoxide, hydrogen cyanide, and low ambient oxygen levels together with dense, irritant smoke which impedes escape attempts are the greatest hazards (Purser, 1992).

Approximately 80% of fire victims die from smoke inhalation rather than burns, as was seen in the MGM Hotel fire in Las Vegas, the Biloxi, Mississippi, jail fire, and the Westchase Hilton Hotel fire in Houston (Alarie, 1985). In analysis of carboxyhemoglobin and blood cyanide concentrations from fatalities in the 1986 Dupont Plaza Hotel fire in Puerto Rico, both toxicants were found in concentration generally lower than those usually associated with death (Levin *et al*., 1990). These authors concluded that in non-burned fatalities, the combination of carbon monoxide and cyanide resulted in incapacitation and death (Levin *et al*., 1990).

In non-human primates exposed to the pyrolysis products of polyacrylonitrile, hydrogen cyanide was considered to be the major toxic product (Purser *et al*., 1984). Effects of inhaling polyacrylonitrile pyrolysis products were hyperventilation, loss of consciousness within 5 minutes, bradycardia, and cardiac arrhythmias and T-wave abnormalities (Purser *et al*., 1984).

Smoke inhalation is also a major factor in non-trauma-related deaths in aircraft crashes and cyanide plays a detrimental role in fatalities from aviation accident fires (Mohler, 1975; Chaturvedi & Sanders, 1996; Chaturvedi *et al*., 2001; Canfield *et al*., 2005; Chaturvedi, 2010).

In an Australian study, of 178 fire-related deaths, blood cyanide levels were measured in 138 (78%) (Yeoh & Braitberg, 2004). There was no measureable blood cyanide in 52 of these 138 cases (29%). The remaining 86 cases had a mean whole blood cyanide level of

1.65 mg/l (above the level generally considered toxic) and in 11 cases, the level was > 3.0 mg/l (generally considered lethal) (Yeoh & Braitberg, 2004).

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CHAPTER 11
Occupational exposure to cyanide

Tee L. Guidotti

At a Glance

- Cyanide is an unusual occupational exposure hazard.
- Firefighters and electroplating workers are at increased risk.
- Cyanide production workers are also at risk.
- Cyanide is a potential chemical warfare and toxic terrorism agent and may be released during industrial accidents; first responders have an increased risk of exposure.
- Occupational exposure to cyanide may also occur during acrylonitrile production, in response to aviation fires, and in processes involving extraction of gold from ores.

11.1 Introduction

Exposure to cyanide is unusual as an occupational hazard, except in firefighting and certain types of electroplating. When it occurs, it is generally recognized as a serious hazard because of the well-known toxicity of the agent. Even so, some egregious situations have occurred in which workers who are poorly educated, untrained, or illiterate in the predominant language of their employer and local commerce have been exposed, on occasion with fatal consequences (Brummer, 1999).

For an agent of such potential hazard and well-reputed risk, occupational exposure standards for cyanide (and cyanide salts expressed as cyanide) may seem unusually high in the United States. The current permissible exposure limit of the U.S. Occupational Health and Safety Administration (OSHA, 2012) is 10 ppm 8-hour time-weighted average (TWA). The immediately dangerous to life and health (IDLH) level is 50 ppm. Although inhalation is the principal route of entry, cyanide carries a "skin" notation from both OSHA and the National Institute of Occupational Health and Safety (NIOSH) in recognition that transdermal exposure may be significant. However, NIOSH has promulgated a recommended short term exposure limit (STEL) of 4.7 ppm 8-hour (corresponds to 5 mg/m^3). This means that the NIOSH-recommended STEL, allowable for 15 minutes no more than once a day, is less than half of the current OSHA allowable exposure level for an entire 8-hour shift, one of the largest discrepancies among chemical exposure standards (CDC, 2012). The recommendations of the advisory scientific committee to the European Commission for occupational exposure limits for cyanide are also much stricter than the current OSHA standard: 0.9 ppm (1 mg/m^3) 8-hour TWA with a STEL of 4.7 ppm (5 mg/m^3). This recommendation is based in large part on studies demonstrating mucosal irritation and central nervous system effects at levels as low as 4.7 ppm in the Egyptian electroplating industry in the 1970s (European Commission, 2010).

On close review it seems clear that the extant occupational exposure limits are implicitly based on the highly irritant effects of cyanide, not primarily on the acute chemical asphyxiant effect, probably on the assumption that prevention of chronic effects necessarily prevents acute toxicity. Occupational exposure to cyanide presents two distinct problems related to different outcomes: the prevention of acute lethal exposure and the prevention of chronic effects that are primarily manifestations of mucosal irritation, together with varying degrees of low-level neurotoxicity. A standard based on a time-weighted average, which

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assumes prolonged exposure, chronic effects, and a concentration × time constant for the effect of concern, has relatively little bearing on acute systemic toxicity, in which the exposure-response curve is very steep and driven by concentration. The prevention of lethal exposure and acute systemic toxicity, such as cardiac insufficiency and neurological signs, depends on staying well below the threshold of response (represented by the IDLH) to ensure the widest margin of safety.

Occupational exposure to cyanide occurs against a background of low exposure levels due to environmental sources, principally motor vehicle exhaust, and personal exposure due to smoking (Dzombak *et al.*, 2006). Levels encountered in the workplace and associated with toxicity are many times and even orders of magnitude greater than background levels in air and water, however, and induced or adaptive tolerance plays no role. In these respects, cyanide behaves quite differently from carbon monoxide and cannot be considered as analogous.

The warning properties of cyanide are poor because the ability to smell it is lacking in most of the population on a genetic basis (Kirk & Stenhouse, 1953). For the approximately 40% who have inherited the trait (studies vary), the odor threshold is about 1 ppm and the odor is described characteristically as "bitter almond." Cyanide is also irritating and therefore has some effects below the lethal level of exposure that could warn users when exposure occurs in isolation. However, the margin of safety with respect to either perception of odor or early irritant effects is too narrow to rely upon for such a toxic agent.

Hydrogen cyanide is not used commercially but is sometimes an intermediate or byproduct in industrial processes. Cyanide salts are relatively more common in industrial use but are only found in certain industries and processes such as electroplating, as described in this chapter. Cyanide salts evolve to cyanide gas when mixed with acid. Cyanogenic compounds are even less often encountered as occupational hazards, with the exception of acrylonitrile. Acrylonitrile is metabolized to cyanide, especially in the presence of bis(2-cyanoethyl)ether (common name: 2-cyanoethyl ether), which is a highly reactive, cyanogenic product of combustion and may coexist with acrylonitrile in fires. The end product is thiocyanate. However, there is little or no evidence that toxicologically significant amounts of cyanide are generated by exposure to acrynitrile at occupationally relevant concentrations. Propanenitrile (propionitrile) and acetonitrile are highly toxic cyanogenic solvents used for specialized purposes when less toxic substitutes are not effective.

Cyanide is often impugned as the responsible agent in situations involving toxic exposure when it is not. Cyanide is not generated by the heating or combustion of cyanoacrylic compounds, which are commonly found in acrylate adhesives. Likewise, it is not generated by welding on or cutting galvanized steel, regardless of whether the zinc coating was originally applied by electroplating in cyanide solution. Furthermore, cyanide is not a hazard in blueprinting, although this process is often listed in tables of processes or occupations involving exposure to the agent.

11.2 Firefighters

The principal occupation of concern for exposure to cyanide is firefighting and there has been evidence for many years that cyanide is a significant hazard (Levine & Radford, 1978; Brandt-Rauf *et al.*, 1988; Guidotti & Clough, 1992). However, acceptance of the risk was slow to develop in part because some early studies reported low levels and made generalized conclusions from limited data. One such study concluded flatly that "HCN does not present an acute health hazard to firefighters" (Treitman *et al.*, 1980). Today the view is altogether different (Riley & Young, 2008; Alarie, 2002; Guidotti, 2006).

Cyanide is produced by the combustion of materials containing nitrogen, including natural materials such as wool, silk, and cotton, and synthetic materials such as nylon, polyurethane, melamine, nitriles, synthetic rubber, and polyacrylics (Alarie, 2002; Riley & Young, 2008). Paper and wood may not be very rich in nitrogen but because much material is combusted in a typical structural fire they contribute a great deal to the total. Certain plants produce disproportionate cyanide when burned, including the California holly, mountain mahogany, elderberry, California chokecherry, bracken fern, chamise, redshank, California buckeye, and most subspecies of oak (Longerich, 2010).

In open fires, cyanide appears to play a relatively minor role in the combustion atmosphere. However, fires in enclosed spaces may accumulate cyanide to dangerous levels. Furnishings that yield particularly

rich sources of cyanide include carpets, cushions upholstered with polyurethane foam, mattresses, and pillows (Riley & Young, 2012; Alarie, 2002; Longerich, 2010).

The stage during which firefighters suppress an active fire is called "knockdown"; it is followed by "overhaul," during which the firefighters examine the scene and walls and uncover debris to find burning embers. In a study conducted to assess the efficacy of self-contained breathing apparatus (SCBA) system, Jankovic *et al.* (1991) measured cyanide and other airborne combustion product levels in a series of 22 fires and compared them against carbon monoxide levels and other studies. Cyanide levels ranged from non-detectable (detection limit was 0.1 ppm) to 23 ppm during knockdown, non-detectable to 0.4 ppm during overhaul, and non-detectable inside the mask of the SCBA unit worn by the firefighter. The knockdown figures were broadly consistent with other studies that did not make the distinction in work phase and explains some of the variation (Brandt-Rauf *et al.*, 1988). Firefighters in the 1990s (when awareness was not as high as today) complied with directives to wear SCBA approximately 70% of the time, at most, during knockdown, because most firefighters removed their masks for some period, usually briefly, during this first phase (Jankovic *et al.*, 1991). Their findings suggest that for a suitably protected firefighter, exposure may occur primarily during overhaul, because compliance has been poor during that second stage, but that only brief periods of noncompliance during knockdown may increase exposure considerably. It is also clear that firefighters who are overcome should have their SCBA kept on until they are removed from the atmosphere.

The hazard of cyanide in fire situations has been recognized for decades (Levine & Radford, 1978; Brandt-Rauf *et al.*, 1988) but was not fully appreciated until a series of clinical studies conducted in Paris demonstrated that aggressive treatment for cyanide toxicity improved survival and post-recovery function of firefighters (Baud *et al.*, 2002; Lipha-Sante, 1996). In cases in which carbon monoxide levels were relatively low, mortality correlated with cyanide levels and seemed to show a threshold for single-cause lethality above 250 μg/dl (threshold for toxicity was about 50 μg/dl). In some fatal cases, however, carbon monoxide and cyanide were elevated but not into the highly toxic range, giving rise to the hypothesis that the two agents may show an interaction. Since cyanide is much more toxic than carbon monoxide (by a factor of about 37 for lethality), and the diminished oxygen transport conferred by carbon monoxide could be irrelevant if toxic levels of cyanide blocked uptake, it is not obvious why this should be the case.

Because cyanide cannot be measured in the emergency room or in the field in real time, its role as a significant inhalation hazard was eclipsed by the obvious hazard of carbon monoxide which is easily measured by co-oximetry. The similarity in cardiovascular response at high concentrations and the lack of a specific treatment for cyanide made apportionment of the relative contribution of the two chemical asphyxiants seem to be of only academic interest.

Given the potential additive toxicity of components of the traditional cyanide kit (vasodilation and hypotension due to nitrites administered for methemoglobin formation and methemoglobin-related depression in oxygen carrying capacity), there was some urgency in identifying cyanide as an independent factor in smoke inhalation but this has not been technically feasible. As a consequence, and given the long time lag required for blood cyanide levels to return from the laboratory, clinicians have had to rely on clinical judgment, sometimes supported by indicators such as signs of smoke inhalation, fundoscopic examination showing red venous blood, elevated blood lactate (Baud *et al.*, 2002), increased mixed venous oxygen levels, and reduced arterio-venous blood oxygen concentration, and sometimes not. The introduction of hydroxocobalamin, a rapidly effective and virtually nontoxic antidote for cyanide, changed the risk-benefit equation, which now favors presumptive treatment if hydroxocobalamin is available (Guidotti, 2006).

Cyanide toxicity is acutely and rapidly incapacitating. Several toxic effects of cyanide therefore assume special importance in the setting of firefighting because they could interfere with escape or rescue inside a burning structure. Cyanide impairs cognitive performance, resulting in stupor and confusion leading ultimately to coma and convulsions. For a firefighter trying to find his or her way out of a burning structure or in search of a victim, even mild impairment in judgment and orientation may be fatally dangerous. The inhibition of oxidative phosphorylation by cyanide causes acute weakness. Acute cardiac toxicity from the same process may result in reduced function, tachycardia, and ineffective perfusion, which may lead to dangerous

arrhythmias and myocardial infarction. Firefighters are already at risk for acute cardiac events occurring during or shortly after fire suppression. Much of this is undoubtedly due to carbon monoxide, heat stress, alarm response, and other exposures. Significant exposure to cyanide would not only be expected to increase the risk greatly itself but also to bring the heart closer to the tipping point of inadequate myocardial oxygen demand in the face of these other factors (Shusterman, 1993; Alarie, 2002).

One of the best-documented recent occurrences of cyanide toxicity among firefighters occurred in 2006 as a result of a fire in a fast food restaurant in Providence, Rhode Island. One firefighter in the Providence case who sustained cardiac arrest was reported to have been successfully resuscitated, suggesting that not all cases occurring during response are associated with intractable cytochrome oxidase inhibition (Varone *et al*., 2008).

Another untoward effect of cyanide in the context of firefighting is that some levels of cyanide exposure may mask cardiac or respiratory insufficiency. Because cyanide inhibits uptake and therefore extraction of oxygen, venous blood returns rich in oxygen and the circulation seems well oxygenated when at the tissue level conditions are actually hypoxic. This, combined with the cherry-red color of carbon monoxide, may take away warning signs such as cyanosis and delay recognition that a firefighter is not just fatigued but in danger of circulatory collapse.

Understanding the literature on firefighters and toxic exposure requires a firm knowledge of the toxicology of combustion products and the work organization of the firefighting occupation. Firefighting as an occupation involves exposure to many respiratory hazards, ranging from irritant gases (such as phosgene and cyanide, both of which are better known for their acute toxicity, and the higher oxides of nitrogen with more intense heat) and products of combustion (polycyclic aromatic hydrocarbons or PAHs and their nitrogen-containing analogs) to incidental exposure to structural components such as asbestos (predominantly chrysotile in North America) and to hazardous materials that may be released due to catastrophic failures (such as polycyclic chlorinated biphenyl compounds or PCBs and their corresponding furans, paraoxons from organophosphate pesticides that may be on site, and various dusts, of which more will be said later) or volatilized (innumerable hydrocarbons, including styrene, benzene, and other compounds more familiar as solvents). These inhaled agents are toxic, to some degree, to virtually every structure in the respiratory tract, from the epithelium of the upper respiratory tract to the alveoli of the deep lung. (It is noteworthy that among the agents specifically listed in this paragraph, even those that are not usually considered to be toxic to the respiratory tract apart from carcinogenicity, such as PCBs and PAHs, have been shown to affect tissues of the respiratory tract acutely.) Exposure during firefighting has changed over decades, with the introduction of synthetic materials (particularly in the 1970s) bringing to the traditional hazards of structural firefighting (in which wood smoke, which is relatively simple toxicologically, has predominated) a wider variety of potential exposures (including cyanide from nitriles and chlorinated hydrocarbon hazards, such as phosgene, from polyvinyl chloride-containing materials) (Guidotti & Clough, 1992; Shusterman, 1993; Alarie, 2002).

Thus, toxicity from cyanide among firefighters is likely to occur in the context of a mixed exposure with combined and possibly interactive effects. The contribution of cyanide to acute cardiac failure and circulatory insufficiency may be complicated by concomitant exposure to carbon monoxide and the respiratory tract irritation may be combined with effects of other irritant gases such as phosgene, and the acute systemic toxicity may reflect multiple exposures, including cyanide, carbon monoxide, paraoxons (if present), and other gases.

11.3 Hazmat and counter-terrorism

Cyanide exposure is possible in the setting of hazardous materials (hazmat) response, including intentional use of toxic agents in terrorist acts.

A major spill of sodium cyanide into the Barskoon River occurred in Kyrghyzstan in 1998, threatening the drinking water supply for population of the area. Although the principal concern was for local residents and environmental impact, the risk to clean up, emergency response, and monitoring workers could have been significant in the immediate aftermath of the spill (Anonymous, 2003).

In recent years, there has been some attention given to cyanide as a potential weapon of mass destruction (Keim *et al*., 2003; Upfal *et al*., 2003; Baud, 2007) The properties

of cyanide do not lend themselves particularly well to use of the chemical as a weapon on the battlefield but it has tragically been put to use in confined spaces as an agent of execution and of genocide. It is conceivable that it could be used in an intentional assault in a building or other confined space. In such cases, emergency response personnel and hazardous materials specialists would be at high risk.

To date, the intentional use of cyanide against civilians for purposes of terrorism is only known to have occurred once, in the adulteration of Tylenol® in the Chicago area in 1982. The perpetrator has never been caught and the motive for the deed is unknown, although an opportunistic extortion attempt took place at the same time (Holstege & Maniscalco, 2011).

11.4 Other occupations

Small amounts of cyanide are produced by some historical and current industrial processes, including coking and gasification of coal, blast furnace reduction of ferrous and nonferrous oxides, alumina reduction, and incineration of municipal and nitrogen-containing industrial waste. These have been an issue more for water and soil contamination than occupational exposure (Wong-Chong *et al.*, 2006). It has also been used as a grain fumigant.

11.4.1 Acrylonitrile production

Historical and contemporary processes for manufacturing acrylonitrile involve cyanide as a byproduct which can be used in the production of acetonitrile, a solvent, and methyl methacrylate.

11.4.2 Aviation fires

Fires onboard aircraft unfortunately appear to be ideal situations for cyanide toxicity, given the synthetic furnishings and confined space. Recent studies confirm this risk and suggest a link between cyanide exposure and fatalities in aircraft fires, independent of carbon monoxide levels (Canfield *et al.*, 2005).

11.4.3 Chemical synthesis of Prussian blue and related technologies

Historically, cyanide was an occupational hazard in the production of Prussian blue (potassium ferrocyanide, $K_4Fe(CN)_6$) as used in pigments, "blue printing" and photography laboratories, using technology that is now obsolete. Prussian blue is essentially nontoxic because the cyanide is bound so tightly to iron but the traditional technology of creating it required nitrogenous animal matter to be reacted with potassium carbonate under reducing conditions and produced cyanide as an intermediate product. It is made today in a process using sodium cyanide.

Although blueprinting frequently appears on lists of occupations at risk for cyanide exposure, this appears not to be the case. The blueprinting technology was cyanotype, which involved treating paper or canvas with ferrocyanide and ammonia ferric citrate to render it photosensitive to ultraviolet light by conversion to Prussian blue, which is then fixed. No free cyanide was involved. Treated areas exposed to ultraviolet radiation would turn blue but lines on an original paper drawing would mask the ultraviolet, producing negative images copies at somewhat limited resolution. Blueprints were mostly used for architectural and engineering drawings. The cyanotype process has a fascinating history, as it was invented by the famous astronomer Sir John Herschel in 1842 as a way of reproducing his notes and calculations and became briefly popular as a method of photography. Today, it is used primarily by photography hobbyists interested in the blue color and enthusiasts in the history of photography (Ware, 1999).

11.4.4 Galvanized metal parts

Galvanization is a process of coating steel or iron with zinc, to prevent corrosion and oxidation. Large objects are usually galvanized by dipping into molten zinc. Galvanized steel cables, wires, bolts, and other small objects are sometimes produced using an electrolytic process that involves a solution of zinc cyanide and sodium cyanide, the process that gave galvanization its name (after Luigi Galvani, an Italian physicist who experimented with electricity). Workers in this specific process, but no other galvanization process, may be at risk of exposure and toxicity from cyanide (Juźwiak *et al.*, 1979). Once deposited on the steel or iron by electrolysis, or any other means, there is no residual cyanide in the zinc coating. However, the belief that it may has given rise to an "urban legend" among metalworkers that welding or cutting galvanized steel gives off cyanide fumes that can be toxic and even lethal. This is incorrect and represents a misunderstanding of the origin of metal fume fever from zinc inhalation.

11.4.5 Cassava processing

Many foods, including pitted fruits such as apricots, are rich in cyanogenic glycosides, which release cyanide when hydrolyzed. In general, however, they are not important constituents of the diet and are not processed in ways that would release cyanide. Cassava is a starchy root crop rich in such cyanogenic compounds which nonetheless is a staple food for many people in Africa and the Amazon region of Latin America. To be edible, cassava must first be soaked, fermented, and allowed to outgas during drying to remove the cyanide, all of which may release cyanide fumes. Traditionally this is done by women in village kitchens. In modern times, this may be done by workers in bulk processing to produce a cassava flour. It is the third most important food crop in tropical regions, although it is low in protein, in part because it is very robust and grows in poor soils. Residual cyanogenic compounds are responsible for a number of neurological syndromes in these areas, particularly in times of famine when cassava may be eaten before it is fully fermented (Sims, 1994).

11.4.6 Electroplating

Cyanide salts are used in electroplating several metals, principally gold jewelry and zinc for galvanization (see above). In industrial electroplating facilities, levels have been found as high as 4.0 ppm in the late 1970s and early 1980s. Exposure levels in the industry are probably somewhat lower today. Artisanal foundries making art castings have also shown elevated levels (ATSDR, 2010).

11.4.7 Health-care workers

Health-care professionals and laboratory technicians may be exposed to a number of agents at issue in the cases they are investigating. Autopsy technicians (prosectors) may be at risk for cyanide intoxication in the course of performing an autopsy on a person with toxicity from ingested cyanide (Nolte and Dasgupta, 1996). The stomach, in such situations, should therefore be opened only under a laboratory hood (Wetli, 2001). It has been suggested that the cyanide content of smoke from electrocauteries, laser scalpels, and similar devices may present a risk both to operating room personnel and, during endoscopic surgery, to patients (Barrett and Garber, 2003).

11.4.8 Gold processing

Cyanide solutions are among the few solutions that easily dissolve gold and so are used for plating, cleaning, recovering, and recycling gold. Cyanide is a major hazard in small-scale gold-plating operations and in gold mining and extraction, where cyanide solution is used to extract gold from ore often illicitly.

11.4.9 Laboratory technicians

The most common chemical agents of suicide among laboratory personnel in the 1990s were reported to be barbiturates, carbon monoxide, cyanide, nitrogen, and methemoglobin-inducing chemicals (Binder & Frederickson, 1991). Women chemists were found in 1985 to have a five-fold excess of suicide notable for the use of cyanide (Walrath *et al.*, 1985). In this situation, cyanide obviously played an instrumental role, probably due to easy accessibility, rather than being an occupational hazard of primary concern. No doubt this profile would be different today.

11.5 Illicit operations using cyanide

Cyanide has a history of misuse in illegal operations. The extreme risk of the agent is more likely to be ignored when illicit profits are high, there is financial pressure that causes managers to disregard safety or where human life is not valued.

Cyanide has been used in the recovery of silver from photographic films. In 1983, negligence on the part of managers of Film Recovery Systems in a suburb of Chicago led to the death of an employee whose job was to clean vats that had contained cyanide solution for the recovery of silver from used photographic and x-ray film. The process was highly unusual, a crude batch process using cyanide solution to solubilize silver. Film recovery operations in customary use in that era or today do not use cyanide. This incident led to the indictment of several executives of the firm for murder and the conviction of three, the first ever obtained for murder in an occupational fatality in the United States. However, the convictions were later overturned on the grounds that murder requires intent and is therefore not compatible with "reckless endangerment," with which the defendants were also charged. This case remains one of the central case studies in modern business ethics (Brummer, 1999).

Cyanide exposure has also been a hazard in the illicit manufacture of phencyclidine, a drug of abuse known as PCP (Burgess & Chandler, 2007). Cyanide may also be

generated by metabolism of a nitrile contaminant, affecting users (Soine *et al.*, 1980). Clandestine PCP workshops, like methamphetamine laboratories, may expose workers in the criminal enterprise, their families (when processing is done in the home), law enforcement officials, hazmat technicians, and other professionals who are called upon to remediate the facility, and subsequent occupants of the space if it is not properly cleaned up.

Fishermen in Southeast Asia attempting to catch live specimens that inhabit coral reefs for ultimate sale to aquariums have illegally used cyanide to stun and immobilize fish, although many die in the process. Toxicity from this use of cyanide has affected some fishermen, their families, and local residents. The cyanide is readily available through illegal mining activities and appears to have been an improvised substitution for illegal dynamiting (Halim, 2002).

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CHAPTER 12
Cyanogenic aliphatic nitriles

Stephen W. Borron

At a Glance

- Aliphatic nitriles exist as mononitriles (with 1 cyanide moiety) or dinitriles (with 2 cyanide moieties).
- Aliphatic nitriles are widely used in industry for a variety of applications.
- While the parent aliphatic nitrile compounds may have their own toxicity, metabolic release of cyanide after systemic absorption by the oral, inhalational, or dermal routes accounts for the majority of their toxicity.
- The onset of cyanide poisoning signs and symptoms is often delayed, and toxic manifestations may recur due to continued metabolic release of cyanide.
- Human poisonings have been reported with acetonitrile, adiponitrile, isobutyronitrile, glycolonitrile, lactonitrile, propionitrile, succinonitrile, and acetone cyanohydrin.
- Specific cyanide antidotes have been efficacious in cases of human aliphatic nitrile poisoning; the most clinical experience has been with sodium thiosulfate, alone or in combination with other cyanide antidotes.

12.1 Overview

This chapter addresses some aliphatic nitriles of industrial importance, essentially excluding acrylonitrile, which is covered in Chapter 13. Cyanogenic glycosides, including those found in foodstuffs, such as cassava, and in drugs, such as laetrile, are not discussed here.

Aliphatic nitriles have the general formula R-CN and exist as mononitriles and dinitriles (one and two functional CN^- groups, respectively). The closely related cyanohydrins have a general formula of $R_2C(OH)CN$ (see Figure 12.1). The nitriles are an extremely important chemical family, employed as solvents (acetonitrile, propionitrile), in the manufacture of plastics and synthetic rubber (acrylonitrile, adiponitrile), in drug manufacture (acetonitrile, glycolonitrile, propionitrile), in petroleum refining and hydrocarbon extraction (acetonitrile, propionitrile) and as intermediates in the manufacture of other chemicals (ketones, esters) and fibers. Some of the important physical properties of nitriles involved in human poisonings are found in Table 12.1. More than 40 nitriles appear in the U.S. Environmental Protection Agency's High Production Volume Information System (HPVIS), indicating that quantities of 1,000,000 pounds or more of these chemicals are produced or imported each year into the United States (Table 12.2). This chapter will highlight those compounds for which acute human poisoning incidents have been published. This should not be construed, however, to preclude the potential for human poisoning by other nitriles.

12.2 Toxicology

12.2.1 Absorption

Nitriles are potentially toxic via oral or dermal absorption or through inhalation (Johannsen & Levinskas, 1986). For all three routes, the toxicity classification varies among nitriles by orders of magnitude. In rats, propionitrile, lactonitrile, and acetone cyanohydrin

Toxicology of Cyanides and Cyanogens: Experimental, Applied and Clinical Aspects, First Edition.
Edited by Alan H. Hall, Gary E. Isom and Gary A. Rockwood.

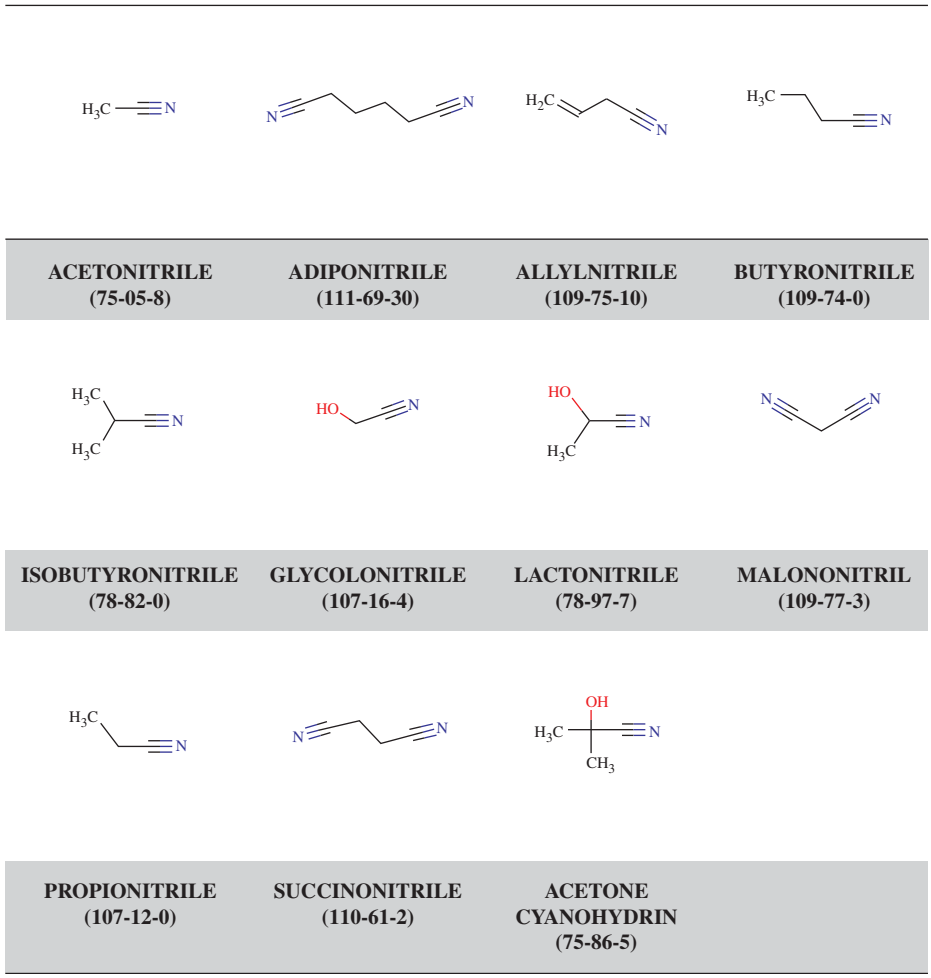

Figure 12.1 Common nitriles and other cyanogenic compounds (courtesy Chemidplus Lite, National Library of Medicine).

are highly toxic (LD50 < 50 mg/kg) by the oral route, whereas butyronitrile, acrylonitrile, succinonitrile, and adiponitrile are only moderately toxic (50 mg/kg ≤ LD50 ≤ 500 mg/kg). Acetonitrile is reported to be only slightly toxic (LD50 > 500 mg/kg) by the oral route. With regard to dermal absorption, propionitrile, butyronitrile, acrylonitrile, lactonitrile, and acetone cyanohydrin have been found to be highly toxic (dermal LD50 < 200 mg/kg) in a rabbit model of acute toxicity. Acetonitrile was found to be only slightly toxic (dermal LD50 > 2000 mg/kg) and adiponitrile moderately toxic (200 mg/kg ≤ dermal LD50 ≤ 2000 mg/kg) through dermal absorption. Finally, in rat acute four-hour inhalation studies, propionitrile, butyronitrile, acrylonitrile, adiponitrile, and succinonitrile were found to be moderately toxic (0.5 mg/l ≤ inhalation LD50 ≤ 5 mg/l), whereas acetonitrile was found to be practically non-toxic (inhalation LD50 > 20 mg/l) (Johannsen & Levinskas, 1986).

12.2.2 Metabolism

Acetonitrile is metabolized by cytochrome P450-2E1 to a hydroxylated compound, then by catalase-H2O2 to cyanide. Cyanide is then converted to thiocyanate and excreted in the urine. A portion of the parent chemical is excreted in expired air (Feierman & Cederbaum, 1989). Thier and colleagues (2002) have postulated that CYP 2E1 polymorphisms might alter individual susceptibility to poisoning with acrylonitrile in humans

Table 12.1 Important physical properties of nitriles.

								Solubility		
Substance	**Chemical abstracts service number**	**State of matter**	**Molecular weight**	**Melting point · °C**	**Boiling point · °C**	**Vapor pressure mm Hg at 25°C**	**Vapor density (Air = 1)**	**Water**	**Ethanol**	**Ether**
Acetonitrile	75-05-8	Colorless liquid	41.05	−45	81.6	88.8	1.42	Miscible	Miscible	Miscible
Acetone cyanohydrin	75-86-5	Colorless liquid	85.105	−19	95	1.1	2.96	Very	Very	Very
Adiponitrile	111-69-3	Colorless liquid	108.2	1–3	295	6.8 × 10E−4	3.73	8.0 × 10E4 mg/l@20°C	Soluble	Low
Allylnitrile*	109-75-1	Colorless liquid	67.09	−87	119	16.3	ND	ND	ND	ND
Butyronitrile	109-74-0	Colorless liquid	69.11	−112	117.5	19.5	2.4	33, 000 mg/l@25°C	Miscible	Miscible
Isobutyronitrile	78-82-0	Colorless liquid	69.1	−71.5	103.9	32.7	2.38	Slightly	Very	Very
Glycolonitrile	107-16-4	Colorless liquid	57.05	−72	183	1	1.96	Very	Very	Very
Lactonitrile	78-97-7	Yellow liquid	71.08	−40	221	1.19 × 10E−1	2.45	Soluble	Soluble	Soluble
Malononitrile	109-77-3	Colorless solid	66.06	32	218–219	0.200	ND	1.33 × 10E5	0.4 g/ml	0.2 g/ml
Propionitrile	107-12-0	Colorless liquid	55.08	−91.8	97.2	47.4	1.9	1.03 × 10E5	Miscible	Miscible
Succinonitrile	110-61-2	Colorless waxy solid	80.09	54.5	266	0.00778	2.8	126.9 g/l@20°C	Slightly	Slightly

ND: no data. All data obtained from Hazardous Substances Databank (http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm) except for allylnitrile, obtained from: www.chemnet.com/cas/supplier.cgi?terms=109-75-1&l=&exact=dict&f=plist&mark=&submit.x=7&submit.y=18 and www.chemicalbook.com/ChemicalProductProperty_EN_CB7169605.htm

Table 12.2 Common nitriles and their roles in human poisoning.

Name	CAS	Life-threatening human poisoning reported?*	2002 production range (pounds)**	Human poisoning references
Acetonitrile	75-05-8	Yes	10–50M	(Amdur, 1959; Caravati and Litovitz, 1988; Dequidt *et al*., 1974; Geller, *et al*., 1991; Jaeger *et al*., 1977; Jones *et al*., 1992; Kurt *et al*., 1991; Losek *et al*., 1991; Michaelis *et al*., 1991; Mueller and Borland, 1997; Turchen *et al*., 1991)
Acetone cyanohydrin (Propanenitrile, 2-hydroxy-2-methyl)	75-86-5	Yes	> 1B	(Thiess and Hey, 1969)
Succinonitrile	110-61-2	Yes	ND	(Contessa and Santi, 1973; Marigo and Pappalardo, 1966)
Adiponitrile (Hexane dinitrile)	111-69-3	Yes	> 1B	(Ghiringhelli, 1955)
Allylnitrile (3-Butenenitrile, Allyl cyanide)	109-75-1	No	ND	
Crotononitrile	4786-20-3	No	ND	
Glycolonitrile	107-16-4	Yes	10–500K	(Patscheider and Dirnhofer, 1973)
Malononitrile	109-77-3	No	10–500K	
3-pyridinecarbonitrile	100-54-9	No	10–50M	
2-pyridinecarbonitrile	100-70-9	No	1–10M	
Propanenitrile (Propionitrile)	107-12-0	Yes	10–50M	(Bismuth *et al*., 1987; Scolnick *et al*., 1993)
Butanenitrile	109-74-0	No	500K–1M	
Propanenitrile, 3,3′-thiobis-	111-97-7	No	1–10M	
9-Octadecenenitrile, (9Z)-	112-91-4	No	1–10M	
2-Pentenenitrile	13284-42-9	No	ND	
Butanenitrile, 2,2′-azobis[2-methyl-	13472-08-7	No	1–10M	
2-Amino-2,3-dimethylbutanenitrile	13893-53-3	No	ND	
3-Butenenitrile, 2-methyl-	16529-56-9	No	100–500M	
Butanedinitrile, ethyl-	17611-82-4	No	10–500K	
2-Pyridinecarbonitrile, 3,4,5,6-tetrachloro-	17824-83-8	No	1–10M	
Propanenitrile, 2-amino-2-methyl-	19355-69-2	No	1–10M	
Dodecanenitrile	2437-25-4	No	1–10M	
2-Pentenenitrile, (2Z)-	25899-50-7	No	10–50M	
Propanenitrile, 3-[(9Z)-9-octadecenylamino]-	26351-32-6	No	ND	
Butanenitrile, 2-amino-2-methyl-	4475-95-0	No	1–10M	
Pentanedinitrile, 2-methyl-	4553-62-2	No	10–50M	
3-Pentenenitrile	4635-87-4	No	500M–1B	
Acetonitrile, 2,2′,2′′,2′′′-(1,2-ethanediyldinitrilo)tetrakis-	5766-67-6	No	10–50M	
4-Pentenenitrile	592-51-8	No	1–10M	

Table 12.2 (*continued*)

Name	CAS	Life-threatening human poisoning reported?*	2002 production range (pounds)**	Human poisoning references
Nitriles, coco	61789-53-5	No	10–50M	
Nitriles, tallow	61790-28-1	No	50–100M	
Nitriles, tallow, hydrogenated	61790-29-2	No	10–50M	
Benzonitrile, 3-methyl-	620-22-4	No	500K–1M	
1,3-Benzenedicarbonitrile	626-17-5	No	1–10M	
Hexadecanenitrile	629-79-8	No	10–500K	
Acetonitrile, [ethyl(3-methylphenyl)amino]-	63133-74-4	No	1–10M	
Octadecanenitrile	638-65-3	No	1–10M	
Propanenitrile, 3-(isodecyloxy)-	64354-92-3	No	ND	
Nitriles, C16 and C18-unsatd.	68002-64-2	No	1–10M	
Nitriles, C16-18	68002-65-3	No	ND	
Nitriles, C16-22	68153-02-6	No	1–10M	
Propanenitrile, 3-(tridecyloxy)-	68239-19-0	No	ND	
Hexanedinitrile, hydrogenated, high-boiling fraction	68411-90-5	No	1–10M	
Nitriles, C14-18 and C16-18-unsatd.	68513-04-2	No	100–500M	
Nitriles, soya	68514-67-0	No	1–10M	
Propanenitrile, 3-(C8-10-alkyloxy) derivatives	68784-39-4	No	1–10M	
Propanenitrile, 3-amino-, N-tallow alkyl derivatives	68784-70-3	No	ND	
Propanenitrile, 2,2′-azobis[2-methyl-	78-67-1	No	1–10M	
Propanenitrile, 2-methyl- (Isobutyronitrile)	78-82-0	Yes	ND	(Thiess and Hey, 1969)

*Obtained from PubMed, Toxnet, and references cited within published papers.

**Non-confidential Inventory Update Reporting Production Volume Information from the U.S. Environmental Protection Agency: http://www.epa.gov/oppt/iur/tools/data/2002-vol.html

ND: No Data

(Thier *et al.*, 2002). As acetonitrile and some other nitriles are likewise metabolized by CYP 2E1, similar differences in susceptibility might be expected. Freeman and Hayes (1985) confirmed the earlier work of Pozzani *et al*. (1959) that acetone augments the toxicity of acetonitrile when given orally to rats. Acetone consistently delayed the onset of acetonitrile poisoning and delayed the metabolism of acetonitrile to cyanide, but furthermore enhanced this metabolism when it did occur (Freeman & Hayes, 1985). Houeto *et al*. (1997) found a plasma half-life of 32 hours for acetonitrile among cigarette smokers. Unpublished

data from the DuPont Company demonstrated that adiponitrile is rapidly absorbed from the gastrointestinal tract, with a biological half-life of 21 hours in rats. Of the administered dose 42% is recovered unchanged, with at least five normal urine components suggesting metabolism (Kennedy, 2004). In guinea pigs, adiponitrile is recovered as urinary thiocyanate, suggesting metabolism to cyanide (Ghiringhelli, 1955). Farooqui and colleagues (1993) have demonstrated in vitro rat studies that allylnitrile is also metabolized to cyanide by cytochrome P450 (Farooqui *et al*., 1993). Mumtaz *et al*. (1997) have implicated cytochrome P450 in the metabolism of propionitrile to cyanide in rats. Silver *et al*. (1982) proposed multiple mechanisms for metabolism of unsaturated nitriles, not all of which would necessarily lead to cyanide release. They investigated two such compounds, acrylonitrile and crotononitrile, which both yielded cyanide but to a smaller extent than saturated nitriles (Silver *et al*., 1982).

12.2.3 Relative toxicity of nitriles

The principal toxicity of nitriles is widely believed to come from cyanide release as a result of their metabolism. However, there are still limitations in our understanding regarding cyanide's role in nitrile(s) toxicity and of their innate toxicity, absent any metabolism. Because knowledge is incomplete regarding the cyanogenic potential of some of these compounds, because some have delayed toxicity, and because they may have other inherent toxicity, caution is advised in treating those exposed to aliphatic nitriles.

Seemingly distinct from cyanide poisoning, several nitriles, including 3, 3′-iminodipropionitrile, crotononitrile, and allylnitrile, have been shown to induce neurotoxicity and behavioral abnormalities in experimental animals (Balbuena & Llorens, 2003; Zang *et al*., 1999; Boadas-Vaello *et al*., 2009). This toxicity may involve GABAergic systems (Tanii *et al*., 2000).

Willhite and Smith (1981) have shown that the extent and toxicokinetics of nitrile metabolism to cyanide varies with individual compounds (Willhite & Smith, 1981). They studied the role of cyanide liberation in a series of six aliphatic nitriles and acetone cyanohydrin in mice. The latter was found to have the lowest LD50 after IP injection (8.7 mg/kg), followed by malononitrile (18 mg/kg), propionitrile (28 mg/kg), n-butyronitrile (38 mg/kg), acrylonitrile (46 mg/kg), succinonitrile (62 mg/kg), and acetonitrile (175 mg/kg). The mean time to death varied from five minutes for acetone cyanohydrin to 1260 minutes for propionitrile. When normal hepatic function was inhibited, the parent nitriles were essentially devoid of acute toxic effects. The authors concluded that their findings are consistent with the hypothesis that aliphatic nitriles are activated by hepatic microsomal enzymes to liberate cyanide *in vivo*, which is responsible for their acute toxicity (Willhite & Smith, 1981).

Ahmed and Farooqui (1982) studied the relative toxicity of nitriles in rats, measuring blood and hepatic cyanide concentrations one hour after administration of an LD50 dose of each of the nitriles tested (Ahmed & Farooqui, 1982). Cyanide concentrations were highest following administration of malononitrile, followed by propionitrile, butyronitrile, acrylonitrile, allylnitrile, fumaronitrile, and finally, acetonitrile. Among the saturated nitriles, signs of cyanide poisoning, including CNS depression, convulsions, and respiratory failure were prominent. Among the saturated nitriles, cholinomimetic symptoms of salivation, diarrhea, and vasodilation were more common. The authors concluded that the toxicity of aliphatic nitriles depends not only on cyanide release but on their degree of unsaturation. With unsaturated aliphatic nitriles, cyanide release played a minimal role in their toxicity (Ahmed & Farooqui, 1982). This study suffered from a major design flaw, in that all animals were sacrificed after one hour, eliminating time to metabolism as a variable. This is underscored by the absence of symptoms in acetonitrile-treated animals. Acetonitrile is known to be metabolized to cyanide. The study of Willhite and Smith (1981) suggests strongly that metabolism to cyanide is delayed for some nitriles. Human toxicity reports seem to confirm this.

Johannsen and Levinskas (1986) reviewed the toxicological properties of nitriles in rats using structural activity relationships. They compared several mononitriles (acetonitrile, propionitrile, butyronitrile, and acrylonitrile) to dinitriles (succinonitrile and adiponitrile), along with two cyanohydrin derivatives (lactonitrile and acetone cyanohydrin). With regard to acute toxicity, the cyanohydrins were found to be most toxic, followed by the mononitriles, and then the dinitriles. Acetone cyanohydrin toxicity resembles that of inorganic cyanide salts in terms of its rapid onset. Acetonitrile was found to have acute toxicity an order of magnitude less than the rest of the series.

Oral toxicity was found to be highest for lactonitrile, acetone cyanohydrin, and propionitrile. Dermal LD50 was lowest for lactonitrile, followed by acrylonitrile and butyronitrile. Inhalation toxicity was greatest with succinonitrile, followed by acrylonitrile and propionitrile. The authors concluded, in agreement with Willhite and Smith (1981), that cyanide release appears to be important in the mechanism of acute toxicity of all these tested nitriles (Johannsen & Levinskas, 1986; Willhite & Smith, 1981).

It is important to emphasize that the toxicity of the nitriles is highly variable, not only among the various compounds, but is likely to differ significantly among various animal species. While acetonitrile has been determined to be virtually nontoxic in rat acute 4-hour inhalation studies, at least two human deaths have been attributed to acetonitrile inhalation (Amdur, 1959; Dequidt *et al*., 1974). And although acetonitrile is considered to be among the least toxic of nitriles by the oral route in animals, it has been responsible for numerous accidental and intentional human deaths. Age may also be an important factor in acute toxicity. Kimura and colleagues found that acetonitrile was an order of magnitude more toxic in 14-day-old rats than in young adult rats (Kimura *et al*., 1971).

12.3 Case reports of human toxicity of specific nitriles

12.3.1 Acetonitrile

Acetonitrile (methyl cyanide, CAS 75-05-8) is a widely used solvent employed in extraction processes, in laboratories as a solvent for high performance liquid chromatography, as a catalyst and intermediate in the manufacture of other compounds, including pesticides, nitrile rubber, and resins (Sax & Lewis, 1987). According to the U.S. EPA, between 10 and 50 million pounds of acetonitrile were produced in the United States in 2002. It was formerly sold as an artificial finger nail remover prior to its regulation by the U.S. Consumer Product Safety Commission.

Industrial exposures

Pozzani *et al*. (1959) studied the mammalian toxicity of acetonitrile in several species before subjecting three men to inhalation of acetonitrile vapor for four hours (Pozzani *et al*., 1959). While none suffered symptoms during the inhalation exposure, the youngest subject experienced slight chest tightness that evening. The two asymptomatic subjects were subsequently exposed to 80 ppm for four hours and later to 160 ppm for four hours. One subject experienced flushing of the face two hours after inhalation and chest tightness five hours later. The authors underscored the variability of subjective responses, suggesting that while a vapor concentration might be selected which would not endanger the health of the majority of workers, it might cause discomfort in some.

Amdur (1959) provided clinical details on a previously reported (Grabois, 1955) collective exposure of 16 men to acetonitrile during the painting of the interior of a chemical tank. One worker died, two additional workers suffered life-threatening toxicity, while the remainder suffered minimal to moderate toxicity. A delay of several hours between exposure and symptoms was noted in all cases. Of note, the two subjects with life-threatening toxicity were treated with oxygen and sodium thiosulfate, whereas the fatal case was not. Significant concentrations of cyanide were recovered in the blood from the three most seriously affected victims.

Dequidt and colleagues (1974) described the fate of a 19-year-old male working in a photographic laboratory who poured acetonitrile, followed by boiling water, onto the floor to clean it. Several hours later he complained of epigastric pain and during the night vomited profusely. He was taken the next morning to the hospital in a coma, interspersed with convulsions. In spite of endotracheal intubation and assisted ventilation, the subject suffered cardiac arrest that evening. He was resuscitated, but no cyanide antidote was administered until the following day when physicians were made aware of the acetonitrile exposure. The patient received 600 mg of dicobalt EDTA at that time. His seizure activity stopped; however, he remained in profound coma. On the following day, he was given 4 g of hydroxocobalamin. The patient was determined to be brain-dead on day five following exposure (Dequidt *et al*., 1974).

Muraki and colleagues (2001) described a 35-year-old chemical plant worker who was hospitalized one day after washing the inside of a reactor kiln with acetonitrile. He presented 15 hours after exposure with severe nausea, vomiting, diarrhea, and muscle weakness. He subsequently developed generalized convulsions with severe metabolic acidosis. He was intubated and underwent mechanical ventilation. He was treated with

sodium bicarbonate and dopamine and then transferred to another hospital for antidotal treatment against cyanide. He arrived awake and restless at the second hospital, complaining of dyspnea. He was hypotensive in spite of dopamine therapy and was tachycardic. He remained acidotic with evidence of acute renal insufficiency. He underwent treatment with sodium nitrite and repeated boluses of sodium thiosulfate. No analyses were performed for blood cyanide. He was subsequently found to have extremely high creatine kinase (CK) levels. He underwent continuous hemodiafiltration and intermittent hemodialysis until CK levels return to normal. He was discharged with persistent renal dysfunction on day 96 (Muraki *et al*., 2001).

Unintentional exposures

In the late 1980s and early 1990s, a spate of pediatric accidental ingestions of acetonitrile occurred in the United States, leading to severe poisoning of several infants, with multiple deaths. Each of these cases involved an artificial fingernail remover containing acetonitrile.

Caravati and Litovitz (1988) reported on two cases of pediatric acetonitrile ingestion. A 16-month-old child ingested 15 to 20 ml of acetonitrile and vomited spontaneously about 20 minutes later. A poison center was called but the product was mistaken for acetone fingernail polish remover. The child was put to bed and found dead 12 h post-ingestion. A 2-year-old boy was brought to the hospital with severe poisoning 9.5 hours post-ingestion of approximately 30 ml of an artificial nail remover product. He was lethargic, pale, tachycardic, hypotensive, and extremely acidotic. Due to rapid response to aggressive supportive care, a decision was made not to administer a cyanide antidote, even though the diagnosis was known. Whole blood cyanide levels reached 600 mcg/dl 12 hours after the exposure. He survived with vigorous supportive care alone (Caravati & Litovitz, 1988).

Kurt, *et al*. (1991) reported a third case of accidental acetonitrile poisoning from artificial nail remover (Kurt *et al*., 1991). A two-year-old child ingested 5 to 10 ml of 84% acetonitrile at home. The child was taken to a minor emergency department where vital signs were stable and the patient was asymptomatic. The child was sent home and fell asleep, awakening the next morning moaning, restless, and vomiting. She subsequently suffered three tonic-clonic seizures and was taken in comatose condition to an emergency department. Arterial blood gases revealed severe metabolic acidosis. Blood cyanide concentrations were 182 mcg/dl, with a serum lactate of 24.3 mmol/l. The child was given oxygen and amyl nitrite by inhalation, followed by intravenous sodium nitrite and sodium thiosulfate. Activated charcoal was administered by nasogastric tube. The child woke up within minutes after receiving the cyanide antidotes and continued to improve following transfer to a children's hospital. She was discharged after two days with no apparent sequelae (Kurt *et al*., 1991).

Geller and colleagues (1991) subsequently reported the case of a three-year-old child who presented asymptomatic to an emergency department 30 minutes after ingestion of 15 to 30 ml of an artificial nail remover product. A poison center was called. The patient underwent nasogastric intubation and gastric lavage with 1l of normal saline. Activated charcoal was given. Blood cyanide concentrations drawn three hours and 45 minutes after ingestion revealed a concentration of 124 mcg/dl. The child was admitted for observation. At 16 hours post-ingestion, the child became less responsive. Sodium thiosulfate was administered and the child returned to a clinically normal state shortly thereafter, remaining so until discharge from hospital at 42 hours (Geller *et al*., 1991).

Losek and colleagues (1991) reported on a 23-month-old boy who ingested approximately 60 ml of an artificial nail remover 12 hours prior to presentation in an emergency department (Losek *et al*., 1991). He was reported to have vomited three times approximately 6 hours post-ingestion, but appeared well. Physical examination was normal. The child underwent testing for blood cyanide, was administered oxygen by mask and admitted to the hospital. At 24 hours post-ingestion, the patient became unresponsive, tachycardic, and tachypneic. A plasma lactate of 50.1 mg/dl was reported. The child was treated with amyl nitrite and later with activated charcoal and intravenous sodium thiosulfate every four hours. A total of five doses of sodium thiosulfate were administered. The patient's level of consciousness returned to normal. The patient was discharged on hospital day three. Whole blood cyanide levels obtained at 12 and 25 hours post-ingestion were 2.1 μg/ml and 3.8 mcg/ml, respectively (Losek *et al*., 1991).

In 1990, the Consumer Product Safety Commission imposed requirements for child-protective packaging for

products containing more than 500 mg of acetonitrile (16 CFR 1700.14). Since 1991, there have been no additional pediatric ingestions of acetonitrile-containing artificial nail remover reported in PubMed.

Jones *et al*. (1992) report an extremely unusual case of a couple: a male of 40 years and a female of 53 years who were found dead in their bed at home (Jones *et al*., 1992). The couple had been drinking what they apparently believed was alcohol brought home from the workplace. Two bottles were found with labeling indicating that they contained ethanol. Indeed, during the initial forensic analysis elevated concentrations of blood and urine ethanol were found. Upon reanalysis using a different headspace gas chromatography and gas chromatography/mass spectrometry methodology, acetonitrile was instead identified. In addition inorganic cyanide was identified in the blood of both victims in elevated concentrations (Jones *et al*., 1992).

Suspected suicidal ingestions

Jaeger and colleagues (1977) described a 26-year-old male who attempted suicide by ingesting 40 g of acetonitrile. After a delay of some three hours, the patient began vomiting and having convulsions. He subsequently developed coma, acute respiratory insufficiency, and severe metabolic acidosis, resulting in cardiac arrest. The patient was treated with artificial ventilation, correction of acidosis and administration of dicobalt EDTA, sodium nitrite, sodium thiosulfate, and hydroxocobalamin. The patient remained in a coma for six days and suffered diverse complications including anemia and hepatic insufficiency. Prolonged elimination of acetonitrile was confirmed by persistence of thiocyanate in the urine up to 20 days post-intoxication. The patient survived, recovering without sequelae after three months (Jaeger *et al*., 1977).

Boggild *et al*. (1990) reported on a fatal case of acetonitrile ingestion by a 22-year-old female who was found semiconscious at work (Boggild *et al*., 1990). She arrived at hospital conscious but refused to provide any history. She remained drowsy for 18 hours and then rapidly progressed from generalized seizures to asystole. She was resuscitated and admitted to intensive care, where she remained in profound lactic acidosis with hypotension, pulmonary edema, and gastritis. The patient received no specific antidotes because cyanide poisoning was not suspected during the clinical course. She died approximately 30 hours post-ingestion (Boggild *et al*., 1990). Interestingly, the authors reported co-ingestion of acetone, previously shown to slow the metabolism of acetonitrile to cyanide (Freeman & Hayes, 1985).

Michaelis and colleagues (1991) reported on the ingestion of 5 ml of acetonitrile by 30 year old male in a suicidal attempt. He was taken to hospital approximately 5 hours later and was given 4-dimethylaminophenol (4-DMAP) and sodium thiosulfate en route. He suffered minimal toxicity, including livid skin color and excitation. Of principal interest in this case, the authors undertook toxicokinetic analysis of serum acetonitrile and blood cyanide. Acetonitrile had an elimination half-life of 32.4 hours, whereas blood cyanide had an elimination half-life of 15.1 hours, the latter suggesting continuing production of cyanide via acetonitrile metabolism. The peak blood cyanide concentration was 17.3 mcg/ml. The authors do not mention whether blood cyanide concentrations were measured before administration of 4-dimethylaminophenol. Induction of methemoglobinemia has been shown to spuriously increase blood cyanide concentrations (presumably due to red cell sequestration), which may explain these extraordinarily high blood concentrations in the absence of significant toxicity (Michaelis *et al*., 1991).

Mueller and Borland (1997) recorded the first case in the United Kingdom of deliberate self-poisoning with acetonitrile (Mueller & Borland, 1997). A 39-year-old woman was admitted to hospital two hours after ingesting 25 g of acetonitrile. Having vomited several times following ingestion, she was symptomatic only for mild dizziness on admission. Eleven hours after ingestion, the patient became nauseated, confused, and sweaty, with Kussmaul respiration and rapid onset of coma. Severe metabolic acidosis was found and the diagnosis of acute cyanide poisoning made. The patient received sodium nitrite and sodium thiosulfate in bolus doses. She suffered generalized seizures, was intubated, and ventilated. Her acidosis improved and she became stable on day 2, but at 32 hours once again became hypotensive and tachycardic with recurrence of metabolic acidosis. She received additional sodium bicarbonate, sodium nitrite, and sodium thiosulfate. Sodium nitrite infusion was continued from day 4 to 5. The patient had an unsteady hospital course complicated by pneumonia, but was discharged well 26 days after admission. Similar to the findings of Michaelis and colleagues (1991), a serum half-life of 36 hours for acetonitrile was found. Likewise the elimination half-life of blood cyanide was markedly prolonged at 44

hours, representing continued generation of cyanide by acetonitrile metabolism (Mueller & Borland, 1997).

Turchen and colleagues (1991) described the case of a 39-year-old woman who ingested 59 ml of 99% acetonitrile in a suicide attempt. Paramedics found the woman vomiting and confused some seven hours after ingestion. On hospital arrival, the patient was tachycardic and tachypneic, awake, and uncooperative. She underwent gastric lavage and was administered activated charcoal. At 11.5 hours after ingestion, the patient became unresponsive. She was intubated and given sodium nitrite and sodium thiosulfate intravenously. During sodium nitrite administration her systolic blood pressure fell briefly to 70 mm Hg. Severe metabolic acidosis was treated with sodium bicarbonate. The patient's condition improved over 30 minutes. A recurrence of toxicity occurred at 26.5 hours post-ingestion. Sodium thiosulfate and sodium bicarbonate were given with rapid improvement in the patient's condition. At 33.25 hours post-ingestion, the patient's condition again deteriorated. She received additional sodium nitrite and sodium thiosulfate intravenously, with recurrence of hypotension during administration of sodium nitrite. Additional sodium bicarbonate was given with rapid improvement in her condition. Nine hours later, the patient required additional sodium thiosulfate, with similar rapid response. The patient continued to have a complicated course but gradually improved and was discharged on hospital day 6. Of significant interest in this case, in addition to recurring toxicity, was a saw-tooth pattern of increasing blood cyanide concentrations which peaked at 59 hours at a value of 1281 mcg/dl. The authors, probably correctly, surmise that the increasing cyanide concentrations reflected increasing sequestration of cyanide by erythrocytes (it is important to note that this was likely due to repeated administration of sodium nitrite) (Turchen *et al*., 1991).

Swanson and Krasselt (1994) reported on a 39-year-old female who was discovered dead in her home. Forensic testing revealed the presence of diphenhydramine and acetonitrile. The blood cyanide concentration was 4.4 μg/ml. No source for the acetonitrile was discovered (Swanson & Krasselt, 1994).

12.3.2 Adiponitrile

Adiponitrile (CAS 111-69-3) is an intermediate in the manufacture of nylon. It is acutely toxic in laboratory animals by oral, dermal, and inhalation routes (Kennedy, 2004). Ghiringhelli (1955) reported on the case of an 18-year-old male, who accidentally ingested a few milliliters of adiponitrile (Ghiringhelli, 1955). Within a few minutes, the patient developed severe asthenia, headache, vertigo, vomiting, chest constriction, and difficulty standing. He was taken to the factory infirmary where he was noted to have cyanosis of the mucosae, tachypnea, tachycardia, marked hypotension, and pupillary mydriasis. He rapidly developed altered consciousness, stertorous respirations and tonic-clonic contractions. Gastric lavage was carried out without improvement. The patient was given intravenous sodium thiosulfate and 40% glucose. Minutes after treatment the patient regained consciousness, the cyanosis and mydriasis improved, and his hypotension resolved. After 4 hours of apparent well-being, poisoning recurred, with symptoms similar to those previously described. Glucose and sodium thiosulfate were again administered, with slow but definitive resolution of the symptoms (Ghiringhelli, 1955).

12.3.3 Allylnitrile

Allylnitrile (3-butenenitrile, allyl cyanide; CAS 109-75-1) has been demonstrated to be metabolized to cyanide via cytochrome P-450 mixed function oxidase systems in a rat liver (Farooqui *et al*., 1993). No human cases of poisoning were identified. Allylnitrile induces neurotoxic effects in several animal species (Boadas-Vaello *et al*., 2009).

12.3.4 Butyronitrile

Butyronitrile (N-butyl nitrile, butanenitrile; CAS 109-74-0) is a basic chemical and pharmaceutical intermediate used in the manufacture of butyric acid and pharmaceutical compounds (Sax & Lewis, 1987). While more toxic than acetonitrile or propionitrile in mice (Willhite, 1981), no published cases of significant human toxicity were identified.

12.3.5 Isobutyronitrile

Isobutyronitrile (2-methylpropanenitrile; CAS 78-82-0) is employed as an intermediate in the manufacture of insecticides (Sax & Lewis, 1987).

Thiess and Hey (1969) reported the case of a 53-year-old male working in a production facility in which isobutyronitrile was poured into a distillation still (Thiess & Hey, 1969). In spite of wearing protective clothing and a respirator, the worker became dizzy and nauseated. He assumed his respirator face mask was leaking and that he had inhaled isobutyronitrile vapors. He rapidly developed vomiting and cold sweats with

unsteady gait. He was taken to the infirmary, where he was noted to be hypertensive at 180/70 mm Hg. He rapidly lost consciousness and developed fixed large pupils with difficulty breathing and thick mucus secretions. He was taken to hospital by ambulance with a physician in attendance, during which time he was ventilated with oxygen. In the hospital, he suffered enuresis and tonic-clonic convulsions. He developed cyanosis and required artificial ventilation and cardiopulmonary resuscitation. He was treated with norepinephrine followed by injection of sodium nitrite and sodium thiosulfate. The cyanosis improved, his pulse became stronger, but respirations remained irregular. He was given lobeline and phenobarbital intravenously. Within 5 to 10 minutes, there was significant improvement. He was transfused with 300 ml of blood. Approximately 4 hours after the intoxication, the patient regained consciousness. His hospital course was complicated by persistent headache and an abnormal electrocardiogram and electroencephalogram. Within 14 days of the poisoning, he left the hospital symptom-free (Thiess & Hey, 1969).

12.3.6 Glycolonitrile

Glycolonitrile (hydroxyacetonitrile, CAS 107-16-4) is employed as a solvent and chemical intermediate in the production of pharmaceuticals and resins.

Glycolonitrile human poisoning has been reported, including fatal poisoning. Patscheider and Dirnhofer (1973) reported ingestion of beechnut meal contaminated with glycolonitrile, which led to symptomatic cyanide poisoning and death. The beechnut meal was apparently contaminated during shipment alongside a container of the nitrile. It was purchased by the victims, who prepared a meal with it and began having symptoms within 15 minutes of ingestion. A 51-year-old woman complained her hands were falling asleep, went to the bathroom, vomited, and slumped to the floor unconscious. She arrived at hospital comatose, gasping for breath, with hypotension, bradycardia, and metabolic acidosis. She was admitted to the intensive care unit but her condition deteriorated steadily and she was pronounced dead two days later. Other than sodium bicarbonate and other alkalizing agents, no antidotes were administered. At autopsy, there were bilateral necrotic lesions in the Nucleus lentiformis. Her two male companions also vomited, one out the window, which allowed for later analysis of the vomitus. Both men survived with only minor symptoms (Patscheider & Dirnhofer, 1973).

An industrial dermal and possibly inhalation exposure involving 70% glycolonitrile resulted in headache, dizziness, and unsteady gait. The exposed worker vomited several times, became confused and unresponsive. He was treated with amyl nitrite, oxygen, and sodium thiosulfate. He returned to work the next day, but continued to have weakness and nausea for five additional days (Clayton & Clayton, 1993–1994: 3151).

12.3.7 Lactonitrile

Lactonitrile (2-hydroxypropanenitrile; CAS 78-97-7) is employed as a solvent and as an intermediate in ethyl acetate and lactic acid production (Sax & Lewis, 1987).

Only a single case of human poisoning was encountered in the recent literature. Nagata and colleagues (1968) reported an industrial exposure in a 36-year-old man cleaning discharge pipes in an acrylonitrile production facility. During work, the patient developed severe headache, nausea, palpitations, and abdominal pain. He became unconscious on his way home from work and was admitted to a hospital, where cyanide poisoning was diagnosed. In spite of "vigorous therapy," the patient died. While acrylonitrile was also present in pathologic samples, the authors concluded that lactonitrile was responsible for the patient's death (Nagata *et al.*, 1968).

12.3.8 Malononitrile

Malononitrile (CAS 109-77-3) is used in the production of herbicides and pharmaceuticals, such as triamterene and methotrexate, and as a leaching agent for gold (Sax & Lewis, 1987). Malononitrile was employed experimentally in the late 1940s in the treatment of schizophrenia and depression. Patients were given an intravenous infusion of 5% malononitrile for 10–69 min, in doses ranging from 1 to 6 mg/kg. Treatments were repeated 2–3 times per week, with at least one day intervals in between doses. Ten to twenty minutes after the beginning of the infusion, all patients experienced tachycardia. In addition, redness, nausea, vomiting, headache, shivering, muscle spasms, and numbness were reported with varying frequency

(Clayton & Clayton, 1993–1994: 3158). No cases of unintentional or suicidal poisoning with malononitrile were identified.

12.3.9 Propionitrile

Propionitrile (CAS 107-12-0) is employed as a solvent, as a raw material in pharmaceutical manufacture, a dielectric fluid, and chemical intermediate (Sax & Lewis, 1987).

Bismuth and colleagues (1987) reported on the case of a 55-year-old male who attempted to repair a leaky pipe fitting which was releasing propionitrile (Bismuth *et al.*, 1987). The worker wore protective gloves but no other protective clothing or respirator. The patient was exposed to liquid propionitrile through both skin contact and inhalation. He rapidly lost consciousness. He was taken 25 minutes later to the company infirmary, comatose and unresponsive. On arrival in the intensive care unit two hours after exposure, the patient was confused and agitated with tachypnea and deep inspirations. Laboratory studies revealed metabolic acidosis with a serum lactate of 10 mmol/l. The patient underwent skin decontamination with soap and water. He was given sodium bicarbonate intravenously, followed by hydroxocobalamin and sodium thiosulfate. During antidote administration, the patient regained consciousness and vomited. Central nervous system depression resolved over the next hour. Blood cyanide concentration was 5.71 mcg/ml prior to antidote infusion and decreased to 0.93 mcg/ml at end of infusion, with a concomitant rise in thiocyanate from 0 to 21.1 mcg/ml. The patient was subsequently transferred to the oncology service for a serendipitously discovered lung tumor (Bismuth *et al.*, 1987).

Scolnick and colleagues (1993) subsequently reported on two cases of accidental industrial exposures to propionitrile. The first case was a 28-year-old male who was treating waste discharge. He was wearing a protective suit, boots, and gloves but wore no respirator. He collapsed after approximately 7 hours of exposure and was found in cardiac arrest. On his arrival at the emergency department, he was comatose with eye deviation, was tachycardic, and bradypneic. He shortly thereafter suffered generalized tonic-clonic seizures. He was treated with atropine and diazepam. Arterial blood gases revealed severe metabolic acidosis, which worsened in spite of treatment with sodium bicarbonate. Poison center consultation was obtained resulting in a recommendation of cyanide antidote kit administration. Approximately 90 minutes after ED arrival, the patient received sodium nitrite, followed by sodium thiosulfate. Shortly thereafter his mental status improved, with the ability to follow commands and respond to questions. Blood cyanide concentration was 5 mcg/ml. Due to clinical evidence of pulmonary edema, the patient was intubated. Plant officials subsequently confirmed the presence of a 1 inch film of unreacted propionitrile overlying the waste slurry. Because of continuing neurologic complaints, the patient was transferred for therapy with hyperbaric oxygen. He was discharged home 48 hours later. He continued to complain of severe headaches and dizziness for the next 30 days, but was symptom-free six months after the exposure. Case two was a 34-year-old male who was performing the same task as the previous patient. He became ill with headache, nausea, and dizziness and left the area. He was subsequently found confused and disoriented approximately 5 hours later. He was taken to the same emergency department, where he complained of nausea and vomiting as well as headache. His vital signs were normal, as was an arterial blood gas and hemogram. However, six hours after admission he was found to have an elevated blood cyanide concentration (3.5 mcg/ml) and was thus administered a cyanide antidote kit of sodium nitrite and sodium thiosulfate. He was discharged 24 hours later with an uneventful recovery (Scolnick *et al.*, 1993).

12.3.10 Succinonitrile

A case of iatrogenic succinonitrile (CAS 110-61-2) poisoning was published in Italy (Marigo & Pappalardo, 1966). A 53-year-old male with polyarthritis and bronchitis was treated for three weeks daily intramuscular injections of succinonitrile 200 mg. Two hours after the last injection, he began vomiting, and had psychomotor agitation and confusion, together with cold sweats. He was admitted to hospital with convulsions and mental disorientation and died two hours later. Because of the smell of bitter almonds when the skull was opened during autopsy, chemical analysis for cyanide was conducted. No cyanide was found in the blood but elevated cyanide concentrations were found

in the liver, brain, kidney, lungs, and urine. It was concluded that his death was due to cyanide poisoning as a result of metabolism of succinonitrile. Contessa and Santi (1973) cite other publications of Marigo and Pappalardo, indicating that they had reported five cases of death in the course of therapeutic treatment with succinonitrile as an antidepressant agent (Contessa & Santi, 1973).

12.3.11 Acetone cyanohydrin

Thiess and Hey (1969) reported a case of acetone cyanohydrin (CAS 75-86-5) poisoning in an industrial worker. A 33-year-old laboratory worker was filling 200-liter steel drums by means of a rubber hose. The worker splashed acetone cyanohydrin on one hand and continued to work. Within five minutes he became suddenly ill, vomiting several times, becoming unconscious and collapsed. He was showered and subsequently treated with sodium nitrite and sodium thiosulfate. He showed initial improvement, but then fell back into an unconscious state. It was discovered that a glove soaked in acetone cyanohydrin was still in his pocket. After this was removed and the patient was completely undressed and decontaminated, the treatment with sodium nitrite and sodium thiosulfate was repeated. Vomiting and convulsions ceased and consciousness gradually improved. After several hours of observation he was released from the infirmary and subsequently, after a few days' rest, returned to work (Thiess & Hey, 1969).

12.4 Antidotal treatment

While supportive therapy alone has been successful in some cases (Caravati & Litovitz, 1988; Zavotsky *et al.*, 2004), it is not always effective (Boggild *et al.*, 1990). Oxygen supplementation is recommended. Hyperbaric oxygen appeared to result in improvement in one case of propionitrile poisoning (Scolnick *et al.*, 1993). Severe cyanide poisoning by nitriles may require specific antidotal therapy. Due to the prolonged and continuous metabolism of nitriles to cyanide, multiple doses or a continuous infusion of cyanide antidotes should be envisaged. The choice of cyanide antidotes in the particular case of nitrile poisonings has not been clearly established; although there is general consensus that sodium thiosulfate is effective.

12.4.1 Animal studies

Bhattacharya and colleagues (2009) have studied the effects of alpha-ketoglutarate in rats poisoned with various nitriles. Significant protection ratios (> 2 × oral LD50) were found for malononitrile and propionitrile. Protection indices were less than 2 × oral LD50 for acetonitrile, acrylonitrile, and succinonitrile (Bhattacharya *et al.*, 2009).

4-Dimethylaminophenol (4-DMAP) has been studied for protection against acrylonitrile poisonings. Buchter and Peter (1984) found limited protection by 4-DMAP in combination with sodium thiosulfate when acrylonitrile was given orally to rats, but not after intraperitoneal or inhalational acrylonitrile administration (Buchter & Peter, 1984). Peter and Bolt (1985) found that DMAP and thiosulfate were protective against methacrylonitrile inhalation toxicity in rats.

Haguenoer and colleagues (1975) studied the effects of hydroxocobalamin in a nonlethal subacute rat model of acetonitrile poisoning. The authors found that administration of hydroxocobalamin decreased the quantity of free cyanide eliminated in the urine, concluding that hydroxocobalamin provides a protective effect (Haguenoer *et al.*, 1975).

Sodium thiosulfate was found to be 92.8% effective in protecting against an LD50 dose of adiponitrile in the guinea pig (Ghiringhelli, 1955). Sodium thiosulfate protected against toxicity associated with a mixture of acetonitrile and acetone in rats (Freeman & Hayes, 1985). Sodium thiosulfate was likewise found to be effective in prophylactic and post-exposure treatment of mice exposed to intraperitoneal acrylonitrile (Mehta, 1995).

12.4.2 Human case reports

Sodium thiosulfate has been successfully used in multiple cases of acute acetonitrile poisoning, both alone (Amdur, 1959; Geller *et al.*, 1991; Ghiringhelli, 1955; Losek *et al.*, 1991) and in combination with other cyanide antidotes (Bismuth *et al.*, 1987; Kurt *et al.*, 1991; Michaelis *et al.*, 1991; Mueller & Borland, 1997; Muraki *et al.*, 2001; Turchen *et al.*, 1991). Sodium thiosulfate

appeared effective in a serious case of human poisoning with adiponitrile (Ghiringhelli, 1955).

Michaelis *et al*. (1991) reported on use of 4-dimethylaminophenol (4-DMAP) and sodium thiosulfate after intentional ingestion of acetonitrile. The patient suffered minimal toxicity.

Bismuth and colleagues (1987) administered hydroxocobalamin and sodium thiosulfate in combination to a 55-year-old male with propionitrile poisoning. He regained consciousness and orientation during the infusion of the antidote combination.

Sodium nitrite has been used in combination with sodium thiosulfate in various nitrile poisonings (Jaeger *et al*., 1977; Kurt *et al*., 1991; Mueller & Borland, 1997; Muraki *et al*., 2001; Scolnick *et al*., 1993; Thiess & Hey, 1969). Kurt *et al*. (1991) reported almost immediate improvement and subsequent discharge without sequelae after administration of this combination in a two-year-old child with acetonitrile poisoning. Scolnick and colleagues (1993) reported similar impressive improvements in the clinical condition with this combination after propionitrile poisoning.

Dicobalt EDTA was employed late in a case of acetonitrile poisoning described by Dequidt and colleagues (1974), with cessation of convulsions. In spite of additional treatment with hydroxocobalamin, the patient succumbed to decerebration. Jaeger *et al*. (1977) reported the use of dicobalt EDTA, sodium nitrite, sodium thiosulfate, and hydroxocobalamin in a suicidal ingestion of acetonitrile. In spite of persistent symptomatology, the patient ultimately survived without sequelae.

12.5 Summary

Nitriles have been responsible, after both accidental and intentional exposure, for toxicity resembling cyanide poisoning. The time to onset of poisoning varies from minutes (acetone cyanohydrin) to hours (acetonitrile) after exposure; thus, close observation of exposed victims over a prolonged period (at least 24 hours) is advisable. All poisoned patients should receive supplemental oxygen and supportive care. Patients with evidence of cyanide poisoning should be considered for specific antidotal therapy. The greatest amount of successful human experience has been with sodium thiosulfate, alone or in combination with other cyanide antidotes. Hydroxocobalamin, sodium nitrite, sodium thiosulfate, 4-DMAP, or alpha-ketoglutarate may be considered, depending on local availability. See specific antidote chapters for additional information.

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CHAPTER 13

The special case of acrylonitrile (CH_2=CH–C≡N)

Dana B. Mirkin

At a Glance

- Acrylonitrile can release cyanide through hepatic metabolism.
- The onset of signs and symptoms of cyanide poisoning is often delayed for several hours after acrylonitrile exposure.
- The most common exposure routes are inhalation of vapor and dermal contact with the liquid.
- Various cyanide antidotes have been used successfully to treat acute acrylonitrile poisoning.
- However, the parent compound and other metabolites have toxicities of their own, notably liver and renal adverse effects.
- N-acetylcysteine (NAC) has been shown to have a protective/therapeutic effect in acute acrylonitrile poisoning.

13.1 Introduction – clinical vignettes

Two workers were brought to the plant health clinic complaining of headaches, dizziness and nausea while containing and cleaning up an acrylonitrile spill. Dressed in Tyvek® coveralls for level C splash protection and using respiratory protection, they had been working in the area of the spill for approximately 2 hours.

They were both treated with 100% oxygen via facemask. Patient #1, a middle age male, appeared anxious with florid facies and was mildly hypertensive. Electrocardiographic monitoring showed a supraventricular tachycardia. He also complained of a burning sensation on his lower legs.

Patient #2, a young man, complained of headache, nausea and dizziness. Vital signs and electrocardiographic monitoring were normal.

Exposure information from plant safety personnel was confusing, as it was believed both employees had been properly protected from exposure.

Concerned about the cardiovascular status of patient #1, the plant physician had him transported directly by ambulance to a local hospital emergency room where an experienced clinician recognized the patient's toxidrome and administered the cyanide antidote, sodium nitrite followed by sodium thiosulfate. Immediately upon infusion of the sodium nitrite solution, the supraventricular tachycardia converted to a normal sinus rhythm and the patient's symptoms resolved. Impressed by this dramatic clinical response to treatment with the cyanide antidote, the emergency room physician immediately contacted the plant physician to advise him of this finding, which supported the diagnosis of cyanide poisoning. The plant physician then carefully crushed an amyl nitrite inhalant pearl under the nose of patient #2 whose headache and nausea promptly disappeared, and he became completely asymptomatic. After a period of observation, this patient was discharged.

Meanwhile, patient #1 was admitted to the hospital where symptoms and signs of cyanide poisoning did not recur during almost 24 hours of observation.

However, the following day upon discharge from the hospital he was found to have a bullous eruption of his lower legs that had the appearance of a partial thickness skin burn. He was taken to the plant health clinic for treatment where the bullae were debrided and

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the skin lesions were then treated like burns, with silver sulfadiazine cream and daily dressing changes until they resolved without sequelae.

13.2 Physical and chemical properties

Acrylonitrile is a clear, colorless, highly flammable, volatile liquid with a mild, unpleasant odor (ACGIH, 2001), also described as a sharp onion-like odor (Leonard *et al*., 1999). The odor threshold is 21.6 ppm. Molecular weight is 53.06. Freezing point is −83.5°C; boiling point is 77.3°C. Conversion factors are $1\ \text{ppm} = 2.15\ \text{mg/m}^3$; $1\ \text{mg/m}^3 = 0.46\ \text{ppm}$ (ACGIH, 2001). It is soluble in water, acetone, and benzene, and miscible with ethanol and ether (Leonard *et al*., 1999).

Acrylonitrile is also known as acrylic acid nitrile, acrylon, carbacryl, cyanoethylene, propenenitrile, 2-propenenitrile, propenoic acid nitrile, propylene nitrile, VCN, ventox, vinyl cyanide, Acrylon®, Carbactryl®, and Fumigrain®. Its chemical abstracts service (CAS) number is 107-13-1 and its molecular formula is C3H3N. Acrylonitrile has explosive properties when mixed with air in certain proportions. Spontaneous exothermic polymerization of acrylonitrile also presents a risk of explosion with the generation of cyanide gas (Long & Meek, 2002). It is reactive and requires stabilization by chemical inhibitors to prevent polymerization reactions (Kirman *et al*., 2008). Acrylonitrile has two chemically active sites, at the carbon–carbon double bond and at the nitrile group, where it undergoes a wide variety of reactions (Long & Meek, 2002).

13.3 History – preparation – manufacture

Acrylonitrile was first synthesized around 1893 by dehydrating acrylamide or ethylene cyanohydrin with phosphorus pentoxide, but commercial production did not start until about 40 years later (Leonard *et al*., 1999).

Acrylonitrile was first produced in Germany and the United States on an industrial scale in the early 1940s. These processes were based on the catalytic dehydration of ethylene cyanohydrin produced from ethylene oxide and aqueous hydrocyanic acid at 60°C in the presence of a basic catalyst. The intermediate was then dehydrated in the liquid phase at 200°C in the presence of magnesium carbonate and alkaline or alkaline earth salts of formic acid. A second commercial route to acrylonitrile was the catalytic addition of hydrogen cyanide to acetylene. The last commercial plants using these process technologies were shut down in 1970 (IARC, 1999).

The growth in demand for acrylic fibers, starting with the introduction of Orlon® by DuPont around 1950, spurred efforts to develop improved process technology for acrylonitrile manufacture. This resulted in the discovery in the late 1950s of a heterogeneous vapor-phase catalytic process for acrylonitrile by selective oxidation of propylene and ammonia, commonly referred to as the propylene ammoxidation or Sohio process. Commercial introduction of this lower-cost process by Sohio in 1960 resulted in the eventual displacement of all other acrylonitrile manufacturing processes. Today, the ammoxidation process accounts for over 90% of the approximately 4000 tonnes produced worldwide each year. In this process, propylene, ammonia, and air react in the vapor phase in the presence of a catalyst (bismuth–iron; bismuth–phosphomolybdate; antimony–uranium; ferrobismuth–phosphomolybdate). Hydrogen cyanide and acetonitrile are the chief byproducts formed. Sulfuric acid is used to remove excess ammonia from the reaction mixture, and the nitrile compounds are removed by absorption in water. High-purity acrylonitrile is obtained by a series of distillations (IARC, 1999).

Reports of the acute toxic effects of acrylonitrile in humans began in the 1940s when the industrial use of acrylonitrile increased dramatically with the production of synthetic rubber (ACGIH, 2001). Until 1977, the standard for occupational exposure to acrylonitrile in the United States and most West European countries was an 8-hour time-weighted average (TWA) of 20 ppm. This standard was mainly based on acute and subacute effects observed in animal experiments (Houthuijs *et al*., 1982).

In 1977, the U.S. Occupational Safety and Health Administration (OSHA) was informed by the Manufacturing Chemists Association of interim results of a 2-year feeding and inhalation study in rats. After 1 year, rats developed tumors in the brain and the zymbal gland, as well as papillomas in the stomach. In the same year, the Du Pont Company reported to OSHA preliminary results of an epidemiological study of a

cohort of acrylonitrile workers in which an increase in the incidence of colon and lung cancers was found (Houthuijs *et al*., 1982). Because of these findings, in 1978 OHSA lowered the permissible exposure limit to 2 ppm (8-hour TWA), with a short-term exposure limit (STEL) value of 10 ppm. Other countries lowered their permissible exposure levels as well. As a result, most manufacturers reduced occupational exposure to acrylonitrile by means of engineering controls and good work practices (Houthuijs *et al*., 1982).

The carcinogenic risks of acrylonitrile have since been assessed many times. In 1979, the International Agency on Research for Cancer (IARC) evaluated acrylonitrile and found "there is sufficient evidence for the carcinogenicity of acrylonitrile to animals. There is limited evidence for the carcinogenicity of acrylonitrile to humans. The agent is probably carcinogenic to humans" (Group 2A) (Leonard *et al*., 1999). In an updated review of the evidence in 1999, however, an IARC working group downgraded the classification for acrylonitrile from "2A (probable carcinogen)" to "2B (possible carcinogen)" (Boffetta *et al*., 2008).

A statement by officials of the Commission of the European Communities was "The possibility that acrylonitrile could be a lung or prostatic carcinogen cannot be excluded" and suggested further epidemiological research on cohorts of workers with case control studies for confounding factors such as smoking and other workplace exposures; it also encouraged industry to make carcinogenicity studies readily available. Finally, the U.S. Department of Health and Human Services determined in 1988 that acrylonitrile may "reasonably be anticipated to be a human carcinogen" (Leonard *et al*., 1999).

Acrylonitrile is as a high-volume commodity chemical with worldwide production of more than 10 billion pounds per year. Asia accounts for approximately 45% of global production, with 30% in the United States and 25% in Europe (Neal *et al*., 2009).

13.4 Occurrence

Exposure to the general population is limited to tobacco smoke, accidental fires, and residual acrylonitrile in commercial polymers (Leonard *et al*., 1999). However, the major source for non-occupational exposure to acrylonitrile is by tobacco smoke (Minet *et al*., 2011). Interestingly, approximately 3.9 times as much acrylonitrile is emitted in sidestream smoke as in mainstream smoke (39 vs. 10 mg/cigarette) (Perez *et al*., 1999), although others have reported the concentration in cigarette smoke to be only 1–2 mg/cigarette. Plastic containers are not allowed to release more than 0.17 ppb of acrylonitrile into food (Leonard *et al*., 1999).

Acrylonitrile in the environment is broken down rapidly in air and somewhat more slowly (1–2 weeks) in water (Leonard *et al*., 1999). Since acrylonitrile has a short environmental half-life, human exposure is thought to occur primarily from occupational sources (Chanas *et al*., 2003). Thus, population exposure to acrylonitrile in the environment is small except near factories or waste sites, with air concentrations near factories generally below 1 ppb. However, exposure to acrylonitrile can also occur from residual acrylonitrile in commercial polymeric material in fibers, usually less than 1 mg/kg; in resins, about 30–50 mg/kg; in styrene–acrylon nitrile resins, about 15 mg/kg; and concentrations in rubber and latex vary widely. Acrylonitrile also has been detected in commercial acrylamide, 25–50 mg/kg (Leonard *et al*., 1999).

Nevertheless, the concentration of acrylonitrile in end-use products is considered low. Therefore, risks due to airborne exposure or dermal exposure to workers during the handling of such products will be minimal. Measured data indicate that exposure levels of acrylonitrile in these situations are below the level of detection (Hazardous Substances Assessment Unit, 2004). Acrylonitrile has been detected at low levels in surgical smoke produced during transurethral resection and vaporization of the prostate (Chung *et al*., 2010).

13.5 Compounds and uses

Acrylonitrile (AN) has been used on a large scale since 1950 (Houthuijs *et al*., 1982) in the production of a variety of polymers (Minet *et al*., 2011; ACGIH, 2001). However, nitrile resins with acrylonitrile are no longer used to make beverage bottles (ACGIH, 2001). Acrylonitrile was formerly used as an insecticide (Bakker *et al*., 1991) and a fumigant (Buchter & Peter, 1984).

Nevertheless, acrylonitrile is one of the 50 most commonly used chemicals in the United States. World consumption of acrylonitrile is over 4 million tons and is growing over 3% a year (Khan *et al*., 2009).

Acrylonitrile is used as a monomer in the production of acrylic and modacrylic fibers, which accounts for approximately 50% of its global use. Acrylic fiber is used for clothing, carpeting, and other fabrics and in the production of rugged plastics for automotive components, computers, and appliances. Acrylic fiber is also used in the manufacture of polyacrylonitrile (PAN)-based carbon fibers, which are increasingly important materials for lightweight, high strength applications in aeronautics, automotive engineering, and so on. Acrylonitrile is used as a co-monomer in the production of acrylonitrile–butadiene–styrene (ABS) and styrene–acrylonitrile (SAN) polymers, which account for an additional 31% of use. These polymers are used in a wide range of oil- and chemical-resistant nitrile rubbers for industrial hoses, gaskets, and seals. Acrylonitrile is also used as an intermediate in the production of other industrial chemicals, such as adiponitrile and acrylamide (Neal *et al*., 2009).

13.6 Hazardous exposures

The preponderant health risks of acrylonitrile are associated with industrial exposure and are connected with its very high acute toxicity, generally by inhalation and by dermal contact and absorption (Diodovich *et al*., 2005). Workers may be exposed to acrylonitrile in an industrial setting during its production, polymerization, and transportation (Benz & Nerland, 2005).

Several retrospective cohort epidemiology studies evaluated a number of health outcomes in workers exposed to acrylonitrile. Most workplace exposure to acrylonitrile was from inhalation, but dermal exposure also occurred. Average inhalation exposure was generally highest during acrylic fiber production, especially in the polymerization and spinning processes where unreacted acrylonitrile was present. The levels in these fiber operations were estimated to average from 7 to 20 ppm in the 1950s and 1960s, falling to 3–9 ppm in the 1970s (IARC, 1999). Other significant exposures may have occurred in monomer production and in the manufacture of acrylonitrile-based resins, nitrile rubbers, carbon fibers, acrylamide, adiponitrile, and acrylic acid. Exposures in these processes were estimated to be in the 1–4 ppm range with some operations having levels up to 15 ppm. Since the early 1980s, exposure levels in these operations have been below 2 ppm for acrylic fiber production and generally below 1 ppm for all other acrylonitrile operations (Cole *et al*., 2008).

The U.S. Occupational and Health Administration limits worker exposure to 2 ppm (4.5 mg/m^3) in air; the short-term exposure limit (STEL) is 10 ppm. The health authorities of the Federal Republic of Germany have placed acrylonitrile in the category of carcinogenic chemicals for which no threshold limits are established (Leonard *et al*., 1999). EPA has classified the "volatile organic compound" acrylonitrile as a "water priority pollutant" and a "hazardous air pollutant" (Leonard *et al*., 1999). In 2010, releases of acrylonitrile in the United States of America amounted to about 10.7 million pounds, of which 3.6% occurred into air, 0.001% into surface water, 0.00007% onto land, 76.3% underground, 1.45% into public sewage, and 18.7% off-site. In 2010, these releases ranked 39th in the Toxic Release Inventory of 488 chemicals and 10th among carcinogens (EPA, 2011).

13.7 Toxicokinetics

13.7.1 Absorption

Exposure to acrylonitrile at the workplace may occur by inhalation of the vapor, by dermal contact and absorption of the liquid, and possibly by oral ingestion (Buchter and Peter, 1984). Human skin permeability is relatively high for acrylonitrile and is an important aspect of exposure leading to health complaints. The permeability of the human skin was determined *in vitro* in a so-called diffusion cell. In the first 30 minutes the penetration rate was 0.033 $mg/cm^2/min$, increasing to 0.066 $mg/cm^2/min$ after 60 minutes (Bakker *et al*., 1991).

In animal studies, acrylonitrile is readily absorbed from the respiratory and gastrointestinal tracts and through the intact skin (ACGIH, 2001). Schettgen and colleagues (2002) demonstrated in a pilot study that acrylonitrile crosses the placental barrier. The results showed that neonates of smoking mothers took up much higher doses of hemoglobin acrylonitrile adducts than those of non-smoking mothers (Diodovich *et al*., 2005).

Acrylonitrile is rapidly absorbed via all routes of exposure and distributed throughout the tissues. There is little potential for significant accumulation in any organ,

with most of the compound being excreted primarily as metabolites in the urine within the first 24–48 hours following administration (Long & Meek, 2002).

13.7.2 Metabolism

Following absorption, acrylonitrile is metabolized in humans and experimental animals via two pathways:

1 Nucleophilic reaction with glutathione (GSH) and proteins, quantitatively the major pathway. This reaction ultimately leads to the formation of a number of mercapturic acids excreted into the urine, the most important of which is 2-cyanoethylmercapturic acid (CEMA) (Minet *et al*., 2011).
2 The alternative pathway is catalyzed by cytochrome P450-2E1 (CYP 2E1) and leads to the epoxide cyanoethylene oxide (glycidonitrile, 2-cyano-oxirane), the primary metabolite thought to mediate genotoxicity by forming DNA and protein adducts. Extensive secondary metabolism of cyanoethylene oxide (CEO) leads to several other metabolites including cyanide (Thier *et al*., 2000).

Acrylonitrile undergoes both oxidative and non-oxidative metabolism (Benz & Nerland, 2005). Available data are consistent with conjugation to glutathione being the major detoxification pathway, while oxidation to 2-cyanoethylene oxide is considered an activation pathway (Long & Meek, 2002). One of the products of the oxidative pathway is cyanide, which is acutely toxic. Metabolically released cyanide has been implicated in acrylonitrile-induced mortality, and human intoxications have been successfully treated with cyanide antidotes (Benz & Nerland, 2005). The formation of the cyanide ion requires the activation of the double bond in acrylonitrile to the active epoxide (Mostafa *et al*., 1999).

There are species differences in metabolism and toxicity of acrylonitrile. The development of toxic cyanide blood levels upon acrylonitrile intoxication in humans highlights an important metabolic difference between laboratory rodents and humans. In rats and mice, the oxidative pathway, which leads to the formation of cyanide, is much less important than in humans. In rodents, roughly 25% of the acrylonitrile is metabolized via the oxidative pathway. In contrast, peak cyanide blood levels in humans exceed those of acrylonitrile (Thier *et al*., 2000).

Both acrylonitrile and its epoxide metabolite can react with reduced glutathione to form conjugates which are further metabolized to mercapturic acids and excreted in the urine (ACGIH, 2001).

In vitro studies with human and rat hepatic microsomes have indicated that CYP2E1 is the major catalyst of acrylonitrile epoxidation, although other cytochrome P450 isozymes can also metabolize acrylonitrile. CYP 2E1 is the only catalyst of acrylonitrile epoxidation in mice (Sumner *et al*., 1999).

Because both parent acrylonitrile and CEO undergo conjugation with endogenous glutathione that prevents further metabolism of either chemical to cyanide, it is likely that glutathione tissue levels and glutathione transferases (GST) may influence the eventual metabolism of acrylonitrile to cyanide (Chanas *et al*., 2003).

Acrylonitrile is rapidly metabolized and excreted in urine. In the period from 1 to 8 hours after exposure, about 60%–70% of the dose has been found in the urine. Excretion of the vinyl group is more rapid than that of the nitrile group (Sapota & Draminski, 1981).

13.8 Mode of action

The metabolism of acrylonitrile has important implications on its mode of action (Kirman *et al*., 2008). Acrylonitrile is metabolized to cyanide *in vivo* but cyanide production alone cannot explain its acute toxicity (Campian *et al*., 2002).

The parent acrylonitrile molecule is considered to be acutely toxic (Benz & Nerland, 2005) and is thought to cause toxicity through three distinct pathways that promote oxidation: (i) conjugation with GSH, a metabolic process representing the major route of detoxification of acrylonitrile that results in a rapid depletion of GSH and an overall decrease in cellular antioxidants; (ii) liberation of cyanide (CN) from acrylonitrile, a potent generator of reactive oxygen species (ROS) production (via inhibition of the mitochondrial respiratory chain) as well as an inhibitor of the activities of several antioxidant enzymes; and (iii) ROS generated as byproducts of acrylonitrile metabolism via cytochrome CYP 2E1 oxidation (Rongzhu *et al*., 2009).

The major pathway of acrylonitrile elimination is its conjugation with GSH to form mercapturic acid. By depleting GSH, acrylonitrile may decrease the antioxidant levels in the cells leading to an overall increase in intracellular reactive oxygen species (ROS)

and oxidative damage. Both enzymatic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST)) and nonenzymatic (GSH) antioxidant defense systems are impaired in acrylonitrile-treated rats (El-Sayed *et al*., 2008).

Metabolism of acrylonitrile results in the production of cyanide, which induces oxidative stress (lipid peroxidation) in the brain of acutely treated mice and cell lines by inhibiting the mitochondrial respiratory chain, CAT and GSH-Px (El-Sayed *et al*., 2008). Additional information points to different modes of action between species. When rats were acutely intoxicated with acrylonitrile, symptoms were observed that were indicative of increased parasympathomimetic or cholinomimetic activity (lacrimation, salivation, miosis), and atropine proved to be an effective experimental antidote. A dose-dependent cholinomimetic effect in the early neurotoxicity of acrylonitrile in rats was confirmed, and a dose-dependent decrease of blood acetylcholinesterase activity was found in rats subchronically treated with acrylonitrile by gavage for 8 weeks. A likely explanation for these findings was that acrylonitrile, owing to its chemical reactivity, interacted with the active center of acetylcholinesterase (Bolt *et al*., 2003).

Cholinomimetic symptoms, comparable to those observed in rat experiments, have not been reported in occupationally exposed humans in Germany, where the clinical status of a large number of people intoxicated by acrylonitrile has been carefully recorded. This is consistent with the concept that the oxidative, CYP2E1-mediated metabolism of acrylonitrile, associated with the formation of cyanide, is much more prevalent in humans than in the experimental rats (Bolt *et al*., 2003).

In contrast, Chinese clinical reports of cholinomimetic symptoms in cases of acute industrial acrylonitrile intoxication pointed out that the cyanide metabolite alone would not explain all observed manifestations of acute acrylonitrile toxicity. While the oxidative metabolism of acrylonitrile, via CYP2E1, appeared more commonly in Europeans (as opposed to the experimental situation in rats), in populations of the Far East with consistently lower CYP2E1 expression levels, different relative proportions of the two major metabolic pathways of acrylonitrile occurred. Thus, the established interethnic variations in CYP2E1 genetics and enzyme expression appeared to be connected with clinically different pictures in acute industrial acrylonitrile poisoning (Bolt *et al*., 2003).

Chronic exposure to acrylonitrile is known to conjugate GSH leading to oxidative stress as demonstrated by elevated levels of malondialdehyde (MDA) and ROS, as well as decreased levels of GSH, CAT, and SOD in the brain. Also, it indirectly leads to oxidative DNA damage in rat brain as indicated by increased levels of 8-hydroxy-2-deoxyguanosine (OH8dG). In *in vitro* studies, acrylonitrile-induced DNA strand breaks and cell transformations were found in Syrian Golden hamster embryo cells (Bhattacharya *et al*., 2009).

Acrylonitrile also induced oxidative stress in normal human astrocytes *in vitro*, albeit at concentrations that produced significant cytolethality. In addition, the antioxidant *N*-acetyl cysteine (NAC) was shown to protect primary rat glial cells against lipid peroxidation and GSH depletion induced by acrylonitrile. Collectively, these data support the notion that oxidative stress occurs following acrylonitrile exposure and may potentially be the mode of action for glial tumor formation by acrylonitrile (Pu *et al*., 2009).

Information available for acrylonitrile suggests that genetic polymorphisms in the enzyme systems responsible for its metabolism are not an important determinant of cancer risk. A study was conducted on CYP2E1 polymorphisms in a cohort of 59 people handling industrial materials and exposed to low levels of acrylonitrile from 1994 through 1999. The individual means and medians of hemoglobin adduct (*N* -(cyanoethyl)valine) levels over the entire observation period were compared with the CYP2E1 variants. No significant influences in the investigated CYP2E1 polymorphisms on *N*-(cyanoethyl) valine levels were found (Kirman *et al*., 2005).

Another pathway of cellular oxidative stress is the activation of the immune system. Acrylonitrile-induced immunotoxicity in mice has been reported. Tumor necrosis factor alpha (TNF – α) is known to play a critical role in immune responses and inflammatory processes, and TNF – α secretion has been used as a marker of oxidative stress (Jacob & Ahmed, 2003).

13.9 Clinical effects

13.9.1 Acute

Acrylonitrile is toxic by the oral, inhalation, and dermal routes and causes neurotoxic effects (Kirman

et al., 2008). Acrylonitrile (AN) is acutely toxic to humans at relatively low levels. Headache, nausea, and dizziness have been reported at exposures between 20 and 150 ppm for short periods (Cole *et al.*, 2008). More serious acute exposures have resulted in tremors, convulsions, unconsciousness, respiratory and cardiac arrest, and even death (Kirman *et al.*, 2008). Exposure in excess of 500 ppm for several minutes is considered lethal (Cole *et al.*, 2008).

Severe acrylonitrile intoxication causes loss of consciousness, convulsions, respiratory arrest, and may lead to death. Other symptoms and signs of intoxication are irritation and inflammation of the respiratory tract and mucous membranes, salivation, nausea, vertigo, vomiting, and diarrhea. Respiratory distress, cyanosis, pulmonary edema, and tachycardia may be seen. Headache and fatigue are common complaints. Liver injury with mild jaundice and liver tenderness may be observed, as well as low grade anemia and kidney dysfunction (Buchter & Peter, 1984).

Correlation has been reported between blood cyanide levels and the acute lethality of acrylonitrile in mice (Neal *et al.*, 2009). Symptoms in humans caused by inhalation of acrylonitrile occur at concentrations in the range of 3–16 ppm, less than the levels that can be smelled (~ 19 – 22 ppm) (ACGIH, 2001; Kirman *et al.*, 2008).

In laboratory animals, the acute toxicity of acrylonitrile is relatively high, with 4-h LC50s ranging from 140 to 410 ppm (300 to 900 mg/m^3) and oral LD50s ranging from 25 to 186 mg/kg body weight. Dermal LD50 values for various species were in the range of 148–693 mg/kg body weight, with the rat being most sensitive. Signs of acute toxicity include respiratory tract irritation and central nervous system dysfunction, resembling cyanide poisoning. Superficial necrosis of the liver and hemorrhagic gastritis of the forestomach have also been observed following acute exposure (Long & Meek, 2002).

In animals, acrylonitrile-induced neurotoxicity following acute exposure via inhalation or ingestion has been described as a two-phase phenomenon. The first phase, which occurs shortly after exposure and is consistent with cholinergic overstimulation, has been likened to toxicity caused by acetylcholinesterase inhibition. Cholinomimetic or parasympathetic signs in rats exposed to acrylonitrile have included vasodilation, salivation, lacrimation, diarrhea, and gastric secretion. These effects are maximal within one hour of dosing. The second phase of toxicity is delayed by four hours or more and includes signs of central nervous system disturbance, such as trembling, ataxia, convulsions, and respiratory failure (Long & Meek, 2002).

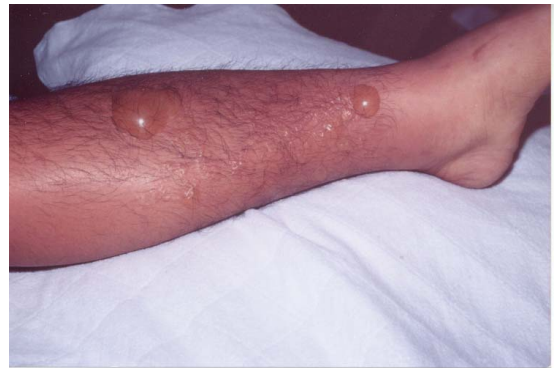

Figure 13.1 Photograph taken 24 hours after skin exposure to acrylonitrile vapor. Courtesy of Dr. Parkes.

Dermal exposure to acrylonitrile and subsequent skin absorption can lead to systemic toxicity and lethality (ACGIH, 2001). After dermal contact, there is a burning sensation followed by erythema, and later by blisters, edema, pruritis, and pain. These skin changes may not occur for up to 24 hours after exposure (see Figure 13.1). Development of allergic contact dermatitis may occur several days after contact (Buchter & Peter, 1984). Several cases of toxic epidermal necrolysis leading to death were described among people exposed to a mixture of acrylonitrile and carbon tetrachloride used for residential fumigation (Radimer *et al.*, 1974). Industrial experience has shown that acrylonitrile, when absorbed in workers' leather shoes, can cause large blisters resembling second-degree burns after several hours (Radimer *et al.*, 1974). Instillation into the eyes causes conjunctivitis and possibly edema and slight necrosis (Buchter & Peter, 1984).

13.9.2 Chronic

With long-term acrylonitrile exposure, several effects reported are headache, decreased work capacity, weakness, poor sleep, irritability, chest pains, nausea, and dermatitis (Buchter & Peter, 1984). Acrylonitrile has been described as a strong sensitizing compound, and Bakker *et al.* (1991) opined that it should be classified and labeled as such.

Chronic workplace exposures to acrylonitrile have been reported to cause neurologic symptoms similar to those reported following acute overexposure, including, nausea, vomiting, general weakness, and symptoms of neurasthenia (Kirman *et al.*, 2008). Pouyatos and colleagues (2005) demonstrated that combined exposure to acrylonitrile and noise could induce hearing loss and otic hair cell (OHC) loss through oxidant stress, while the exposure to the same noise alone did not induce hearing loss nor cochlear damage (Pouyatos *et al.*, 2005).

Acute acrylonitrile administration produced a loss in auditory threshold sensitivity that reached a maximum 10–20 min following subcutaneous injection. Auditory thresholds returned to control levels 75–100 min following exposure. In the study of permanent auditory threshold shifts, acrylonitrile plus noise increased auditory threshold impairment relative to rats receiving noise only when thresholds were assessed 3 weeks following exposure. Acrylonitrile by itself did not produce permanent hearing threshold impairment 3 weeks following administration (Fechter *et al.*, 2003).

Information from a large body of epidemiology data does not support a relationship between long-term workplace acrylonitrile exposure and increased mortality from any cause. No increase in overall or specific mortality has been reported for acrylonitrile workers in numerous epidemiological studies. No consistent relationship has been noted in terms of increasing dose or duration of exposure (Kirman *et al.*, 2008).

13.9.3 Cancer

Acrylonitrile is a relatively potent carcinogen in rats and mice, producing cancers at multiple sites at exposure levels that might occur in the workplace (Cole *et al.*, 2008).

Tumors of the CNS were the predominant type in long-term bioassays of rats exposed through inhalation or drinking water. The CNS tumor excess in rats occurred at exposures as low as 20 ppm in an inhalation study. It was noted that daily average acrylonitrile exposures in excess of 20 ppm had been reported in workers in the past. A cancer bioassay was conducted in mice exposed to acrylonitrile via gavage. Although no brain tumors were observed, several tumor types were identified as being potentially related to acrylonitrile exposure (Cole *et al.*, 2008).

The first study of lung cancer mortality among workers who were exposed to acrylonitrile in a textile factory in the United States, reported in 1978, showed a fourfold increased risk, based on six deaths from lung cancer. An International Agency for Research on Cancer (IARC) working group cited this study in its review of acrylonitrile in 1979 and concluded that "while confirmatory evidence in experimental animals and humans is desirable, acrylonitrile should be regarded as if it were carcinogenic to humans." Following the initial report, a total of 16 partially overlapping occupational cohort studies provided results on lung cancer risk among acrylonitrile-exposed workers, including an expanded analysis of the first study, which identified two additional observed and 2.9 additional expected lung cancer deaths (Boffetta *et al.*, 2008).

The early studies included many workers with high exposure to acrylonitrile (Cole *et al.*, 2008). However, multiple mortality studies on populations of workers exposed to acrylonitrile failed to associate increased risk of cancer with exposure to acrylonitrile (ACGIH, 2001). IARC later downgraded acrylonitrile from "probably carcinogenic" to "possibly carcinogenic to humans" in 1999 finding that "the earlier indications of an increased risk among workers exposed to acrylonitrile were not confirmed by the recent, more informative studies" (Cole *et al.*, 2008).

Limitations of early epidemiologic studies included the lack of quantitative acrylonitrile exposure estimates as well as the few cancer deaths occurring in cohorts with small sample sizes. These factors contributed to an inability to conduct useful exposure–response analyses (Symons *et al.*, 2008).

The largest and most comprehensive single epidemiologic study to date was performed by the National Cancer Institute (NCI) and the National Institute for Occupational Safety and Health (NIOSH). With the possible exception of lung cancer, the NCI–NIOSH study revealed no evidence of elevated site-specific cancer risks in relation to several indicators of acrylonitrile exposure. For lung cancer mortality, the overall relative risk based on internal comparisons (acrylonitrile exposed to acrylonitrile unexposed workers) was only slightly elevated (rate ratio (RR) 1.2, 95% confidence interval (95% CI) 0.9–1.6), and there was no strong or consistent evidence of a linear exposure-response relationship for any of the acrylonitrile exposure indicators. However, the lung cancer excess was largest in the highest quintile of cumulative acrylonitrile exposure (RR 1.5, 95% CI 0.9–2.4) and for the subgroup of

workers at risk ≥ 20 years since their first exposure (RR 2.1, 95% CI 1.2–3.8) (Marsh *et al*., 2001). Several subsequent analyses, including a nested case-cohort study that included smoking histories, failed to elucidate the reasons for the elevated risks for lung cancer. The findings for lung cancer led Blair *et al*. (1998) and his co-workers to conclude that there may be evidence of carcinogenic activity at the highest level of acrylonitrile exposure (Marsh *et al*., 2001).

It was counter-argued that 67% of workers in the highest quintile originated from one plant, with a prior history of exposure to asbestos. However, having reviewed 18 published cohort studies, Sakurai (2000) arrived at the conclusion that, although there was inadequate evidence in humans for carcinogenicity of acrylonitrile, the possibility of a causal association between high exposure and lung cancer in humans could not be excluded (Bolt, 2003).

Based on this considerable number of reported epidemiological studies, the carcinogenic potential in humans appears to be weak (if any). By contrast, the preponderant health risks of industrial handling of acrylonitrile appear connected with its very high acute toxicity, in combination with its clear potential for skin penetration, which leads to a high danger of even fatal accidents (Bolt, 2003).

13.9.4 Reproduction and development

In general, when acrylonitrile exposures were less than those causing overt maternal toxicity, no developmental toxicity was observed (ACGIH, 2001). Acrylonitrile was given to Sprague–Dawley rats by gavage at 10, 25, or 65 mg/kg/day or by inhalation at 40 or 80 ppm, 6 h/day, from gestational day (GD) 6 to GD15. Decreases in maternal weight gain were noted after oral administration of 65 mg/kg/day or inhalation exposure to 40 or 80 ppm. A significant increase in the incidence of fetuses with a short tail and missing vertebrae was observed after oral administration of 65 mg/kg. A lower incidence of the same alterations was seen at 25 mg/kg/day by gavage and at 80 ppm by inhalation. These findings were considered suggestive of a teratogenic effect. Pregnant golden hamsters received a single intraperitoneal injection of 1.51 mmol acrylonitrile/kg (80 mg/kg) on GD8. Fetal malformations including exencephaly, encephalocele, and rib defects were produced in the presence of severe maternal toxicity (Saillenfait & Sabate, 2000).

A single study demonstrated that acrylonitrile lead to a decrease in sperm quality among acrylonitrile-exposed workers. It was determined that acrylonitrile or its metabolites could induce reproductive defects as an *in vivo* multipotent genotoxic agent by inducing DNA strand breakage and sex chromosome non-disjunction in spermatogenesis (Xu *et al*., 2003).

Nevertheless, a weight-of-the-evidence evaluation of the reproductive and developmental toxicity data for acrylonitrile did not support concern for exposures below those producing overt maternal toxicity, and did not demonstrate a significant reproductive hazard, or unique susceptibility of the fetus or offspring (Neal *et al*., 2009).

13.10 Diagnosis – toxicity

Clinical effects of incidental industrial acrylonitrile intoxication are mostly ascribed to the metabolic formation of cyanide and manifest themselves as a delayed form of cyanide poisoning (Bolt *et al*., 2003). A threshold for clinical symptoms exists in the range of 3 to 16 ppm (ACGIH, 2001; Kirman *et al*., 2008). A NOAEL of 3 ppm is supported by the absence of clinical signs in exposed workers (Kirman *et al*., 2008). Acrylonitrile is readily absorbed through intact skin; dermal exposure may result in systemic toxicity (BASF, 2010). Systemic symptoms after skin exposure occur typically 1 ½ hours post exposure, even after decontamination.

The toxic effects occur after a latent period (in which formation of cyanide occurs) and involve the respiratory, cardiovascular, and, in particular, the central nervous system. Symptoms are initially non-specific and may include nausea, vomiting, flushing, headache, dyspnea, dizziness, weakness, asphyxia, tachycardia, chest tightness, drowsiness, convulsions, loss of consciousness, cardiac arrhythmias, hypotension, and jaundice (Graham, 1965; BASF, 2010). All routes of exposure to acrylonitrile can result in systemic effects. CNS symptoms can evolve rapidly or be delayed. Symptoms of cyanide poisoning may recur several times after exposure and require repeated treatment. The initial onset of systemic symptoms has been reported as long as 12 hours after exposure (BASF, 2010). These symptoms are reported to be independent of ethnicity (Bolt *et al*., 2003).

Acrylonitrile is toxic if inhaled, swallowed or in contact with the skin. Skin contact causes blistering; the eyes and mucous membranes are particularly at risk. Skin exposure to acrylonitrile vapors has caused blistering of the skin 24 hours post exposure (see Figure 13.1). If direct contact with acrylonitrile has taken place or is suspected, immediate medical attention is strongly recommended (CEFIC, 2009).

Acrylonitrile's odor does not provide adequate warning of hazardous concentrations. The odor threshold is 21.6 ppm (ACGIH, 2001). Olfactory fatigue develops rapidly. Acrylonitrile is heavier than air and may cause asphyxiation in poorly ventilated, low-lying, or enclosed spaces. Concentrations as low as 16 ppm for 20–30 minutes may produce headache, nausea, and irritability (BASF, 2010).

13.11 Treatment – antidote

As with other aliphatic nitriles, the cyanide toxicity associated with the metabolism of acrylonitrile has been treated effectively with the various cyanide antidotes. However, there are alternative mechanisms of acrylonitrile toxicity that do not respond as readily, if at all, to the usual cyanide antidotes.

After a single injection of acrylonitrile in rabbits, a small amount of hydrogen cyanide appeared in the blood. When the rabbits were pretreated with sodium thiosulfate, a significant reduction in the hydrogen cyanide in the blood with a decrease in symptoms was observed. However, the animals were still not protected effectively from poisoning. At higher dose levels of acrylonitrile, death was merely delayed (Hashimoto & Kanai, 1965).

Hydroxycobalamin reduced the immediate toxicity of acrylonitrile in mice. The protection afforded was more obvious if sodium thiosulfate was added, but most of the animals died hours later despite treatment (Graham, 1965).

The lessened impact of the cyanide metabolite in other laboratory animals such as rats was underscored by the differences in efficacy of the various cyanide antidotes. It was demonstrated that the cyanide antidotes 4-dimethylaminophenol and sodium thiosulfate were essentially ineffective in rats intoxicated by acrylonitrile via inhalation, while N-acetylcysteine (NAC) proved highly effective (Thier *et al.*, 2000).

Consequently, in Germany high intravenous doses of NAC were recommended for treatment of accidental poisoning of acrylonitrile workers. By contrast, antidotal measures in the United States continued to focus upon the cyanide metabolite (Thier *et al.*, 2000).

The understanding of acute acrylonitrile toxicity suggests that three antidotal mechanisms, the nonenzymatic reaction with acrylonitrile, replenishment of endogenous GSH and protein sulfhydryls, and detoxification of cyanide, should be of benefit. For L-cysteine and NAC, two of the more effective acrylonitrile antidotes studied, all of these mechanisms are possible (Benz, *et al.*, 1990).

N-Acetyl-l-cysteine (NAC) is the acetylated precursor of both the amino acid l-cysteine and GSH. The biological activity of NAC is attributed to its sulfhydryl group; while its acetyl substituted amino group affords its protection against oxidative and metabolic processes. Animal and human studies of NAC have shown it to be a powerful antioxidant by reducing extracellular cystine to cysteine, or by acting intracellularly as a source of sulfhydryl groups. Thus, NAC stimulates GSH synthesis, enhances glutathione-*S*-transferase activity, and is a powerful nucleophile capable of scavenging free radicals (Esmat *et al.*, 2007).

As a free radical scavenger, NAC significantly inhibits acrylonitrile-induced cyanide production by glial cells and prevents subsequent ATP depletion. It is thus able to neutralize acrylonitrile and/or its reactive metabolites, preventing its conversion into cyanide (Esmat *et al.*, 2007). NAC is a clinically used medicine with an extensive safety record over many years. NAC (Acetadote®, Mucomyst®) has also passed the stringent safety requirement for U.S. Food and Drug Administration approval for acetaminophen overdose, countering the liver damage induced by glutathione (GSH) depletion and consequent oxidative stress. In addition, NAC is almost devoid of serious adverse side effects (Pouyatos *et al.*, 2007).

The recommended antidotal treatment for acrylonitrile is (Thier *et al.*, 2000):

- Mild intoxication (patient conscious, only mild CNS symptoms and/or skin exposure): administer NAC 150 mg/kg IV (over 15 minutes).
- Severe intoxication (patient unconscious or severe CNS symptoms, e.g., seizures): administer NAC 300 mg/kg IV (150 mg/kg as above, then 50 mg/kg over 4 hours, followed by 100 mg/kg over 16 hours).

- Treat cyanide poisoning if it appears, with the usual standard cyanide antidote (sodium nitrite, hydroxycobalamin, sodium thiosulfate, etc.), and monitor for recurrence.

13.12 Biological monitoring

Accidental industrial acrylonitrile intoxications occur via inhalation and/or skin contact while the currently prevailing workplace concentrations of acrylonitrile in the air remain well below official standards. In view of the skin permeability of acrylonitrile, biological monitoring strategies are important for the surveillance of exposed workers (Thier *et al.*, 2000).

Hemoglobin adduct measurement is an extremely sensitive method for monitoring exposure to acrylonitrile (Thier *et al.*, 1999; Long & Meek, 2002). These adducts are stable and their elimination depends on the life span of the erythrocyte (120 days in humans); they represent the accumulated effect of the previous 3–4 months of exposure (Ogawa *et al.*, 2006). Therefore, hemoglobin adducts of acrylonitrile are valuable parameters of internal exposure and biochemical effects, which make it possible to estimate the individual health risk (Schettgen *et al.*, 2002).

The hemoglobin adduct N-cyanoethyl-valine (CEV) is a widely recommended parameter for this purpose (Thier *et al.*, 2000). A correlation has been found between the concentration of acrylonitrile in air and the CEV level in the globin of erythrocytes. Acrylonitrile exposure levels cover a range between 0.14 ppm and 3 ppm, corresponding to 655–17,200 pmol/g CEV (Bader & Wrbitzky, 2006).

There was a study of six Japanese acrylic factories in which acrylonitrile exposure was measured by personal air sampling as well as biological monitoring. Acrylonitrile was determined in the urine collected at the end of the workday via azeotropic distillation and analysis by gas chromatography. The 8-hour TWA acrylonitrile concentration in the breathing zone of the workers with the highest exposures was 4.2 ppm. The mean urinary acrylonitrile concentration was 360 mcg/l. For the group of workers with the lowest exposures these values were 0.1 ppm and 3.9 mcg/l. No acrylonitrile was detected in the urine of 22 controls (lower detection limit 5 mcg/l), although considerable amounts of acrylonitrile in cigarette smoke were reported. It was concluded that urine acrylonitrile concentrations might be a useful indicator of individual low-level exposure (Houthuijs *et al.*, 1982).

N-Acetyl-S-(2-cyanoethyl)-L-cysteine (2-cyanoethylmercapturic acid, CEMA) is a urinary metabolite of acrylonitrile. The analytical method used for its determination is sufficiently sensitive and specific to detect differences between smokers and non-smokers. Urinary CEMA levels show a clear dose-response relationship to the smoking dose, such as daily cigarette consumption. CEMA can also discriminate between smokers of different tar yield cigarettes. The method is therefore appropriate to assess the quantitative changes in exposure associated with the use of tobacco products, including the switch to reduced exposure tobacco products (Minet *et al.*, 2011).

13.13 Exposure limits

The American Conference of Governmental Industrial Hygienists (ACGIH) gave acrylonitrile a TLV-TWA of 2 ppm (4.3 mg/m^3) with a skin notation and an additional A3 notation as a "confirmed animal carcinogen with unknown relevance to humans" (ACGIH, 2001). The 2 ppm TLV was intended to minimize the potential for headache, nausea, respiratory difficulties, and central nervous system effects and for "cancer reported only in laboratory animals" (ACGIH, 2001). Systemic toxicity in rats from dermal absorption of acrylonitrile warranted the skin notation. Sufficient data were not available to recommend a SEN (sensitization) notation or a TLV-STEL (ACGIH, 2001). NIOSH has recommended that TWA exposure limits for acrylonitrile be set at 1 ppm with a short-term ceiling value set at 10 ppm (NTP, 2002).

OSHA, in its acrylonitrile standard, 29CFR1910.1045, established a permissible exposure limit (PEL) of 2 ppm (4.3 mg/m^3) as an 8-hr TWA with no eye or skin contact. This standard requires personal protective equipment, training, medical surveillance, signs and labeling, and engineering controls. A short-term exposure limit (STEL) (15 minutes) value was set at 10 ppm by OSHA. OSHA regulates acrylonitrile as a chemical hazard in laboratories under the Hazard Communication Standard, 29CFR1910.1450 (NTP, 2002). The U.S. Department of Labor (1978) stated the 2 ppm limit had been set by OSHA because it would provide significant

employee protection, and it was the lowest technically feasible level of exposure for most employers affected by the standard. No data was available to demonstrate the existence of a "safe" level of exposure to acrylonitrile. In the absence of a demonstrated safe level, and because of the irreversibility and long latency period for cancer, OSHA felt compelled to conclude, for regulatory purposes, that no safe level existed (U.S. Dept. of Labor, 1978).

The assessment of acrylonitrile by IARC was that it was possibly carcinogenic to humans (Group 2B). A review of available epidemiological findings still suggested the possibility of a causal association between very high exposure to acrylonitrile and lung cancer in humans. Therefore, it was recommended that the occupational exposure limit (OEL) for acrylonitrile should still be set with due regard to carcinogenicity, although OELs for many other chemicals classified by IARC as possibly carcinogenic (Group 2B) have generally been recommended without consideration of their carcinogenicity (Sakurai, 2000).

Exposure-response relationships for health effects other than carcinogenicity are not yet sufficiently known. Acute subjective symptoms, including headache, vomiting, and weakness, had been known to occur after exposure to acrylonitrile but were not reported by volunteers exposed to 4.6 ppm. Chronic effects such as subjective symptoms and abnormal findings in multiple clinical laboratory test results could not be detected in workers regularly exposed to 0.53 ppm. Teratogenic effects were not found in rats exposed to 40 ppm. Only slight inflammatory changes of the nasal mucosa were observed in rats chronically exposed to 20 ppm. These data were not inconsistent with an OEL of 2 ppm. Overall, it was concluded that the current OEL for acrylonitrile at 2 ppm was a reasonable value, and there was no need for its revision (Sakurai, 2000; Bolt, 2003).

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CHAPTER 14
Cyanide in chemical warfare and terrorism

René Pita

At a Glance

- While earlier potential military uses were proposed, the first use of cyanide-containing munitions was by the French in WWI in response to a German attack with phosgene.
- In the interwar period (between WWI and WWII), the U.S. military studied the potential use of cyanide as a chemical warfare agent (CWA), which also lead to the use of cyanide gas chambers for judicial executions.
- However, the discovery of various organophosphate nerve agents during this time rendered cyanide basically obsolete as a CWA because of difficulties in maintaining an incapacitating or lethal airborne concentration on the battlefield.
- During WWII, the U.S., Great Britain, and Canada conducted tests of a number of chemical warfare agents, including cyanide, on the Panamanian island of San José; however, cyanide-containing munitions were not deployed by the Allies.
- As a chemical terrorism agent, cyanide has been used for extortion, in contaminated over-the-counter capsules, in State terrorism for assignations, by Nationalist and separatist groups, by left- and right-wing terrorist groups and "lone wolves", by apocalyptic cults, and potentially by jihadi terrorists.

The high toxicity of cyanides is their most important property as weapons, while the difficulties of effective dissemination to cause a large number of casualties are the most important drawbacks. This chapter aims at explaining the history of cyanide as a weapon since World War I until the present, when there is a high concern of possible terrorist attacks with weapons of mass destruction (WMD), especially by al-Qaeda and other jihadist terrorist groups.

14.1 Cyanides as chemical warfare agents

In the 19th century, there were different proposals to use cyanide as a weapon. In 1813, a pharmacist proposed the use of cyanide salts on bayonets to the Prussian General Bülow, an idea also attributed to Napoleon III during the Franco-Prussian war (Croddy, 2005; Medema, 2006). These proposals were rendered infructuous because, at that time, poisoning the enemy was considered inhuman. Even the Lieber Code of 1863 during the American Civil War established that, "the use of poison in any manner, be it to poison wells, or food, or arms, is wholly excluded from modern warfare. He that uses it puts himself out of the pale of the law and usages of war." This code considered that "modern warfare" was an issue between professional military men who should act in accordance with their traditions of honor and abilities. Also, the 1899 Hague Declaration 2 agreement prohibited the use of projectiles whose sole object was to diffuse asphyxiating or deleterious gases. This principle was embodied in the 1907 Hague Convention IV which prohibits the use of poisoned weapons and arms, projectiles, or material calculated to cause unnecessary suffering. The convention was signed by France, Germany, Russia, the United Kingdom and the United States. But in 1914, a patent to use hydrogen cyanide (military code name AC) in shells was being

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prepared in the United States. In the UK, the scientist William Ramsay (a future Nobel Prize winner) proposed the use of AC munitions to the War Office, although it was rejected as it was considered to be contrary to the Hague Convention (Coleman, 2005; Pita, 2008).

14.1.1 World War I

At the beginning of World War I (WWI), the main chemical warfare agents (CWAs) used were chlorine and phosgene, both lung damaging ("choking") agents. Cylinders with these agents were used in trench warfare. But from the start of chemical warfare (CW) in WWI, the French also studied the use of AC, perhaps because it was readily available in the French chemical industry (Foulkes, 1934; Haber, 1986). British Major Victor Lefebure, liaison officer between the French and the UK Armies' chemical services in WWI, described the effects of AC as "kill or cure," which means that affected combatants would either die, or recover without residual effects that could affect their tactical capability (Lefebure, 1923). In WWI, cyanide agents were named "blood agents," something that may cause confusion, leading one to think that cyanides affect the oxygen transportation in the blood. The reason for naming cyanides as blood agents was because chlorine and phosgene were CWAs with local effects in the respiratory tract, while cyanides had to be absorbed in the lungs and pass to the blood to produce systemic effects. Although the toxicological mechanism of action of cyanides is nowadays well known, it is not rare to see them listed or described as "blood agents" when mentioned in the CW or chemical terrorism context.

France developed 75mm "Special Shells No. 4" with AC (as well as "Special Shells No. 5" with phosgene) which were available from the end of 1915, but the French were reluctant to use them, perhaps being afraid of violating the Hague Convention (Prentiss, 1937). However, this justification seems strange as the Germans were already using shells with lacrimating riot control agents and had already carried out their first mass casualties attack with chlorine cylinders on April 22 at the Ypres battle. But the French thought that introducing their 75mm cyanide or phosgene shells in war could be considered as an escalation in CW, as these chemicals were more toxic substances than riot control agents. In fact, the first use of the French shells would not take place until Germany used phosgene shells against them for the first time on June 22, 1916 at Fleury. Soon after that attack, on July 1, France used their Special Shells No. 4 in the battle of the Somme (Figure 14.1).

The main problems observed in WWI when France used AC as a weapon were: its low persistence, due to the fact that its density is lower than air; and its low thermal stability, which made its use in shells not very effective as most of the AC charge in the shells would be inactivated by the thermal effect of the explosion of the shell (see, for example, Johnston, 2003). As France also had phosgene shells available and these did not have the drawbacks of AC, it may seem strange that France decided to keep using AC shells during the war. One of the explanations is that France thought that AC could be more effective as the German gas mask filters were prepared for phosgene, but not for AC. However, the German intelligence services discovered that France had AC shells and deployed filters with pulverized silver oxide scattered through potash layers for its troops in the battle of the Somme (Prentiss, 1937).

The French tried to solve the problems of AC by using a mix of arsenic trichloride as a stabilizer with

Figure 14.1 French WWI 75mm shell (courtesy of Jeff Osborne).

two additives, stannic chloride and chloroform, the first to increase the AC density and decrease its rapid volatilization, while the second was intended to avoid cyanide polymerization inside the shell (Brophy *et al.*, 1959; Fries & West, 1921; Stockholm International Peace Research Institute, 1971). This mix was called Vincennite because it was first tried in Vincennes. A mix of 50% of AC and 50% of arsenic trichloride called Manganite was also used. However, neither of these mixes solved the problem of the low persistence of AC. In fact, they added a new problem, as the size of the shells had to be increased in order to maintain an adequate load of AC in the new mix. The inefficiency of the French attacks to produce high-toxicity AC concentrations among enemy troops is reflected in the fact that German soldiers actually liked the almond odor and did not use their protection masks during the attacks (Medema, 2006). Even then, it is estimated that about 4000 tons of AC were used in WWI (Croddy, 2002).

Contrary to the idea of the French Army, the British Army did not consider AC shells an effective weapon (Foulkes, 1934). Scientists in Great Britain also tried to increase persistence of AC by dissolving it in chloroform and thickening it with cellulose acetate. This preparation was named Jellite (Haber, 1986). A production plant was built in Stratford, but in December 1917 production was finished and Jellite would not be used in combat. The British scientists finally decided that the low persistence of AC was a difficult problem to solve and it was difficult to produce a lethal concentration of AC using the artillery shells available at that time. One of these scientists was Joseph Barcroft, who was sure that there was an important interspecies variation in AC toxicity when he compared French *in vivo* experimental data with dogs and that obtained by the British who used the goat as an experimental model. To solve this issue, Barcroft decided to do a test that consisted of himself and a dog entering a chamber where they would be exposed to an estimated AC concentration of about 500 mg/m^3 (Marrs *et al.*, 1996). This is how Barcroft describes his experience.

> In order that the experiment might be as fair as possible and that my respiration should be relatively as active as that of the dog, I remained standing, and took a few steps from time to time while I was in the chamber. In about thirty seconds the dog began to get unsteady, and in fifty-five seconds it dropped on the floor and commenced the characteristic distressing respiration which heralds death from cyanide poisoning. One minute thirty-five seconds after the commencement the animal's body was carried out, respiration having ceased and the dog being apparently dead. I then left the chamber. As regards the result upon myself, the only real effect was a momentary giddiness when I turned my head quickly. This lasted for about a year, and then vanished. For some time it was difficult to concentrate on anything for any length of time. It is hard to say to what extent this was due to the experiment. (Haldane, 1925)

Prime Minister Lloyd George and even King George V showed great admiration for Barcroft's experiment in which he risked his own life. It is important to note that although it was believed that the dog died, the dog recovered and was found alive next morning (Evans, 2000).

Another line of investigation in trying to solve the AC dispersion problems consisted of searching alternative cyanide compounds with higher density (Prentiss, 1937). For example, in September 1916 Austria introduced cyanogen bromide shells, but they were very corrosive which made storage quite difficult. One month later, France started using cyanogen chloride (called Mauguinite and later given the military codename CK). Its main problem was its tendency to polymerize, and thus a mix of arsenic trichloride, known as Vitrite, was produced.

14.1.2 The interwar period

During the interwar period, cyanides were part of a U.S. campaign developed by the Chemical Warfare Service (CWS) and the American Chemical Society to promote CW as a more "humane" killing option than conventional munitions. One of the activities in this campaign was the use of "gas chambers" for death penalty executions. On February 8, 1924, Gee Jon was the first prisoner executed using AC at the Nevada State prison (Garrett, 1999). Jon was told to hold his breath when a strange smell was detected, then to count to 10, and finally to take deep inhalations to secure a fast death without suffering. But once in the gas chamber, Jon could not follow the instructions given and it took about six minutes until he finally died. But even after this, the CWS campaign had a San Francisco newspaper describe this execution that it "was like watching a man fall asleep in a chair" (Garrett, 1999).

While in WWI the use of AC in combat was limited by the difficulties of obtaining toxic concentrations in the field with shells, the later developments in aerial

warfare were seen as a possible solution as it would be possible to use aerial bombs with a big load of AC. In fact, the United States made several trials with 1,000-lb aerial bombs loaded with 200 lb of AC that gave good results (Brophy *et al*., 1959). Vaporization of large liquid AC payloads produced a cooling that increased the density of the agent, and thus its persistence in the field.

Between 1927 and 1928, Japan built small-scale production facilities for different CWAs, including AC (Tanaka, 1988). In 1927, Okumura Island was chosen to build a large-scale production facility. To shield its secret activities, the island's location disappeared from the maps. Its inauguration took place on May 19, 1929, but it would not be operative until August. At first it produced mainly mustard gas, but in 1935 it started the production of other CWAs, including AC. Liquid AC was produced for glass grenades designed to attack bunkers and tanks, as well as for chemical mortar shells (Figure 14.2). Japan produced about 255 tons of AC (Stockholm International Peace Research Institute, 1971). Different Japanese CW units would make trials with CWAs during the war. For example, it has been described that the Ei 1644 Unit in Nanking, established in 1939, had a gas chamber for AC trials (Harris, 2002: 146). A document discovered in 2004 also shows that in 1948, two Japanese Imperial Army officers were sentenced to death for killing two prisoners of war by using AC grenades in 1944 (Guthrie *et al*., 2005).

Figure 14.2 Japanese TB-10 glass grenade (courtesy of Jeff Osborne).

14.1.3 World War II

During WWII, to recreate the use of chemical weapons in scenarios similar to the Pacific Ocean islands occupied by Japan, the United States carried out trials on the Panamanian island of San José after reaching an agreement with the Panamanian government (Brophy & Fisher, 1959; Lindsay-Poland, 2003). Personnel from Great Britain and Canada also participated in the trials. It is estimated that more than one hundred trials with mustard gas, phosgene, AC and CK took place. The trials used mainly animals, but also American troops. These trials ended in December 1947 and the island was abandoned by the U.S. Army in early 1948. Some of the chemical weapons were taken from the island, some dumped at sea, while others were abandoned and are possibly still there.

Cyanide compounds were thought to be a good option in case CW was decided to be used against Japan. Filters of military gas masks were initially not useful against cyanide compounds because they are not adsorbed by activated charcoal. Later, a treatment of activated charcoal with copper, chromium, and silver impregnants (ASC whetlerite charcoal) was developed to make military filters effective against cyanide (Brophy *et al*., 1959). Japanese gas masks did not have this treatment, and thus it was decided that an attack with cyanide agents against them would be effective. While there were some proposals by the U.S. CWS to use chemical weapons against Japan during WWII, all these were rejected, concern sometimes being expressed that a chemical attack in the Pacific could trigger the use of chemical weapons by Germany in the European WWII area of operations (Pita, 2008). In case CW against Japan was finally authorized, the trials in San José led to the decision that 500-lb and 1,000-lb aerial bombs (e.g., Figure 14.3), loaded with 165 lb and 332 lb of CK respectively, would be used (Brophy *et al*., 1959; Stockholm International Peace Research Institute, 1971), not only because the Japanese masks were not effective against it but, also because it showed good persistence in the jungle (Johnston, 2003). For the Army, 4.2 inch mortar shells were selected for CK use, although none were actually loaded with the agent. During WWII, the United States produced a total of

Figure 14.3 M79 1,000-lb aerial bomb abandoned at San José island, Panama (courtesy of Jeff Osborne).

33,347 M78-500-lb bombs and 55,851 M79-1,000-lb bombs (Brophy *et al.*, 1959; Stockholm International Peace Research Institute, 1971).

During WWII, the German intelligence services discovered that the Soviet Union had developed aircraft capable of effectively dispersing AC. They tried to replicate them, but unsuccessfully. The problem observed by the Germans was that, once dispersed by the aircraft, the AC liquid droplets would evaporate before reaching the ground (Croddy, 2002; Medema, 2005). In 1942, the Germans seized a Soviet aircraft and saw that these were in fact effective in AC dispersion. The trick was to disperse large droplets of AC. Cooling and freezing of the droplets due to initial volatilization produced a solid/liquid mix known as AC "snow." This snow could reach the ground and then volatilization in the target would start. But, again, not only were high toxic concentrations difficult to obtain, but also the need to fly at low attitudes was seen as another drawback by the Germans, as this made aircraft vulnerable to enemy anti-aircraft artillery. It is estimated that during WWII the Soviet Union produced about 11,100 tons of AC (Perera, 1997).

From 1939, the Nazis started using carbon monoxide as part of their "Final Solution" plan to kill Jewish prisoners. In July 1941, the Auschwitz concentration camp received the order to implement the Final Solution. But the SS officer in charge of the camp, Rudolf Hoess, decided to employ Zyklon B instead (Goldensohn, 2004; Hoess, 1959). Zyklon B was an AC-based insecticide used in the camp to prevent typhus transmission through lice. The first trials took place in August, followed by the killing of 600 Russian war prisoners on September 3. However, some authors mention that the Nazis may have already used Zyklon B in January 1940 on 250 gypsy children at the Buchenwald concentration camp (McCamley, 2006). Hoess was sentenced to death by a Polish tribunal and hanged in April 1947.

Zyklon B consisted of pellets of diatomaceous earth impregnated with a stabilized solution of AC and was licensed by the Degesch firm. To prevent poisoning of users, a warning odorant additive was included in the formula, although special lots without the additive were produced to be used in the camps. Based on the Nuremberg War Crimes Tribunal documents, between May 1940 and December 1943 more than two million people were gassed at Auschwitz (Stockholm International Peace Research Institute, 1971). It is estimated that Degesch supplied around 19,653 kg of Zyklon B between 1942 and 1943 to Auschwitz, which means that the number of victims could have been higher.

14.1.4 The Cold War

At the end of WWII the Allies discovered nerve CWAs. This group consists of very toxic compounds, easy to disperse in available munitions, and with a high versatility of physico-chemical properties that allowed the selection of non-persistent agents (e.g., sarin and tabun) or persistent agents (e.g., VX) based on the tactical need. After the discovery of nerve agents, cyanides became obsolete as chemical weapons. However, after the end of WWII there was some cyanide-related news in military conflicts.

During the Cold War and even nowadays it is not unusual to see accusations of CW in military conflicts. Most of the time these accusations are just propaganda

to discredit the enemy in front of the international community. For example, in the 1980s there were accusations of cyanide agents being used in Angola (Pita, 2008). Some authors elaborate that poisonings among UNITA soldiers could have been caused by cooking food with non-alimentary industrial oil that contained tri-*o*-cresyl phosphate (Medema, 2006). Between 1986 and 1987, during the Iraq–Iran war, UN missions received information about alleged cyanide attacks (Pita, 2008). Cyanide was also present in analyzed samples taken by UN inspectors. However, the origin of cyanide could be tabun, a nerve CWA, whose use was confirmed in the Iraq–Iran war and has a cyano group in its molecule. Iranian medical personnel who treated chemicals casualties in the war nowadays also doubt that cyanide was used, and believe that alleged cyanide poisoning cases were in fact tabun or sarin intoxications. In 1995, during the conflict between Ecuador and Peru, high cyanide levels in the blood of indigenous people led to the suspicion that CWAs were being used (Pita, 2008). Finally, it was realized that this was caused by the frequent ingestion of cassava, rich in the cyanogenic glycosides linamarin and lotaustralin.

Some cyanides are covered by the Chemical Weapons Convention (CWC) (www.opcw.org). The CWC entered into force on April 29, 1997 and prohibits the development, production, acquisition, stockpiling, retention, transfer, or use of chemical weapons by states parties. AC and CK are explicitly included in the CWC Schedule 3 of chemicals for the application of verification measures. Basically, Schedule 3 includes chemicals that have been produced, stockpiled, or used as weapons, but may be produced in large commercial quantities for industrial use. For Schedule 3 chemicals, production plants in CWC state parties have a declaration threshold of 30 tons per year and a verification inspection threshold of 200 tons per year. Verification inspections are carried out by inspectors of the Organization for the Prohibition of Chemical Weapons (OPCW), responsible to implement the provisions of the CWC.

14.2 Cyanide and chemical terrorism

14.2.1 Extortion activities

In 1984, the "Man of 21 Faces," a Japanese extortion organization, contaminated candies with cyanide, which made a manufacturing company reduce its production to half (Simon, 1989). At the end of 1982, there were seven fatal cases of cyanide poisoning after the ingestion of acetaminophene-tampered capsules (Hall *et al*., 1987). The McNeil Consumer Products company had to destroy about 22 million units and changed the production from capsules to tablets that were more difficult to tamper with (Dunea, 1983; Wolnik *et al*., 1984). The ones responsible were never identified, and some imitators in the United States started contaminating medicines and food products with toxic chemical substances, which marred the traditional Halloween trick-or-treat celebration. In 1986 a similar incident occurred when two persons died of cyanide poisoning after the ingestion of tampered analgesic capsules (Varnell, 1987). Again, many pharmaceutical companies started producing pills or tablets instead of capsules to avoid tampering. But even then, in 1991, another two persons would die of cyanide poisoning in Washington, DC after the ingestion of capsules for symptomatic treatment of nasal congestion (*MMWR*, 1991).

In some cases, blackmailers have even attacked governments. For example, on March 2, 1989 an anonymous person called the U.S. and Japanese embassies in Santiago, Chile, alerting that cyanide had been injected in fruit destined to be exported to those countries (Grigg & Modeland, 1989; Spiers, 2000; Wilkening, 1999). Although initial measures were not taken, after a second call to the U.S. embassy on March 17, the U.S. Food and Drug Administration (FDA) decided to start inspections at the Philadelphia port, entrance of about 80% of the fruit from Chile. After an extensive search, only two grapes that had been injected with a small quantity of sodium cyanide were found. However, as a preventive measure, on March 13 all fruit coming from Chile was placed on quarantine. Subsequent searches did not find more tampered fruit and on March 17 the quarantine ended. Although the event ended without any poisoning cases, it meant losses of about $300 million for Chile's fruit export market.

14.2.2 State terrorism

Cyanide has also been employed in the assassination of selective targets by secret services. For example, the defection of KGB's Capt. Nikolai Khokhlov in February 1954 brought to light some of the devices created for this type of attack. In fact, Khokhlov defected in the middle of a mission to assassinate Georgi Okolovich,

a Soviet dissident living in West Germany, by using a cigarette pack that concealed an AC ampoule (Andrew & Gordievsky, 1990; Melton, 2002). Similar cases include the killing of two Ukrainian exiles in Munich in 1957 and 1959 by a KGB agent who used a special gun device to disperse AC. Based on a 2001 book by Vadim Birstein, from the 1920s and at least until the late 1970s, different laboratories in the Soviet Union were in charge of the development of assassination devices for their secret services (Birstein, 2001). Some of these devices can be seen in H. Keith Melton's book *Ultimate Spy*, which include KGB devices for the dispersion of AC like canes, pens, or wallets (Melton, 2002). The killers were even provided with a small box that contained a sodium thiosulfate pill and an amyl nitrite ampoule in case of self-poisoning during the attacks.

But the former Warsaw Pact secret services were not the only ones to carry out such programs, as seen during the appearances of CIA officials in the Senate Committee, known as the Church Committee that took place during September 16–18, 1975. Former CIA director William Colby explained that cyanide pills, known as L-pills, were employed in WWII to give the opportunity to the agent to kill himself and avoid torture by the enemy. Although saxitoxin (a toxin produced by marine dinoflagellate algae) had been substituted for cyanide pills, the CIA stock of chemical weapons at that time included 18 L-pills (Select Committee to Study Governmental Operations with Respect to Intelligence Activities of the United States Senate, 1976).

14.2.3 Nationalist and separatist terrorist groups

Secular terrorist groups have not showed special interest in CWAs, perhaps thinking that its use may be rejected by their own followers (although many of them have carried out terrorist attacks with conventional explosives that produced a high number of casualties). There are some reported cases where nationalist and independent groups have used chemical substances in sabotage actions. For example, in 1992 the Kurdistan Workers' Party (PKK) allegedly contaminated several water tanks with sodium cyanide in an air base near Istanbul. The attack was aborted when the recipients of 25 kg of potassium cyanide were found next to the tanks (Karasik, 2002).

Another example can be found in the Liberation Tigers of Tamil Eelam (LTTE) in Sri Lanka. Although the LTTE was a secular terrorist group, their members showed a level of veneration for their leader, Velupillai Prabhakaran, akin to that of a religious cult, and even employed tactics used by jihadist terrorist groups. LTTE members were known to carry sodium cyanide capsules that had to be used in case of capture. There are also some reports about the use of sodium cyanide against Sri Lanka's economic interests (Carus, 2002; Wilkening, 1999). The first report is from 1986 and mentions attempts to sabotage tea export by informing different embassies that some lots were contaminated with cyanide. U.S. authorities analyzed different lots, but did not find anything amiss. The second report is from December 1996, when potassium cyanide was allegedly applied to stamps used by the Sri Lanka Army.

14.2.4 Left-wing terrorist groups

Colombian guerrillas (initially left-wing terrorist groups but nowadays considered narcoterrorism groups due to their association with organizations that deal with illicit traffic of drugs of abuse) have carried out small attacks with CWAs. On December 2, 2001, members of the Revolutionary Armed Forces of Colombia (FARC) attacked a police station in San Adolfo (Huila) probably with CK, killing four policemen. Since then, munitions charged with cyanide have been found in raids against FARC camps. In fact, in November 2007, Colombia's National Police showed its concern after finding a clandestine FARC laboratory near the frontier with Ecuador that was working on the filling of rockets with toxic chemicals (Pita, 2008). The chemicals included ammonia, chlorine, and cyanide compounds.

14.2.5 Right-wing terrorist groups and "lone wolves"

It is not uncommon to find criminal activities using cyanide linked with right-wing groups or sympathizer "lone wolves." Actually, AC production is commonly found in "cookbooks," publications that are popular among members of white supremacist groups and in "amateur terrorist" circles. Some of these titles include *Assorted Nasties*, *The Preparatory Manual of Chemical Warfare Agents*, *The Poisoner's Handbook*, and *Silent Death*. Christian-identity right-wing groups and lone wolves have also carried out terrorist attacks that have caused a large number of casualties, like the April 19, 1995 bombing of the Alfred P. Murrah Federal Building in

Oklahoma that caused 168 fatalities and more than 500 nonlethal casualties.

In the mid-1980s, a group named The Covenant, the Sword, and the Arm of the Lord obtained potassium cyanide to contaminate water supplies of different U.S. cities (Stern, 2000). In a raid that took place on one of their facilities, authorities found a drum with 30 gallons of potassium cyanide. When one of its members was told that with the attack not only those he considered enemies (Jews and "mud-people") would be killed but also other people, including members or sympathizers of the group, his reply was: "We felt that God would take care of this [and] that those who were meant to die would be poisoned" (Stern, 2000). And when he was told that 30 gallons were not enough to obtain a toxic concentration in the city's water reservoir he answered: "God would … make sure the poison got to the town" (Stern, 2000). He even explained that they had decided to act because there were signs of the arrival of the Armageddon: "You get tired of waiting for what you think God is planning" (Stern, 2000). These statements make clear that terrorist groups with religious motivations most of the time have no moral restraints in carrying out attacks that might cause a large number of casualties.

Other examples include the plans made in the 1960s by the "Minutemen" to disperse AC through the ventilation system of the UN building in New York (Jones, 1968). In 1987, members of the Confederate Hammer Skins were arrested when planning to disperse AC through the ventilation system of a Dallas synagogue (Spiers, 2000; Wilkening, 1999). More recently, in 2004, an arms trader with white supremacist group connections was sentenced to 11 years in prison (Kosal, 2006). In April 2003, authorities raided his Texas warehouse arsenal and found that he was building an improvised chemical device (ICD) based on the mixing of sodium cyanide and an acid.

Cases of lone wolves with intentions of using cyanide are also not strange. For example, in the mid-1970s authorities found 25 pounds of sodium cyanide at the apartment of Muharem Kurbegovic, the "Alphabet Bomber" (Simon, 2000). Joseph Konopka, arrested in March 2002, had 0.45 kg of cyanide salts that he planned to use to contaminate water tanks in Chicago (*CNN*, 2002). A year later he was the first person to be sentenced for the violation of the U.S. Chemical Weapons Statute.

14.2.6 Apocalyptic cults

In 1978, more than 900 followers of Jim Jones' People's Temple died in Jonestown (Guyana) after the ingestion of a beverage that contained a cyanide compound (Thompson *et al*., 1987). After the Tokyo sarin terrorist attacks in 1995, Aum Shinrikyo cult members also tried an attack with AC. On May 5 they used an ICD in Shinjuku's Tokyo subway station which consisted of two plastic bags, one with sodium cyanide suspended in 2 liters of water and another one with 1.5 liters of a sulfuric acid solution (Dolnik & Gunaratna, 2008; Tu, 2002). The activation system consisted of two condoms, one with sodium chlorate and another with sulfuric acid, so when the latter ate through the latex, the fire produced would break the plastic bags and allow the mixing of the cyanide salt and the acid, producing AC. However, the quantities were not well calculated and the fire destroyed the ICD. On July 4 and 5 another two attacks took place in Tokyo, this time using an ICD where the activation of an electrical device with blades would break the plastic bags. One ICD failed, while the other only produced one case of mild poisoning.

14.2.7 Jihadi terrorism: al-Qaeda

After the September 11, 2001 (9/11) terrorist attacks in the United States, there is a general concern of the possibility that al-Qaeda or its affiliated groups may use WMD in terrorist attacks. As a terrorist group with religious motivations, al-Qaeda does not fit the assumption made by terrorism analyst Brian Jenkins in 1975 that "terrorists want a lot of people watching and a lot of people listening, and not a lot of people dead" (Jenkins, 1975). This statement fits better with secular terrorist groups. But for religious terrorist groups like al-Qaeda, "divine duty" results in disappearance of moral restraints that would justify "a lot of people dead" in their terrorist attacks, such as the 9/11 ones.

Al-Qaeda's WMD intentions

There are three phases in the statements of al-Qaeda members related to WMD. In the first phase, al-Qaeda tended to justify the acquisition and possession of these weapons from the point of view of deterrence. This phase goes as far back as 1998, when Osama bin Laden had stated that acquiring WMD was a "religious duty" (Pita, 2007). This and similar statements were made by bin Laden in different interviews after the U.S. attack on the Al Shifa Pharmaceutical Industries

factory in Khartoum on August 20, 1998. This attack was part of Operation Infinite Reach in retaliation to the bombings of the U.S. embassies in Kenya and Tanzania on August 7, 1998, for which the bin Laden terrorist network was blamed by U.S. officials. The Al Shifa target was justified in the finding of *O*-ethyl methylphosphonothionate (EMPTA), a precursor of the nerve agent VX, in soil samples outside the factory, and in the financial contributions of bin Laden to the production of chemical weapons. Soon after 9/11 and the mailings of envelopes with *Bacillus anthracis* spores in the United States, bin Laden was interviewed again, and when asked about reports claiming that he was trying to acquire WMD, he answered: "We have the weapons as deterrent." (Pita, 2007).

The second phase of statements of al-Qaeda members related to WMD began soon after the overthrow of the Taliban regime in Afghanistan. Al-Qaeda's reasoning was that the Coalition Forces had used conventional weapons (e.g. missiles) that had caused a large number of casualties and destruction, and for this reason these weapons could be considered WMD. This interpretation justified the use of chemical, biological, radiological, and nuclear (CBRN) weapons as retaliation for similar attacks. The most well-known statement in this second phase was made by Suleiman Abu Gheith, al-Qaeda's spokesman, who wrote in his 2002 electronic article "In the Shadow of the Lances," that, based on this reasoning, they had the "right to kill four million Americans – two million of them children – and to exile twice as many and wound and cripple hundreds of thousands" (Pita, 2007).

The third phase started in May 2003 when Shaykh Naser bin Hamad Al Fahd, a Saudi cleric who supports the global jihad movement, issued a fatwa justifying and authorizing the use of WMD (Paz, 2005). Al Fahd used arguments based on reciprocity, stating that the United States had used weapons that caused a large number of casualties and mass destruction. But what was new in this fatwa was that Al Fahd's arguments were also based on Islamic texts that supposedly justify that it is permissible to use WMD if those engaged in jihad decide there is benefit in using them. And this is the case of al-Qaeda's influential strategist Mustafa Setmarian Nasar, better known as Abu Musab Al Suri, who posted a letter on the Internet in December 2004 stating that the use of WMD was "a necessity."

Al-Qaeda's interest in WMD may be also based on their important psychological effects. Indeed, one of the objectives of using these weapons in a military scenario is not only to cause physical casualties, but also to demoralize troops. Similarly, in a terrorist attack on civilians, one of the primary goals is to create a general sense of panic and fear, resulting in psychological trauma and disruption of economic and social activities (Zanders, 2003). For these reasons WMD can also be regarded as "weapons of mass *disruption*." For instance, it is frequently asserted that the Aum Shinrikyo sarin subway attack caused more than 5000 casualties, but actually only about 1000 patients had clinical signs of sarin exposure (Woodall, 1997). That means that about 4000 people who sought attention in medical facilities were, in fact, mainly psychological casualties with psychogenic symptoms. Based on a book by Abu Walid Al Misri, editor of a magazine for the Taliban, quoted in Peter Bergen's book *The Osama bin Laden I Know* (2006), al-Qaeda has been aware of the psychological effects of WMD since they first thought about acquiring them.

Al-Qaeda's chemical weapons capabilities

Since October 2001, reporters and military forces in Afghanistan have found written and electronic documents with rudimentary procedures for the production and use of toxic chemicals (Pita, 2007). These procedures are similar and, in some cases, word-for-word translations from the ones included in the cookbook publications mentioned earlier. Actually, two well-known cookbooks were found in Afghanistan, *Assorted Nasties* and *The Poisoner's Handbook*. This material came mostly from the Abu Khabab camp located in the Darunta training camp complex, which specialized in explosives and toxic chemicals training (Gunaratna & Acharya, 2006). This camp was named after the man who ran it, the Egyptian Midhat Mursi, commonly known as Abu Khabab, who was killed in a U.S. air strike on the Pakistan–Afghan border in July 2008.

Ahmed Ressam, an Algerian arrested by U.S. authorities for carrying explosives that he intended to use in a bombing against the Los Angeles International Airport (LAX), explained in court in July 2001 that he had been trained in the Darunta training camp complex in 1998 on how to prepare AC by mixing a cyanide salt and sulfuric acid (Pita, 2007). He was told to release it near the air intake vents of buildings and even participated in

live training exercises using dogs. In August 2002, Cable News Network (CNN) aired several al-Qaeda videotapes obtained in Afghanistan that revealed experiments with chemical agents on dogs. One of those tapes showed several men – apparently after having mixed several chemical reagents – rushing out of an enclosure inside which a dog was tied up. Soon, a white vapor appeared, and a few moments later the dog started showing the first clinical signs of exposure. The quality of the images was not good enough to identify a toxidrome, but the videotape was reminiscent of what Ahmed Ressam said about his training with AC in Afghan camps. These experiments were allegedly recorded by Abu Khabab in the Darunta training camp complex. In November 2006, a book published by Omar Nasiri, pseudonym of an alleged informer of the British and French intelligence services, explained his participation in experiments with animals using cyanide at the Khalden training camp (Nasiri, 2006).

One of the most relevant discoveries in Afghanistan regarding the chemical and biological weapons program was made by *Wall Street Journal* reporter Alan Cullison, who obtained two computers from a looter who allegedly stole them from al-Qaeda's central office in Kabul on November 12, 2001 (Cullison, 2004; Cullison & Higgins, 2001). The looter told Cullison he had found the computers in the office of al-Qaeda's military commander Muhammad Atef (aka Abu Hafs), a strong supporter of al-Qaeda's acquisition of WMD, who was killed in a U.S. air strike that same month. Computer files included information of al-Qaeda's effort to start a chemical and biological weapons program code-named "Al Zabadi" ("Yogurt") in May 1999 with an initial budget of only $2000–4000. Based on Cullison's analysis of the computer files, Ayman Al Zawahiri – al-Qaeda's second-in-command at that time – and Abu Hafs (assisted by Abu Khabab) started the program after studying different books and articles from biomedical journals.

According to former CIA Director George Tenet, al-Qaeda became interested in chemical and biological weapons after Aum Shinrikyo's 1995 sarin attack on the Tokyo subway (Tenet with Harlow, 2007). But an electronic message sent by Al Zawahiri to Hafs in 1999 stated that it was "the enemy" who brought these weapons to his attention, possibly U.S. Secretary of Defense William Cohen (Leitenberg, 2004, 2005). In November 1997, Cohen appeared on television showing a 5-lb sugar package and saying that if it were to contain spores of *B. anthracis* and were spread over Washington, DC, half the city's population would die. A photograph of Cohen holding the 5-lb sugar package was allegedly also found in Afghanistan (Leitenberg, 2004).

No public report of sophisticated CBRN biological means or production facilities found in Afghanistan has yet been made. Only a centrifuge and an "oven" found near Kandahar have been presented by the U.S. Department of Defense as the equipment al-Qaeda intended to use to produce CBRN weapons (Miller, 2002). This material was part of a laboratory that was being built for the production of *B. anthracis* spores (Pita & Gunaratna, 2009).

Based on intelligence assembled from collected documents, detainee interviews, and reconnaissance of al-Qaeda facilities during Operation Enduring Freedom, the Commission on the Intelligence Capabilities of the United States Regarding Weapons of Mass Destruction (U.S. WMD Commission, 2005) concluded in its unclassified report that al-Qaeda did not have a large-scale chemical and biological weapons capability. Still, past and current chemical and biological programs are said to be not fully understood, especially because of difficulties in penetrating the terrorist network and, therefore, in collecting human intelligence.

After the disappearance of the Afghan training camps, Internet and jihadi websites have acquired more relevance. Al Suri's book, *The Global Islamic Resistance Call*, posted on the Internet in 2005, suggests an asymmetric approach that includes the use of WMD, as well as a decentralized and diffused global jihad in which autonomous cells play an important role. Autonomous cells should be self-sufficient, including having training capabilities. For these reasons, jihadi websites are important tools providing autonomous cells with training manuals as well as lessons learned from attacks by other cells. These electronic training manuals include information and procedures about toxic chemicals similar to those found in Afghanistan, that is, similar to the information included in cookbooks (Figure 14.4). Some of these websites offer scanned copies of these cookbooks. Salama and Hansell (2005) and Stenersen and Lia (2007) have published detailed studies of electronic jihadi chemical and biological manuals. AC is the most popular toxic chemical whose production is included in these electronic manuals (Stenersen & Lia, 2007).

Figure 14.4 Jihadi manuals like this are available on the Internet containing procedures for production of hydrogen cyanide.

Transporting and mixing the reagents without being discovered constitutes one of the biggest hurdles in using AC in terrorist attacks. ICDs for cyanogen agents that try to solve this problem have also been developed by groups related to al-Qaeda, information about which is available on the Internet. This is the case of the ICD named al-Mubtakkar (Figure 14.5). Basically, the al-Mubtakkar device is supposed to work as a crude binary munition. (A binary chemical munition is one in which chemical substances held in separate containers react when mixed or combined as a result of being fired, launched, or otherwise initiated to produce a CWA.) It produces AC when a barrier that separates the cyanide salt (potassium cyanide) and the acid (hydrochloric acid) is broken. Potassium permanganate is also included for the device to potentially produce a mix of AC, CK, and chlorine. The device can be activated manually or by using the explosive triacetone triperoxide (commonly known as TATP) and a detonator. The detonator can be activated remotely or with a temporizer, allowing terrorists to escape, but the probability is high that, if not well regulated, the explosion will inactivate the chemical reagents and obviate production of the cyanogen agent.

Other examples of ICDs to deliver AC can be found on a Jemaah Islamiyah (an al-Qaeda–affiliated terrorist organization in Southeast Asia) manual obtained in 2003 (Dolnik & Gunaratna, 2008). The manual mentions crude devices for the production of AC by mixing a cyanide salt (potassium or sodium) and sulfuric acid. The ICDs consisted of a tennis ball injected with the acid which, when eaten by the acid, contact with the salt would take place; or the simple use of a glass separating plate that when mechanically broken would allow the contact.

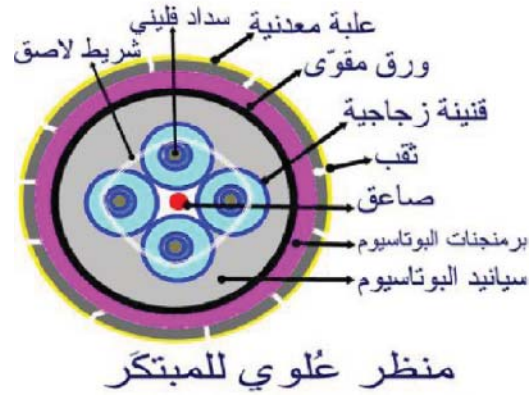

Figure 14.5 Instructions for the manufacture of the Al Mubtakkar device.

Al-Qaeda plots with cyanide

A detailed study of incidents with CWAs linked to al-Qaeda shows that cyanide is among the main choices of jihadi terrorists (Pita, 2007). Curiously, the first case of an al-Qaeda terrorist attack linked to chemical terrorism is the February 1993 World Trade Center bombing as it is commonly believed that the explosives were mixed with a cyanide compound. The reason is that during the trial the judge stated that:

> You had sodium cyanide around, and I'm sure it was in the bomb. Thank God the sodium cyanide burned instead of vaporizing. If the sodium cyanide had vaporized, it is clear what would have happened is the cyanide gas would have been sucked into the north tower and everybody in the north tower would have been killed. That to my mind is exactly what was intended. (Parachini, 2000)

This was based on the idea that the terrorists considered the tactic of using a toxic chemical in the attack and on the fact that the FBI found a small bottle of a sodium cyanide solution in a storage shed. However, AC was never detected in the attack and even a detailed case study of this event concludes that no chemical toxic was mixed with the explosives (Parachini, 2000).

On February 19, 2002, four Moroccans, members of the Salafi Group for Preaching and Combat (GSPC) – now al-Qaeda in the Islamic Maghreb – were arrested in Rome (Pita, 2007). They had about 4 kg of potassium ferrocyanide that they intended to use to contaminate the water supplies near the U.S. embassy. Nevertheless, that particular substance is widely used as a food additive (E 536) and, because of its toxicological properties, was probably not the best choice to use in a chemical attack.

Based on information in Ron Suskind's *The One Percent Doctrine*, an al-Qaeda cell based in Saudi Arabia planned an attack on the New York City subway with al-Mubtakkar devices (Suskind, 2006). The cell members had traveled to New York City from North Africa in the fall of 2002, and had even carried out reconnaissance missions to identify targets. Surprisingly, when al-Qaeda's leader in Saudi Arabia, Sheikh Yousef al-Ayiri, told al-Zawahiri about the plot in January 2003, al-Zawahiri decided to cancel the operation. Suskind's book claims that this decision was made just 45 days before the intended attack. Some analysts hypothesize that al-Zawahiri realized it could not match the results of the 9/11 attacks, as al-Qaeda's intention was that each attack be more destructive than the previous one (Scheuer, 2006). Also, and although not confirmed by official sources, an al-Mubtakkar device could have already been used in Afghanistan, but did not work (Smith, 2006).

Jordanian authorities announced in April 2004 that they had broken up an al-Qaeda plot to employ explosives and large quantities of toxic industrial chemicals (TICs) such as sulfuric acid, cyanide salts, and pesticides against the U.S. embassy in Amman, the Jordanian prime minister's office, and the headquarters of the Jordanian General Intelligence Department (Levitt & Sawyer, 2004).

14.3 Conclusions

With the development of nerve agents in WWII, cyanides became obsolete CWAs. For this reason, a state actor that decides to start a chemical weapons program would probably not choose cyanides. On the other hand, the difficulty of producing nerve agents and the ready access to cyanide products in the chemical industry makes cyanides more attractive to non-state actors. The threat of insiders who work with toxic chemicals and who may be accessed by terrorist groups is one of the main concerns of intelligence services nowadays. In fact, we have described several cases in which terrorist groups were interested in and even acquired cyanides to use in chemical attacks. For this reason, medical personnel should consider cyanide poisoning in differential diagnosis in case of suspected cases of chemical terrorist attacks.

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CHAPTER 15

Cyanide-induced neural dysfunction and neurodegeneration

Gary E. Isom and Joseph L. Borowitz

At a Glance

- The primary target organ of cyanide toxicity is the central nervous system.
- Cyanide distributes within seconds from the blood compartment to the brain to reduce oxidative metabolism by inhibiting cytochrome oxidase.
- In the central nervous system cyanide produces a series of complex cellular and neurochemical effects that lead to brain dysfunction and eventually neuronal death.
- In the brain a number of toxic effects are independent of cytochrome oxidase inhibition, including dysfunction of neuronal calcium regulation, oxidative stress, and neurochemical secretion, followed by activation of specific neurochemical pathways.
- The neurochemical dysfunction contributes to brain injury and neuronal degeneration.
- A number of neurological disorders have been associated with both acute and chronic cyanide toxicity.

15.1 Introduction

The central nervous system is a primary target organ in cyanide toxicity, in which a variety of responses have been observed, ranging from suppression of centrally regulated vital functions to marked degeneration of select brain areas (Way, 1984). The action of cyanide on the nervous system is complex and results from interaction with a number of different enzymes and cellular regulatory processes (Agency for Toxic Substances and Disease Registry, 2006). In acute intoxication, systemic cyanide distributes rapidly to the CNS to inhibit oxidative phosphorylation and disrupts aerobic metabolism within seconds, followed by immediate neurological dysfunction. The extreme sensitivity to cyanide is assumed to be due to the brain's low anaerobic capacity and limited energy reserve critical for maintenance of neurological function. On the other hand, in low level chronic exposure and following severe acute intoxication, cyanide may produce other actions in the CNS which can lead to degeneration of select brain areas (Kato *et al.*, 1985).

The manifestations of toxicity resulting from nervous system dysfunction vary, depending on the duration of exposure (acute vs. chronic), dosage (exposure concentration), and predisposing health conditions. The nervous system toxicity can be broadly classified as acute (immediate dysfunction), delayed post-intoxication sequelae, and chronic exposure. Even though the underlying mechanism of toxicity is inhibition of cytochrome oxidase, it appears that the different modes of toxicity are associated with damage to different brain areas and in turn the manifestations vary. It is becoming increasingly apparent that cyanide's action on the nervous system is complex and toxicity cannot be simply attributed to inhibition of cytochrome oxidase, but multiple toxic mechanisms occur concurrently and continue after removal of cyanide from the systemic system (Borowitz *et al.*, 1992).

Toxicology of Cyanides and Cyanogens: Experimental, Applied and Clinical Aspects, First Edition.
Edited by Alan H. Hall, Gary E. Isom and Gary A. Rockwood.

15.2 Cyanide exposure and manifestations of toxicity

In acute exposure to cyanide, the cardiovascular and central nervous systems are the two primary target organs directly influenced by cyanide (Borowitz *et al*., 1992). In many instances, death is delayed, but after a high dose of cyanide, death may occur within a few minutes. The rapid cessation of aerobic metabolism in the nervous system leads to a broad range of clinical manifestations (Nelson, 2005). In acute intoxication, the early signs and symptoms are attributed to both cardiovascular and central nervous system responses, with the loss of central regulation of vital functions a primary component. In these cases, it is common for the individual to initially display increases in blood pressure and heart rate, hyperventilation, shortness of breath, and headache. As the aerobic metabolism deficit continues, depression of cardiovascular, respiratory, and CNS function progresses, leading to loss of consciousness, seizures, and hemodynamic shock and cardiorespiratory arrest. The time required to progress to severe symptoms is dependent on dose (concentration) and duration of exposure. If death occurs within the first 60 minutes in an acute intoxication, it is usually associated with respiratory arrest as a result of suppression of neural activity.

In many fatal, acute intoxications a deep coma may develop rapidly and the patient may remain comatose for days with progressive deterioration of central function, eventually leading to death (Holstege *et al*., 2011). In some individuals surviving a severe, acute exposure to cyanide, there are clinical reports showing development of delayed, progressive degeneration of the basal ganglia (Rosenberg *et al*., 1989). The post-toxicity sequelae can be characterized by memory deficits and a Parkinson-like condition accompanied by marked dysfunction of central dopaminergic transmission.

Chronic exposure is associated with a number of neurological conditions (Kato *et al,* 1985; Wilson, 1987) and disorders of cyanide metabolism predispose to severe neuropathological responses (see chapter on cyanide metabolism). In chronic occupational exposure, workers reported a variety of symptoms, including fatigue, dizziness, headaches, tinnitus, paresthesias of extremities, hemiparesis, hemianopia, and syncope (Sandberg, 1967; El Ghawabi *et al*., 1975; Chandra *et al,* 1980; Blanc *et al*., 1985). In some individuals, neurological symptoms persisted up to 10 months after being removed from the contaminated environment. Cognitive and psychomotor analysis of workers exposed chronically showed that over 30% of the workers had loss of delayed and immediate memory, and a decreased psychomotor ability and visual learning (D'Mello, 1987; Chandra *et al,* 1980; Borgohain *et al,* 1995). Cognitive impairment can range from moderate to severe degree (Di Filippo *et al*., 2008).

15.3 Cyanide-induced histotoxic hypoxia and metabolic dysfunction

15.3.1 Cyanide distribution to brain

Following systemic absorption from the site of exposure (gastrointestinal tract, lungs, skin, etc.) cyanide undergoes rapid distribution to the tissues. Tissue distribution of free cyanide from the blood is non-energy dependent and driven by the concentration gradient from the blood to the tissue deposition site. Cyanide is a small lipid soluble molecule with a $pk_a \sim 9.2$ in which over 98% of cyanide in solution at pH 7.4 is in the form of HCN. HCN is miscible with ethanol and slightly soluble in ether with a lipid partition coefficient ~0.15 (Oldendorf, 1974). Based on the chemistry, it would be expected that HCN rapidly distributes across biological membranes by passive diffusion (Borowitz *et al*., 1994). Additionally, cyanide is a reactive chemical that complexes with metalloenzymes and forms Schiff-base intermediates with carbonyl, aldehydes, and keto functional groups of proteins, thus producing a CN-sink that facilitates distribution across the blood brain barrier and other biological membranes (Devlin *et al*., 1989). Cyanide is detectable in the brain within seconds of systemic dosing and brain levels continue to increase over the first several minutes of exposure. Interestingly, mouse whole-body autoradiographic studies show the brain had the lowest levels of all tissues examined (Clemedson *et al*., 1960). Post-mortem analysis of sheep killed by intramuscular injection of cyanide showed that brain levels were lower than blood (Ballantyne, 1975).

Following distribution across the blood brain barrier, cyanide is taken up into neural tissue by passive, non-energy dependent movement (Borowitz *et al*., 1994). The distribution of C^{14} – cyanide in mouse brain areas is uneven and found in greater amounts in

hypothalamus that in hippocampus, cortex, or cerebellum. This observation is unexpected since studies of hypothalamic function after cyanide treatment do not reveal significant neuropathologies in this brain area. It is possible that uneven brain distribution is attributed to different levels of cyanide complexation with metalloenzymes and adduct formation.

Movement of cyanide into neural cells may involve mechanisms other than passive diffusion. In neural tissue and isolated neural cells, a substantial portion of total cyanide moves into cells via a chloride channel (Borowitz *et al*., 1989). Modulation of chloride channels by a selective channel blocker reduced intracellular accumulation of cyanide, whereas activation of the channel by GABA enhanced cyanide movement into the cells. It is possible that the cyanide anion selectively moves through the channels since the anion has a number of physical characteristic in common with Cl^- (ion hydration size, ionic charge). The subcellular distribution of cyanide plays a role in the toxicity since the toxic site of action is in the mitochondrial matrix. Subcellular fraction studies of neural cells incubated with C^{14}-cyanide showed that the mitochondrial fraction contained the highest level of cyanide, as would be expected with rapid and high affinity binding to cytochrome oxidase.

In acute, lethal intoxication, the brain levels of cyanide are high irrespective of the exposure route, which is consistent with the brain being a primary functional target organ (Ballantyne, 1987). At autopsy, brain cyanide levels are generally high immediately after death, but rapidly decrease to undetectable levels within 7 days (Troup & Ballantyne, 1987). Djerad *et al*. (2001) showed that respiratory acidosis and alkalosis influence distribution of cyanide. This is an important consideration, since toxic doses of cyanide produce both intracellular acidosis and acidemia. Cyanide is a weak acid in which the ratio of non-ionized (lipid soluble species) to ionized forms can significantly change, depending on the local pH. Respiratory acidosis increased the rapidity of distribution of intravenously administered cyanide into 7 of 10 brain areas examined.

15.3.2 Inhibition of brain cytochrome oxidase and aerobic metabolism

The extreme sensitivity of the CNS to cyanide has traditionally been attributed to inhibition of cytochrome oxidase producing histotoxic hypoxia (Way, 1984). Cyanide binds to and inhibits mitochondrial cytochrome oxidase aa_3, the terminal oxidase in the electron-transport chain which utilizes molecular oxygen to generate ATP. To maintain normal neurological function, the brain has a high aerobic energy demand, but a low anaerobic capacity and limited energy reserve. Thus cyanide produces an immediate reduction of brain ATP generation, followed within seconds by a marked energy deficit (Maduh *et al*., 1991). The result is loss of neural function, leading to the impaired central regulation of vital functions and manifestations of acute intoxication.

Animal studies have demonstrated that at toxic doses, cyanide produces a rapid reduction in brain oxidative metabolism. Decorps *et al*. (1984) implanted surface coils on the skull to measure the P^{31}-nuclear magnetic resonance (NMR) spectra in un-anesthetized rats. Intraperitoneal injection of KCN produced a dose-dependent decrease of brain phosphocreatine, inorganic orthophosphates, and pH. Low doses (3–5 mg/kg) had minimal effects on brain ATP levels, but at doses higher than 6 mg/kg, ATP levels dropped significantly. Lee *et al.* (1988) demonstrated cyanide blockade of brain cytochrome oxidase using reflectance spectrophotometry in rats in which the blood was replaced by an oxygen-carrying isotonic, iso-oncotic fluorocarbon emulsion. Intravenous infusion of cyanide produced a rapid, progressive reduction of cerebrocortical cytochrome oxidase activity, accompanied up to 200% increase in cerebral blood flow. However, cerebral O_2 metabolism rate was not significantly altered from normal levels until a fall in oxygen delivery occurred due to reduced cerebral blood flow. Both inhibition of cytochrome oxidase and reduced availability of O_2 to brain mitochondria accounted for the reduced aerobic brain metabolism. It is apparent that responses of the cardiovascular system contribute to the CNS toxicity.

15.3.3 Actions independent of histotoxic hypoxia

A number of studies have shown that in the CNS, cyanide produces actions independent of cytochrome oxidase inhibition and the subsequent reduction of brain oxidative metabolism. Petterson and Cohen (1985) reported a disparity between the dose-response relationship of cyanide lethality and brain and heart cytochrome oxidase inhibition. Yamamoto (1989) reported in mice rendered unconscious by cyanide,

brain ATP levels were not significantly reduced from control, whereas liver ATP decreased to 60%. Electrophysiological studies in isolated neural tissue also showed that cyanide has direct actions on neurons not mediated by metabolic inhibition. In transverse slices of guinea pig hippocampus, cyanide between 10 and 200 μM rapidly depressed synaptic transmission between Schaffer collateral commissural fibers and CA1 pyramidal cells (Aitken & Braitman, 1989). The effect was reversed within seconds of washout, supporting a direct action of cyanide on neurons not mediated by metabolic inhibition since oxidative metabolism requires a longer period to recover. It is apparent that cyanide's action on the CNS is complex and that multiple toxic mechanisms are activated concurrently.

15.4 Neurochemical actions of cyanide in the nervous system

The vulnerability of the nervous system to cyanide appears to be related to its ability to activate select neurochemical processes in addition to inhibition of oxidative metabolism. Many of cyanide's neuronal effects precede depletion of cellular ATP and a number of enzymes are more sensitive than cytochrome oxidase (Petterson & Cohen, 1985; Aitken & Braitman, 1989). Cyanide inhibits neuronal antioxidant defenses, initiates a Ca^{2+}-dependent release of transmitters (glutamate, dopamine, adenosine) and enhances receptor mediated influx of Ca^{2+} (Ardelt *et al*., 1989; Kanthasamy *et al*., 1991; Cassel, 1995). These actions produce a rapid loss of neuronal homeostasis and dysfunction vital for maintenance of central regulation of vital functions. The onset of neuronal dysfunction manifests as onset of convulsions and seizures characteristic of acute intoxication and delayed onset neurodegeneration characteristic of excitotoxicity. The loss of neuronal Ca^{2+} homeostasis is a critical component of cyanide-induced neurotoxicity that underlies CNS dysfunction and eventually neuronal degeneration.

15.4.1 Mobilization of cytosolic calcium and neuronal injury

In neurons, cyanide produces a rapid rise in intracellular free Ca^{2+} which activates a series of cellular regulatory processes leading to immediate neuronal dysfunction and activation of cell death cascades. In mice, doses of cyanide that caused tremors also elevated total brain Ca^{2+} (Johnson *et al*., 1986, 1987). Elevation of brain Ca^{2+} and accompanying tremors were blocked by a calcium channel blocker, strongly supporting a link between altered brain Ca^{2+} homeostasis and neurological dysfunction. Studies in cultured neuronal models have confirmed that cyanide elevates cytosolic Ca^{2+} independent of inhibition of cytochrome oxidase (Johnson *et al*., 1987; Leavesley *et al*., 2008). Microfluorescence analysis of single neural cells shows that intracellular free Ca^{2+} levels rise immediately (1–2 seconds) after exposure to cyanide and precede cyanide inhibition of oxidative metabolism (Patel *et al*., 1994). Additional studies have shown similar responses in brain slices, synaptosomes, and subcellular organelles of dopaminergic cells (Gibson *et al*., 1989; Huang & Gibson, 1989; Zhang *et al*., 2010).

The rise of cytosolic-free Ca^{2+} is sustained after exposure to cyanide and is due to multiple alterations in neural Ca^{2+} handling mechanisms. Cyanide induces an influx of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+} stores (Rajdev & Reynolds, 1994). In electrically excitable cells, an immediate increase in Ca^{2+} conductance results from voltage-sensitive Ca^{2+} channels (VSCC) activation, reversal of the Na/Ca exchanger and activation of the NMDA receptor (Sato *et al*., 1991; Kiang & Smallridge, 1994; Patel *et al*., 1993). Contributing to the loss of Ca^{2+} homeostasis is an inositol trisphosphate (IP_3)-mediated mobilization of intracellular Ca^{2+} stores (Yang *et al*., 1996). Finally, cyanide impairs the ability of cells to buffer excessive Ca^{2+} loads. The depletion of ATP slows the Ca^{2+} extrusion pump, thereby impairing sequestration of Ca^{2+} by mitochondria and endoplasmic reticulum (Mattson *et al*., 1993).

The loss of Ca^{2+} homeostasis is linked temporally to biochemical and morphological responses to cyanide (Maduh *et al*., 1990). Elevation of neuronal Ca^{2+} has been correlated with neurotransmitter release, thus showing the functional outcome of this action (Gibson *et al*., 1989). Also, sustained elevation of free cytosolic Ca^{2+} is well known to activate critical membranous and cytoplasmic events in concert produce cell dysfunction and if not controlled, to cell injury and death (Tymianski & Tator, 1996). This includes activation of endonucleases, phospholipases, and proteases which mediate formation of cell surface blebs and fragmentation of genomic DNA (Maduh *et al*., 1990; Mills *et al*. 1996). In neural cells, cyanide-induced activation of PLA_2

also stimulates plasma membrane phospholipid breakdown (arachidonic acid formation) and mitochondrial dysfunction (Yang *et al.*, 1994). In addition cyanide interaction with the glutamate metabotropic receptor stimulates IP_3 formation (Yang *et al.*, 1996). A number of Ca^+-dependent kinases play a role in the neuronal responses by producing a prolonged activation of the NMDA receptor and activation of nitric oxide synthase (NOS) and PLA_2 (Okada, 1996). The rise in Ca^{2+} also stimulates secretion of cellular glutamate by a calmodulin kinase II mediated process, thereby activating the NMDA receptor and initiating an excitotoxic response.

15.4.2 NMDA receptor activation

It is well characterized that excessive stimulation of NMDA receptors in neuronal models can produce excitotoxic cell death. In pathological conditions, excessive NMDA receptor activation and subsequent intracellular Ca^{2+} overload lead to cellular injury (Dubinsky & Rothman, 1991). Cyanide neurotoxicity is linked to activation of the NMDA receptor by inducing release of glutamate (the receptor agonist) from the cytosolic pool, which then binds to the NMDA receptor (Patel *et al.*, 1993, 1994; Dubinsky & Rothman, 1991). Also, cyanide directly interacts with vicinal cysteine residues in the NMDA receptor redox-regulatory site to enhance the agonist action of glutamate and inhibit voltage-dependent magnesium blockade of the receptor (Sun *et al.*, 1999). Cyanide activation of the receptor by glutamate release produces simultaneous generation of nitric oxide and reactive oxygen species, leading to cellular oxidative stress and cytotoxicity (Gunasekar *et al.*, 1996). It is concluded that cyanide-mediated changes in NMDA receptor function can accelerate or potentiate the excitotoxic response and plays an important role in cyanide-induced neurotoxicity.

15.4.3 Role of oxidative stress in cyanide-induced neurotoxicity

Exposure of neural cells to cyanide produces an immediate intracellular generation of reactive oxygen species (ROS – superoxide, hydroxyl radical and nitric oxide radical). The sudden, intense burst of ROS plays a critical role in cyanide-induced cytotoxicity by promoting peroxidation of membrane lipids and activation of cell death pathways (Ardelt *et al.*, 1994). ROS are generated as a result of inhibition of oxidative phosphorylation chain in the mitochondrial matrix. When transfer of electrons down the chain is blocked as in the case of cyanide inhibition of cytochrome oxidase, excessive levels of oxygen radicals are generated, which then can damage mitochondria and initiate mitochondrial-dependent death pathways (Van de Water *et al.*, 1994). In addition, sustained elevation of cytosolic Ca^{2+} increases and activates a series of biochemical reactions that generates additional ROS and NO (Gunasekar *et al.*, 1996, 1998).

In addition to stimulating generation of ROS, cyanide also inhibits cellular antioxidant defenses to accentuate the action of the oxidant species (Ardelt *et al.*, 1989). Cyanide inhibits brain catalase, superoxide dismutase and glutathione perioxidase, enzymes critical for modulation of cellular ROS and maintenance of the cellular redox state. Inhibition of the antioxidant defense and enhanced cellular free radical generation lead to oxidative damage to lipids, proteins, and DNA and this damage has been proposed as a final mediator of cyanide neurotoxicity (LeBel & Bondy, 1991).

15.4.4 Interaction of cyanide with central neurotransmitters

Cyanide alters the activity of a number of central transmitter systems that contributes to both acute and chronic toxicity (Persson *et al.*, 1985). In acute toxicity, cyanide produces a sudden release of central transmitters which produces overstimulation and dysfunction of the CNS (Dawson *et al.*, 1995). Central transmitter systems are highly sensitive to cyanide as manifested as increased secretion and turnover. This is attributed in part to the increase in free cytosolic Ca^{2+} which stimulates transmitter secretion and alteration in transmitter synthesis and metabolism (Gibson *et al.,* 1989). In the periphery, catecholamines are secreted from the adrenals producing a rapid increase in blood levels of norepinephrine and epinephrine which mediate changes in peripheral cardiovascular function (Kanthasamy *et al.*, 1991).

Persson *et al.* (1985) examined brain neurotransmitters levels and their turnover in rats after administering sodium cyanide (5–20 mg/kg, ip). Within 60 seconds, dopamine levels were decreased dose-dependently and the metabolite homovanillic acid was reduced in stratum, olfactory tubercle, and hippocampus, but dihydroxyphenylacetic acid was not changed. These results are consistent with cyanide producing long lasting changes in turnover of dopamine (synthesis, secretion, and metabolism) (Cassel *et al.*, 1995). Low doses of

cyanide increased glutamate in cerebellum, striatum, and hippocampus, whereas high doses decreased glutamate in cerebellum, frontal cortex, and striatum. Cyanide interacts with pyridoxal phosphate, a cofactor for all decarboxylation reactions, and would be expected to increase glutamate levels by blocking its decarboxylation. Gamma-aminobutyric acid (GABA) was reduced in all brain areas examined by the high doses of cyanide. Subsequent studies by Cassel *et al.* (1991) showed that reduced GABA levels can be attributed to inhibition of GABA synthesizing enzymes by cyanide. On the other hand, acetylcholine and choline were not altered.

Dysfunction of the dopaminergic system plays an important role in the neurotoxic responses. Central dopaminergic pathways are linked to the post-intoxication sequelae manifested as a Parkinson-like syndrome and possibly the neurodegenerative changes observed in chronic exposures (Kanthasamy, Borowitz, *et al.,* 1994; Jones *et al.,* 2003). In rats, a lethal dose of cyanide depleted striatal dopamine (DA), possibly due to inhibition of energy-dependent granular uptake and enhanced release of DA (Cassel & Persson, 1992). Matsumoto *et al.* (1993) showed by *in vivo* microdialysis that local perfusion of cyanide in the striatum produces a transient and massive increase of extracellular DA which was possibly related to an excess influx of extracellular Ca^{2+}. In the PC12 neural cell model, cyanide produces a Ca^{2+}-dependent secretion and depletion of DA and altered DA synthesis and metabolism (Kanthasamy *et al.,* 1991). In rats, lethal doses of cyanide partially inhibit brain DOPA decarboxylase as measured by increased levels of DOPA (Persson *et al.*, 1985). Cyanide also reacts with the DA metabolite 3,4-dihydroxyphenyl acetaldehyde to generate a neurotoxic cyanohydrin (Kanthasamy, Rathinavelu, *et al.*, 1994). This compound selectively produces hippocampal CA1 lesions independent of glutamate receptor activation (Bitner *et al.*, 1997). However, the role of this cyanide-DA metabolite adduct in the cyanide-induced neurotoxic syndrome remains to be determined.

15.5 Cyanide-induced brain injury and neurodegeneration

Neuropathological responses to cyanide vary, depending on length and dose of exposure, and a number of predisposing factors, including dietary state, underlying disease, and environmental conditions. If death occurs within 24 hours of an acute exposure, the neuropathology generally resembles non-specific asphyxial changes, whereas a delayed, progressive neurodegenerative condition may be observed if the patient survives for several weeks (Wilson, 1987). In chronic exposure, more complex central neuropathology is produced which may involve motor, visual, and cognitive systems (Soto-Blanco *et al.*, 2002). These toxic responses and the underlying mechanisms have been difficult to study since adequate animal and isolated neuronal models have not been available. Recent studies have shown in brain that cyanide initiates a series of complex intracellular death cascades to produce neuronal death and brain degeneration (Zhang *et al.*, 2009). Even though cyanide is widely known as a potent intoxicant, it does not produce immediate cell death, rather intracellular death cascades are activated over an 18–24 hour period after initial exposure. On the other hand, rapid death from acute intoxication is attributed in part to CNS dysfunction leading to apnea and impaired central regulation of cardiovascular function.

15.5.1 Mechanism of cyanide-induced brain injury

Multiple mechanisms underlie the CNS pathology observed in both acute and chronic toxicity. The toxic pathways are initiated by mitochondrial dysfunction (inhibition of cytochrome oxidase), altered dopaminergic function, and an excitotoxic-like response which produce increase oxidative stress (Mills *et al.,* 1999). These actions activate select cell death cascades in different brain areas, cumulating in the characteristic neurologic insult of acute toxicity or chronic neurodegenerative-like disorders (Mattson & Furukawa, 1996).

The selective, delayed dopaminergic toxicity of cyanide has been studied in a mouse model (Kanthasamy, Borowitz, *et al.*, 1994). Following treatment with cyanide over a 7-day period, mice exhibited marked decreases in striatal and hippocampal DA levels, which were accompanied by significant peroxidation of lipids in these brain areas. Tyrosine hydroxylase (TH) immunohistochemical examination showed reduced number of TH-positive cells in the substantia nigra, indicating loss of dopaminergic neurons. Over one-third of the mice exhibited decreased locomotor activity and akinesia which were suppressed by L-dopa. A similar

syndrome has been reported in humans exposed to cyanide in which select degeneration of dopaminergic pathways in basal ganglia occurred and patients exhibited a Parkinson-like syndrome (see details below).

Excessive stimulation of glutamate receptors is associated with a number of neurodegenerative conditions, such as idiopathic Parkinson's disease (Dawson *et al*., 1995). Spencer *et al*. (1992) proposed that cyanide is involved in basal ganglia disease through an excitotoxic effect and disruption of energy metabolism. Toxicants that inhibit oxidative phosphorylation, including MPTP, carbon monoxide, and manganese, can produce basal ganglia dysfunction that manifested as locomotor and cognitive impairment. In primary cultured neurons cyanide produces an excitotoxic-like cell death blocked by glutamate receptor antagonists (Patel *et al*., 1992). In hippocampal neurons, cyanide stimulated release of endogenous glutamate which activated NMDA and metabotropic glutamate receptors. Cyanide also interacts directly with redox regulation of the NMDA receptor to enhance the action of glutamate (Sun *et al*., 1997). Activation of the NMDA receptor-ionophore stimulated influx of Ca^{2+} and activation of the metabotropic receptor mobilized intracellular stores of Ca^{2+}. The sustained elevation of cytosolic free Ca^{2+} and accompanying mitochondrial dysfunction then produced an excitotoxic-like, delayed cell death over a 18–24 hour period.

Mitochondrial dysfunction is a primary contributor to the neurological injury produced by cyanide. In the mitochondrial matrix, cyanide binds to cytochrome oxidase to block intracellular oxygen utilization and reduce mitochondrial ATP generation (Leavesley *et al*., 2008). The bioenergetic failure produces a loss of mitochondrial membrane potential, onset of mitochondrial permeability transition and a burst of ROS generation (Li *et al*., 2006). This is accompanied by a rapid rise of mitochondrial Ca^{2+} due to influx via the mitochondrial Ca^{2+} uniport. This activates Ca^{2+}-sensitive mitochondrial generation of nitric oxide (NO). Recent studies show these mitochondrial events underlie the initiation cell death pathways and the subsequent neuronal degeneration (Zhang *et al*. 2009).

15.5.2 Cyanide-induced cell death and selective vulnerability of the brain

In mice administered sublethal doses of cyanide, region-specific neuronal degeneration has been observed (Mills *et al*., 1999). In parietal and suprarhinal regions of motor cortex, cell loss occurred by apoptosis and in the substantia nigra a progressive necrotic lesion, accompanied by marked astrogliosis, was observed bilaterally. Cell loss in basal ganglia is selective for tyrosine hydroxylase-positive cells and the dopaminergic pathways display a high sensitivity to cyanide (Jones *et al*., 2003). Based on differential responses to antioxidant treatment, it was concluded that neuronal loss in different brain areas involves divergent mechanisms.

Molecular initiation signals and execution pathways underlying the apoptotic and necrotic modes of cell death have been studied in cells from different brain areas (Prabhakaran *et al*., 2002; 2004). The differential response to cyanide was studied in primary cultured cells in which the dominant mode of cell death in mesencephalic cells was necrosis and apoptosis in cortical cells, similar to that observed *in vivo*. Oxidative stress and altered mitochondrial dysfunction are common initiation stimuli in the different types of neuronal death (Li *et al*., 2006), but divergent pathways dictate the dominant mode of death for each specific cell type and hence the characteristic pathological lesion observed. Selective vulnerability of different brain areas to cyanide can be explained by triggering region-specific toxic pathways. This appears to account for the difference in sensitivity of different brain areas and perhaps the different lesions observed in acute and chronic exposures.

15.6 Endogenous cyanide generation in CNS

Under normal conditions in which individuals are not exposed to cyanide through dietary ingestion or smoking, a low constant level of ~3 μM cyanide is detectable in the blood (Anderson & Harland, 1980). This low level is thought to be a byproduct of catabolic metabolism and generated by myeloperoxidase in leukocytes (Stelmaszynska & Zgliczynski, 1981). Recently, it has been demonstrated that cyanide is generated in brain via a receptor mediated process (Borowitz *et al*., 1997; Gunasekar *et al*., 2000). In rat brain cyanide generation was enhanced by opiate agonists and specifically blocked by naloxone, an opiate antagonist.

Biochemical analysis in neural tissue showed that cyanide was generated by a peroxidase-mediated

reaction (Gunasekar *et al*., 2000). Loading brain tissue with glycine enhanced cyanide generation and a myeloperoxidase inhibitor (aminobenzoic acid hydrazide) blocked cyanide generation. This pathway was isolated to the mitochondrial fraction of the cells. It was concluded that cyanide is generated in the neural tissue by a peroxidase enzyme using glycine as a substrate.

It is interesting to note that brain areas that generate the highest cyanide level also contain the highest levels of rhodanese in mitochondria. It appears that specific pathways for regulating cyanide production and removal are present in brain. Endogenous generation of cyanide is region specific in the brain; the hypothalamus and hippocampus generated higher levels than cortex, medulla, and cerebellum (Gunasekar *et al*., 2000). It was estimated the average brain cyanide level is ~6.9 μM (Borowitz *et al*., 1997) which is within the range that activates neurochemical pathways in rat cerebellar granule cells (Sun *et al*., 1999). For instance, in cerebellar granule cells, this concentration of cyanide potentiated the rise in cytosolic Ca^{2+} produced by activation of the NMDA receptor. Cipollone and Visca (2007) speculated that cyanide may serve as a neuromodulator, similar to other small molecule modulators (CO, H_2O and NO). Additional work is necessary to establish if endogenous generation of cyanide plays a role in neurodegenerative disorders, particularly in light of the observation that defects in cyanide metabolism coexist with several neurodegenerative conditions (Isom *et al*., 2010).

15.7 Cyanide-induced neurological disorders

The type and extent of neuropathologic lesions produced by cyanide are dependent on route, duration, and concentration of exposure. Since the human exposure paradigm can be complex, it has been difficult to develop adequate animal models of the human pathology. A number of predisposing factors influence the neuropathology, including dietary state, age, and underlying disease (Wilson, 1987). In acute intoxication alterations in cardiovascular function (delivery of oxygen to brain) and acid-base balance contribute to the type and level of brain injury (Baud, 2007).

In acute exposure, death occurs so rapidly that brain lesions may be limited to vascular congestion (Rosenberg *et al*., 1989). Following the acute episode, survivors may develop cerebral hypoxia/ischemia-like lesions. The brain areas with high oxygen requirements, including the basal ganglia, may develop hemorrhagic necrosis and/or apoptotic degenerative lesions. Lesions have also been detected in the cerebellar/sensorimotor cortex, whereas the hippocampus displays only moderate damage (Mills *et al*., 1999).

In animals, a variety of brain pathologies has been observed in acute, sublethal toxicity models. In dogs surviving more than 3 hours after acute exposure, Haymaker *et al*. (1952) reported almost exclusive gray matter pathology in which the most susceptible areas were the cerebral cortex, caudate nucleus, putamen, globus pallidus, thalamus, and cerebellar cortex. Levine and colleagues (Levine & Stypulkowski 1959; Levine & Wenk, 1959) produced in rats a cyanide encephalopathy of the mid-sagittal brain characterized by demyelination and necrotic lesions. Other rat models show lesions in the hippocampus, corpus callosum, and corpus striatum (Ashton *et al*., 1981; Brierley *et al*., 1976). In a subchronic exposure mouse model, Kanthasamy, Borowitz, *et al*. (1994) used immunohistochemical analysis to show selective loss of dopaminergic neurons in the substantia nigra.

Chronic cyanide exposure is associated with a variety of neuropathologies, including many of the same lesions described for acute intoxication. In an early clinical study, Collins and Martland (1908) demonstrated anterior horn cell degeneration and segmental demyelination in peripheral nerves in chronically exposed rabbits and observed similar pathology in an individual exposed to cyanide. In chronic exposures, humans manifest a complex array of both central and peripheral neuropathies involving motor, sensory, and cognitive function.

15.7.1 Delayed neurologic sequelae of acute intoxication: Parkinson-like syndrome

Several clinical reports show that in patients who survive a severe, acute intoxication, a delayed, progressive Parkinson-like syndrome and dystonia may develop. The incidence of the syndrome is not known and a majority of patients who survive an acute poisoning do not develop extrapyramindal symptoms (Hall *et al*., 1987). In many cases, the patients are comatose for varying times, followed by apparent recovery from

the life threatening intoxication. Symptoms develop over several days to weeks after the acute episode, but subtle changes may be noted within a few days. A progressive dystonia may develop and progress to a severe Parkinson-like state. This may be accompanied by a range of toxic encephalopathies including memory deficits, neuropsychiatric symptoms, and personality changes. The pyramindal manifestations may resolve over time and levodopa therapy produces variable improvement.

At the time of onset of pyramindal manifestations, computerized tomography (CT) and magnetic resonance image (MRI) examinations have shown lesions in the basal ganglia, cerebellum, and cerebral cortex (Carella *et al.*, 1988). Radiologic changes may appear several weeks after onset of symptoms (Holstege *et al.*, 2011). For instance, in a male surviving a suicide attempt, extensive bilateral symmetric globus pallidus and posterior putamen lesions were observed and positron emission tomography with 6-fluorodopa revealed marked dysfunction of dopaminergic transmission in posterior regions of the basal ganglia, similar to that observed in Parkinsonism (Rosenberg *et al.*, 1989). In a similar case, an individual experienced an acute neurobehavioral disturbance that manifested as primary psychosis (Kales *et al.*, 1997). The MRI was consistent with striatal-nigral degeneration. Cyanide intoxication can show anatomical differences from idiopathic Parkinsonism. In cyanide poisoning cell loss can occur in the retricular zone of the substantia nigra with preservation of the compact zone. In idiopathic Parkinsonism, the area affected is the compact zone of the substantia nigra (Utti *et al.*, 1985).

15.7.2 Neurologic disorders associated with chronic cyanide exposure

Several insidious neuropathies are associated with a variety of different chronic cyanide exposure paradigms. In many of these conditions, the etiologic role of cyanide has been difficult to prove and animal studies have not consistently duplicated the syndromes (Soto-Blanco *et al.*, 2002). Long-term, low-level exposure to cyanide has been associated with sensory and cognitive disorders (Blanc *et al.*, 1985) and recently it was shown in rat studies that cyanide can potentiate noise-induced hearing loss (Fechter *et al.*, 2002). In many cases, the neurological conditions are attributed to toxico-nutritional interactions in which it is presumed that cyanide biotransformation is abnormal, resulting in accumulation of cyanide in target tissue or inadequate removal of metabolites. Under normal metabolic conditions, rhodanese catalyzes the primary pathway using a sulfane sulfur co-substrate to convert cyanide to the nontoxic metabolite thiocyanate. The capacity of this pathway can be reduced in nutritional deficiencies of sulfur-containing amino acids. Under these conditions, low level, dietary cyanide exposure may lead to neurotoxicity.

Tropical ataxic neuropathy

In tropic and subtropic areas, consumption of cassava (*Monihot esculenta*) is associated with a neurological syndrome characterized by co-existence of sensory ataxia, optic atrophy, bilateral sensori-neural deafness and symmetrical spastic paraparesis in varying combinations and severity (Osuntokun, 1968, 1972; Wilson, 1987). Tingling of the extremities and blurring of vision are the most typical symptoms. As the condition progresses, wasting of the lower limbs can occur. The neuropathy continues to be prevalent in the endemic areas (Adamolekun, 2011). Appropriately one-third of the patients display stomatoglossitis, consistent with a toxico-nutrition etiology. As the condition progresses, wasting of the lower limbs can occur.

The condition has been reported in all tropic areas, but the clinical manifestations can vary by locality. The neuropathy continues to be prevalent in the endemic areas (Adamolekun, 2011). The pyramidal dysfunction is more severe in the Caribbean areas than in African endemic areas, perhaps due to differences in dietary conditions and level of cyanide intake. The incidence is highest among the elderly (5th–6th decades) and rarely observed in pre-pubertal –children (Wilson, 1987). The neuropathology has been associated with long-term ingestion of cassava, protein deficiency, and low vitamin B_{12} intake. Under certain conditions such as drought in which there may be reduced agriculture production, cassava can become the main dietary food stuff. Cassava contains linamarin, a cyanogenic glycoside that liberates cyanide on decomposition (curing). Improper preparation of cassava can lead to ingestion of low levels of cyanide. It has been proposed that ingestion of cassava (cyanide) and in combination with reduced intake of sulfur substrate (protein deficiency) predisposes to the neuropathy (Oluwole *et al.*, 2002; Soto-Blanco *et al.*, 2002).

The etiologic role of cyanide in the neuropathy continues to be questioned (Wilson, 1987). Recent studies have linked the condition to ingestion of cassava containing neurotoxic compounds such as nitriles, perhaps in combination with thiamine deficiency (Adamolekun, 2011; Llorens *et al*., 2011). However, studies have shown that in many patients that systemic cyanide levels are slightly elevated and that plasma concentrations of sulfur-containing amino acids are reduced (Oluwole *et al*., 2002). This may be accompanied by signs of avitaminosis. Clinical improvement is noted with removal of cassava from the diet and polyvitamin (vitamin B_{12}) administration.

Konzo – an upper motor neuron disease

Konzo is a myeloneuropathy associated with high exposure to cyanide as a result of dietary cassava consumption. The neuropathy manifests as sudden onset of non-progressive, spastic paraparesis in which there is symmetric, isolated bilateral involvement of upper motor neurons (Howlett *et al*., 1990). MRI scans of brain and spinal cord of severely affected individuals show the long axon upper motor neurons are selectively damaged (Tylleskar *et al*., 1992, 1994). The syndrome is permanent and varies in severity, ranging from hyperreflexia in the legs to severe spastic paraparesis with accompanying weakness of the trunk and arms. These manifestations can be accompanied by dysarthria, optic atrophy, and mild sensory symptoms.

A number of epidemics have been reported in rural, tropical Africa (Cliff *et al*., 1985). In Mozambique, over a thousand cases of abrupt onset, symmetrical spastic paraparesis was observed mainly in children and young adults. A strong association was observed with protein deficiency in combination with high cyanide intake resulting from cassava as the dietary mainstay. Epidemiological studies showed a causal role of sustained high blood cyanide levels and impaired metabolism (Mlingi *et al*., 1993).

Cyanide and motor neuron disease

Abnormalities of cyanide metabolism have been observed in patients exhibiting signs of motor neuron disease (MND) and it has been proposed that accumulation of cyanide from exposure or endogenous generation contributes to progressive degeneration of upper and lower motor neurons (Kameyama, 1980; Mimori *et al*., 1984). Whole blood and urine cyanide levels were examined in 83 amyotrophic lateral schlerosis (ALS) patients displaying signs motor neuron lesions and progressive spinal or bulbar muscular atrophy with only lower motor neuron symptoms (Kato *et al*., 1985). In non-smoking subjects, cyanide levels of MND patients was only slightly elevated (non-significant) when compared to non-neurologic non-smoking controls, whereas in smoking individuals, the MND group displayed a marked elevation in whole blood cyanide. Since the cyanide load was higher in smokers, it was postulated that MND patients may have a lower capacity to metabolism cyanide which would accumulate higher levels than non-smoking subjects. It was concluded that in individuals with pre-existing motor disease, the elevation of cyanide may contribute to the degeneration.

Additional studies show a link between altered cyanide metabolism and motor neuron disease. In a study of cyanide metabolizing enzymes distribution in the CNS in the MND, it was observed that spinal cord rhodanese activity was lower in autopsied cases of ALS as compared to controls (Mimori *et al*., 1984). The most significant decrease of rhodanese activity was in the posterior column of the cervical and thoracic cord of individuals with a history of MND, but the anterior horn did not different significantly between controls and NMD patients. This observation is consistent with cyanide accumulating at these sites and possibly exasperating the neurologic disorder.

Tobacco amblyopia

Tobacco amblyopia is associated with heavy smoking in elderly men in which there is an insidious loss of visual acuity, but cases of rapid onset have been reported (Darby & Wilson, 1967). Heavy use of alcohol is a contributing factor, hence the condition is also known as tobacco-alcohol amblyopia. Individuals gradually develop a symmetric bilateral loss of visual acuity with centro-scotomas, diminished color vision, and degeneration of the ganglion cells of the retina (Wilson, 1987). Susceptible individuals may exhibit a vitamin B_{12} deficiency as result of a defect in vitamin B_{12} metabolism or its absorption (Heaton *et al*., 1958). It has been postulated that cyanide from tobacco smoke may reduce the concentration of active vitamin B_{12} by binding with hydroxocobalamin to form the metabolically inactive cyanocobalamin (Foulds *et al*., 1969).

Treatment with hydroxocobalamin can produce full recovery of vision, even in some patients who continue smoking (Chisholm *et al*., 1967).

Epidemics of optic neuropathy, associated with cyanide exposure due to heavy tobacco smoking, have been reported (Lincoff *et al*., 1993). In 1991–1993, over 50,000 individuals in Cuba were afflicted with varying degrees of amblyopia and many cases also showed peripheral neuropathies with painful dyesthesias, diminished ankle reflexes, and increased vibratory and thermal threshold in the legs (The Cuba Neuropathy Field Investigation Team, 1995). Mitochondrial DNA mutations or genetic defects associated with Leber's hereditary optic neuropathy were not observed in the cases (Newman *et al*., 1994). Analysis showed that the total cyanide load was elevated due to heavy tobacco use, particularly cigar smoking, and high cassava consumption. It was concluded that the primary risk factors were heavy tobacco smoking and protein-micronutrient deficiencies. In support of this conclusion, the incidence of the disease in the general population was effectively treated with parenteral vitamin B_{12} and oral multiple vitamins.

Tobacco amblyopia and retrobulbar neuritis have common features that may result from abnormalities of cyanide metabolism and vitamin B_{12} regulation (Baumeister *et al*., 1975). It has been noted that in both conditions, there is a history of heavy tobacco smoking in male subjects (Freeman & Heaton, 1961). In retrobulbar neuritis, which occurs occasionally in Addisonian pernicious anemia, there may be altered incorporation of cyanide into the 1-C metabolic pool. The abnormal metabolism of cyanide may contribute to the neurological complications (Wilson, 1987).

Leber's hereditary optic atrophy (LOA)

In 1871 Leber described a subacute, progressive visual loss, characterized by circumpapillary teleangiectatic micropathy, tortuosity of the retinal vessels and selective degeneration of the papilla-macular bundle of the optic nerve (Berninger *et al*., 1989). The visual impairment can be accompanied with varying degrees of pyramidal dysfunction and diffuse encephalopathy. The features are similar to retrobulbar neuritis of pernicious anemia. Up to 85% of patients are post-adolescent males in the late teens or early twenties, but women can be afflicted (Wilson, 1963).

It has been proposed that LOA is due in part to a hereditary inborn error of cyanide metabolism (Wilson, 1965). This conclusion is based on the observations that in non-smoking normal (control) and LOA subjects, the plasma and urine levels of thiocyanate, the primary cyanide metabolite, were in the same concentration range. However, in a smoking population, LOA patients had reduced levels of thiocyanate as compared to normal subjects possibly due to reduced ability of rhodanese to metabolize the increased cyanide load from smoking. Subsequent analysis of rhodanese activity in liver biopsy and rectal mucosa samples showed reduced rhodanese levels in LOA patients (Cagianut *et al*., 1981, 1982).

The inheritance pattern of LOA is familial, but not easy to explain by classic Mendelan genetics (Parker *et al*., 1989). The inheritance appears to be mitochondrial in which there is a sex link with male preponderance that is transmitted by females to offspring of both sexes. Enlarged subsarcolemmal mitochondrial have been observed and are associated with a defect in one or more of the enzymes in the mitochondrial electron-transport chain. These enzymes are encoded by mitochondrial DNA. In all patients observed, there is a marked decrease in catalytic activity of Complex I of the mitochondrial electron-transport chain. This is attributed to a point-mutation in the mitochondrial DNA resulting in reduced NADH dehydrogenase activity and decreased in cellular energy charge similar to that produced by cyanide (Singh *et al*., 1989).

The relationship of defects in cyanide metabolism to LOA has not been definitively established. Rhodanese is a mitochondrial enzyme encoded in the nucleus, translated on cytoplasmic ribosomes and then transported into the mitochondrion. In the mitochondrial matrix, rhodanese is a multiple functional enzyme that regulates mitochondrial sulfur balance necessary for oxidative phoshorylation and metabolizes cyanide (see Chapter 4 on cyanide metabolism). Reduced mitochondrial rhodanese activity would not only perturb regulation of metabolism, but increase mitochondrial levels of cyanide when exposed to cyanide from exogenous sources (smoking, diet) or endogenous generation. The increase of mitochondrial cyanide levels would reduce the aerobic capacity of susceptible cells and increase the level of oxidative stress, thereby accelerating neuronal degeneration.

15.8 Conclusion

Cyanide produces a variety of complex effects on the nervous system in which the manifestations vary, depending on the exposure dose, length of exposure, and predisposing health conditions. The nervous and cardiovascular systems are the primary toxic targets in which inhibition of aerobic metabolism is an initiating event of the toxicity. The nervous system toxicity results from activation of multiple pathways that lead to neuronal dysfunction and selective degeneration of the brain areas. The dopaminergic tracts are extremely sensitive to cyanide and permanent neuronal loss can occur, leading to impairment of centrally mediated function. Acute intoxication is associated with impairment of central respiratory drive and disruption of central regulation of cardiovascular function.

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CHAPTER 16
Cyanides and cardiotoxicity

J.-L. Fortin, T. Desmettre, P. Luporsi and G. Capellier

At a Glance

- The epidemiology of cardiovascular signs of cyanide poisoning is poorly defined.
- Hypertension may be noted early in the poisoning, rapidly followed by severe hypotension.
- Cardiac disorders reported in cyanide-poisoned patients have included: 1) Dysrhythmias (supra-ventricular or ventricular tachycardia), 2) Impaired intra-cardiac conduction abnormalities, 3) Impaired repolarization (myocardial ischemia, sub-endocardial injury), and 4) Cardiorespiratory arrest (asystole, ventricular fibrillation).
- Of 161 patients with suspected cyanide poisoning from fire smoke inhalation, 135 (84%) had cardiac disorders.
- Of 61 such patients found in cardiac arrest 5 (~1%) survived without neurological sequelae. Adrenalin was administered to only 1 of these 5 patients; the other 4 responded to oxygen administration and external cardiac massage.
- Hemodynamic status and cardiac rhythm stabilized in these 4 patients following hydroxocobalamin administration.

16.1 Introduction

People can be exposed to acute cyanide poisoning in a civilian context, but also in a military or terrorist context. The inhalation of fire smoke and, more rarely, the ingestion of cyanide salts are the two circumstances in which it can occur in a civilian context. The military or terrorist contexts corresponds to the possible use of hydrogen cyanide (prussic or hydrocyanic acid) as a gas in warfare (something actually banned since the Convention and Organization for the Prohibition of Chemical Weapons (OPCW) was signed in Paris on April 29, 1997) and the contamination of water supply systems with cyanide salts.

However, armed forces can also be confronted by a risk of cyanide contamination outside the arena of CBRNE conflicts and in the theaters of external operations when camps are set up near disused industrial sites containing stocks of sodium or potassium cyanide. This risk was encountered when United Nations armed forces were deployed in operations in Bosnia-Herzegovina during the Yugoslavian civil war.

The physiopathology, clinical expression and treatment of cyanide poisoning all remain rather obscure. In addition to the conventional respiratory and neurological clinical signs, there are also cardiovascular signs of the poisoning, the epidemiology of which is poorly documented. Cardiorespiratory arrest and hypotension can be recognized rapidly, but other cardiac signs also have to be identified, such as arrhythmia, impaired conduction and impaired repolarization, particularly in patients exposed to low doses.

16.2 Physiopathology

Cyanide-containing agents that can be used for military or terrorist purposes take two main forms:

- Hydrogen cyanide (prussic acid), which is a very volatile, colorless liquid with a boiling point of 26°C.

Toxicology of Cyanides and Cyanogens: Experimental, Applied and Clinical Aspects, First Edition.
Edited by Alan H. Hall, Gary E. Isom and Gary A. Rockwood.

The gaseous form is also colorless and gives off a smell of "bitter almonds."

- Cyanide salts: sodium cyanide and potassium cyanide.

After ingestion or inhalation, cyanide rapidly enters the blood compartment. At low concentrations, 93–99% is bound to methemoglobin ($Fe3^{+}$) to form cyanomethemoglobin. Oxyhemoglobin ($Fe2^{+}$), which is responsible for oxygenation, is protected due to its low affinity for cyanide.

At high concentrations, like any transporter, methemoglobin is saturated and cyanide is found in free form in the plasma (Antonini & Brunori, 1971). Cyanide is distributed throughout all the tissues of the body and, in particular, to the myocardial cells. Within the cell, hydrogen cyanide is bound and inhibits mitochondrial oxidase cytochrome. The inhibition of this enzyme prevents the cellular consumption of oxygen, and the cell switches to anaerobic metabolism. Energy depletion with reduced adenosine triphosphate (ATP), intracellular acidosis, notably lactic acidosis, and cell death ensue (Figure 16.1).

Thus, during fires which are the most common circumstance under which acute cyanide poisoning occurs (Baud *et al.*, 1991), the severity of the smoke inhalation is related to the combined toxicity of both carbon monoxide and hydrocyanic acid. The hydrogen cyanide reinforces the action of carbon monoxide by blocking the cell's ability to use oxygen. Carbon monoxide is bound to hemoglobin to produce carboxyhemoglobin, which compromises oxygen transportation, thus resulting in blood hypoxemia.

The energy blockade of the cardiac cytochrome oxidases produced by cyanide poisoning leads to the early disruption of calcium homeostasis, notably in cardiac cells (O'Flaherty & Thomas, 1982). Malis and Bonventre (1986) have shown that during an ischemic phenomenon or during poisoning calcium and oxygenated free radicals reduce the activity of ATPase by 55%, and that of adenine nucleotide transferase by 66%.

Animal studies suggest that there is a dose-effect relationship, which makes it possible to define the increase in calcium in the intracellular medium after exposure to cyanide. There is a correlation between the rise in the intracellular calcium and the severity of the clinical signs, in particular cardiac signs (Franchini & Krieger, 1993; Allen *et al.*, 1985; ATSDR, 2006).

During fires, whether the smoke contains hydrogen cyanide or not will depend on the composition of certain materials (Tuovinen & Blomqvist, 2003; Hertzberg *et al.*, 2003). The release of hydrogen cyanide can result from the combustion of natural fabrics (wool, cotton, silk) as well as from that of synthetic materials (polyurethane, polyacrylonitrile, polyamide). The more nitrogenous compounds the involved substance contains, the more hydrogen cyanide it will give off during its combustion (Figure 16.2) (Ballantyne, 1987).

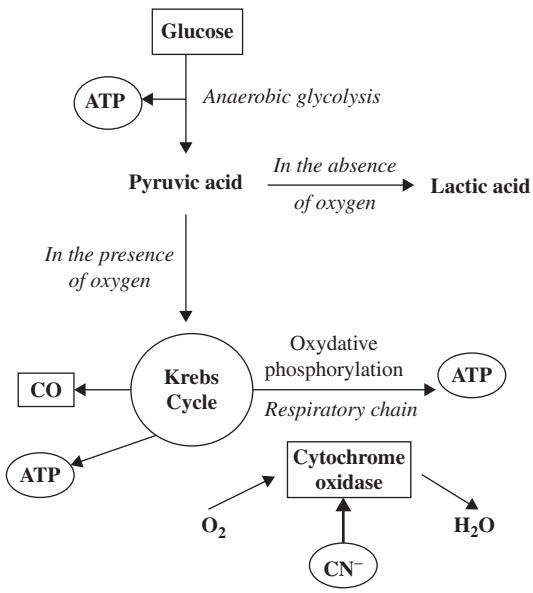


Figure 16.1 Glucose and cellular respiration metabolism and the site of action of the cyanide ion, according to Dehon and Lhermite (1998).

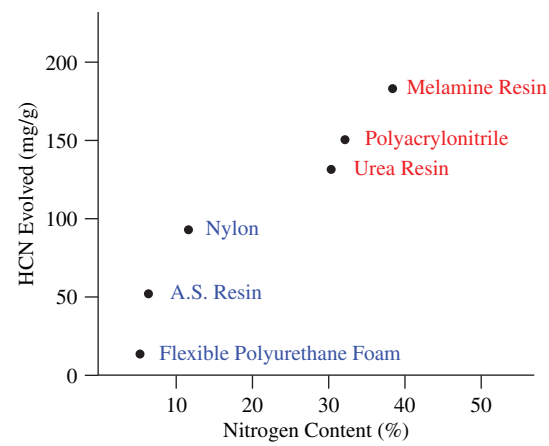


Figure 16.2 Release of cyanide depending on the composition of the material (Ballantyne, 1987).

16.3 Clinical aspects

Recent studies, in particular those of Baud (2009) have defined the toxidrome and the incidence of the clinical signs encountered during hydrogen cyanide poisoning. At low levels of cyanide in the blood, between 0.2 and 2 mg/l, the following, non-specific clinical signs are seen: muscular weakness, ocular irritation, vertigo, nausea, vomiting, sweating, tachycardia. At higher concentrations of between 2 and 6 mg/l, the following clinical signs are observed: coma of various degrees, mydriasis, convulsions, disorders affecting the respiratory rate, or even apnoea or cardiocirculatory arrest. Table 16.1 indicates the incidence with which these clinical signs occur.

As a result of the blockade of aerobic metabolism, cyanide poisoning leads to a marked rise in plasma lactate. In France, portable analyzers for plasma lactate levels have been available for some years. These instruments are used in the medicalized ambulances of the mobile emergency services, which makes it possible to carry out a quick assessment before the patient reaches the hospital, thus providing information required to decide whether antidotal treatment for cyanide poisoning should be administered. As Baud showed in 2007, the level of plasma lactate is correlated with the level of blood cyanides.

With regard to the cardiac disorders that follow acute cyanide poisoning, several studies have identified various clinical cardiac signs. Tachycardia appears at blood cyanide levels of over 0.5 mg/l, whereas hypotension, electrocardiographic disorders of repolarization (appearance of ischemia or myocardial injury), impaired conduction, and perturbed heart rate generally appear at concentrations in excess of 2 mg/l. Cardiocirculatory arrest, usually with asystole, is an ominous sign and can occur rapidly at concentrations even only a little higher than 2.5 mg/l (Fortin, Desmettre, *et al.*, 2009). Blood cyanide levels of 2.5 mg/l correspond to the lethal concentration 50 (LC50). At this concentration, 50% of the subjects exposed to the poison are predicted to die.

The performance of an electrocardiogram and continuous cardiac monitoring is mandatory whenever a patient who has been poisoned with hydrogen cyanide is being evaluated and treated. A recent multicenter study has showed that out of 161 patients suspected of having suffered acute cyanide poisoning during fires, 135 had cardiac disorders as evidenced by ECG monitoring or ECGs (i.e., approximately 84%) (Table 16.2) (Fortin, Desmettre, *et al.*, 2010).

This study demonstrated the usefulness of looking for these disorders in the context of acute cyanide poisoning by systematically performing an electrocardiogram or continuous ECG recording. These electrocardiographic signs can be corrected by administering hydroxocobalamin. These rhythm or conduction disorders are therefore additional indications for rapidly administering treatment with this antidote. Antidotal treatment must be provided without delay at the incident site, and before the victim is transported to the hospital.

Of 61 patients discovered in cardiocirculatory arrest, 5 survived with no neurological sequelae. In 4 of these 5 patients, adrenaline (norepinephrine) had not been administered: these patients were discovered in a state of cardiorespiratory arrest by the first emergency service teams to arrive and spontaneous heart activity was restored after external cardiac massage and oxygen therapy. Hydroxocobalamin was not involved in the restoration of spontaneous heart activity, but after the

Table 16.1 Incidence of clinical signs in cyanide poisoning depending on the blood levels of cyanide (according to Baud, 2009).

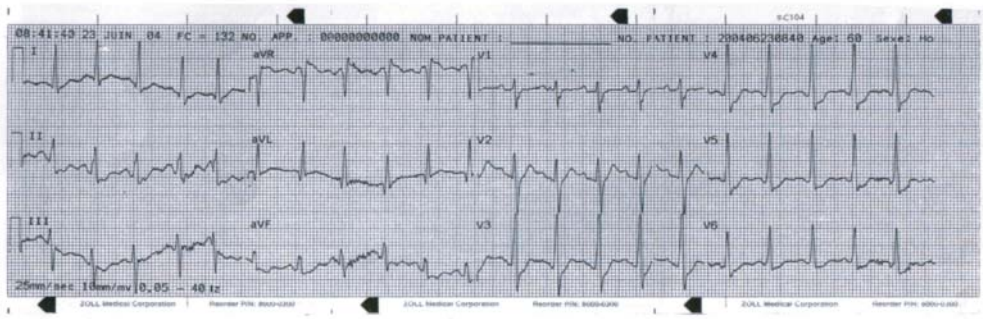
Clinical signs	
Low concentration (between 0.2 and 2 mg/l)	**High concentration (between 2 and 6 mg/l)**
Muscular weakness	Coma (83%)
Ocular irritation	Mydriasis (77%)
Vertigo	Convulsions (26%)
Sweating	Respiratory arrest
Tachycardia	Cardiac arrest

Table 16.2 Incidence of cardiac disorders (according to Fortin, Desmettre, *et al.*, 2010).

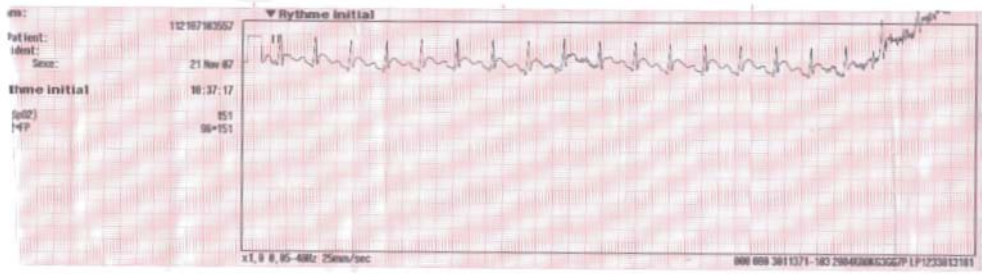
Total number of cardiac problems observed (n=135)		
Cardiac problem		**Number**
Cardiorespiratory arrest	Asystole	58
	Ventricular fibrillation	3
Impaired repolarisation	Myocardial ischaemia	5
	Sub-endocardiac injury	7
Impaired conduction	Intra-cardiac	5
Rhythm disorders	Supra-ventricular tachycardia	56
	Ventricular tachycardia	1

Table 16.3 Examples of electrocardiographic disorders during acute cyanide poisoning.

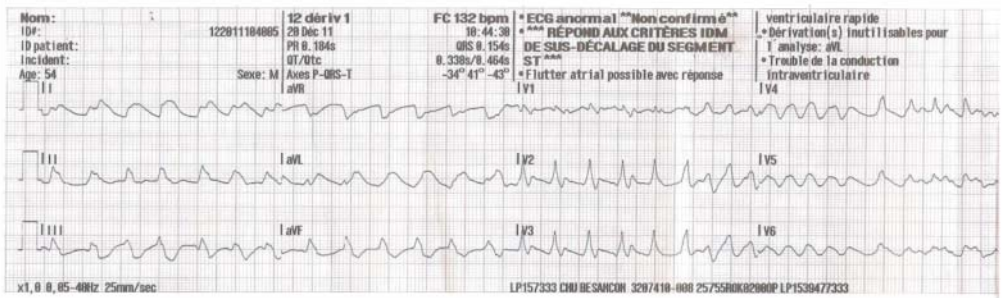
Example 1
48 year old man
Ingestion of potassium cyanide
Blood cyanide concentration = 3.64 mg/l
ECG : Myocardial lesion approximately 40 minutes after cyanide ingestion



Example 2
23 year old man
Fire of matress – Jail of Besancon
Blood cyanide concentration = 6,12 mg/l
ECG = Myocardial lesion approximately 20 minutes after smoke inhalation



Case report 3
27 year old man
Home Fire – Besancon
Blood cyanide concentration = 3,2 mg/l
ECG = Atrial flutter



antidote had been administered, hemodynamic and rhythmic stability was observed in these four patients.

Of the 12 patients who presented with impaired repolarization, 7 underwent a cardiac troponin assay when admitted to hospital. All but one of these patients had a troponin value below the positivity threshold (<0.15 ng/ml), which seems to indicate that the rapid restoration of normal metabolic conditions in the myocardial cells by the early administration of the antidote had made it possible to restrict the duration of myocardial damage.

Table 16.3 shows some examples of electrocardiographic disorders, such as arrhythmia or cardiac repolarization disorders as observed during acute cyanide poisoning.

16.4 Treatment

Specific treatment: although there are several different antidotes that can be used in this situation, they are not all equally effective and some have significant side effects. Historically, from the 1930s, the protective effect of methemoglobin was demonstrated, which is linked to its affinity for the cyanide ion, leading to the development of methemoglobin-inducing substances for the treatment of cyanide poisoning, notably in the United States, Argentina, and Germany (amyl nitrile, sodium nitrite, and 4-dimethylaminophenol (4-DMAP)). The use of these substances is currently being phased out due to the methemoglobin formation with reduced oxygen transport, and the possibility of severe side effects (sudden-onset hypotension or even states of shock). Methemoglobin induction can also compromise or even drastically reduce oxygen transport, which has already been impaired by carboxyhemoglobin as a result of the carbon monoxide poisoning that is combined with cyanide poisoning in fire smoke.

The use of sodium thiosulfate for its role in metabolizing cyanide to form a relatively non-toxic substance, thiocyanate, can theoretically be useful in the treatment of acute cyanide poisoning (Hall *et al.*, 2007; Jang *et al.*, 2010). However, the length of time before it acts makes this an adjuvant treatment that may be used as a

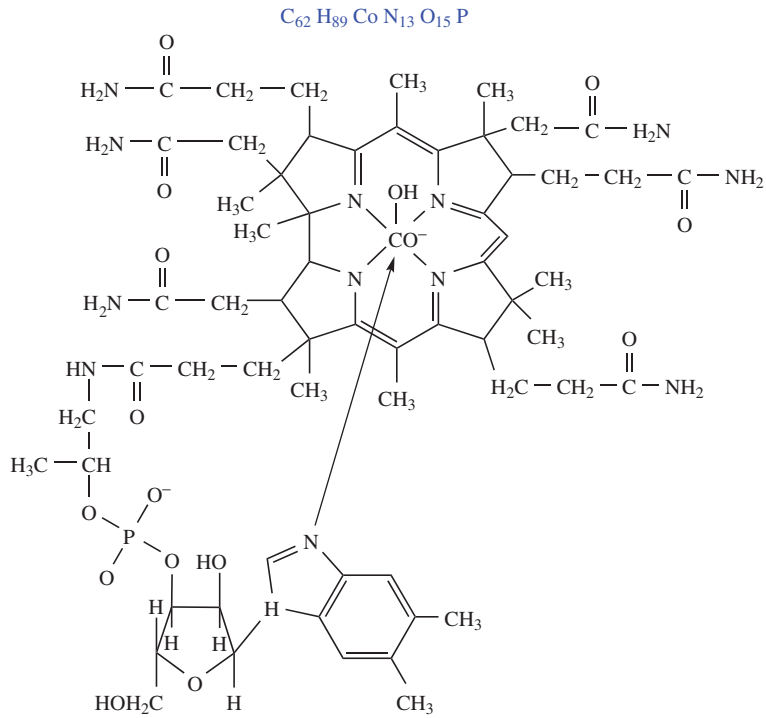


Figure 16.3 Structure of hydroxocobalamin.

Table 16.4 Examples of evolution electrocardiographic changes after administration of the antidote.

Example 1 48 year old man Ingestion of cyanide potassium – Paris	
Before administration of hydroxocobalamin Blood cyanide concentration = 3.64 mg/l ECG : Myocardial lesion approximately 40 minutes after cyanide ingestion	After administration of hydroxocobalamin (Hydroxocobalamin 10 g) ECG : Disappearance of repolarization disorder
Example 2 23 year old man Fire of matress – Jail of Besançon	
Blood cyanide concentration = 6,12 mg/l ECG = Myocardial lesion approximately 20 minutes after smoke inhalation	After administration of hydroxocobalamin (Hydroxocobalamin 10 g) ECG : Disappearance of repolarization disorder

follow-on after an initial injection of hydroxocobalamin in the hospital if correction by means of an antidote is still necessary.

Dicobalt ethylene diamine tetra-acetate (EDTA) acts by binding the cyanide ion and forms a non-toxic complex that is eliminated in the urine. However, it induces significant cardiovascular side effects (sudden-onset hypotension or hypertension, tachycardia, extrasystoles) sometimes combined with nausea, vomiting, diarrhea, profuse sweating, and an anaphylactic reaction which may include laryngeal edema. These adverse effects are even more severe if the subject has not in fact been poisoned with cyanide. This is particularly important because the use of antidotes in the context of fires is always based on presumptions with no formal laboratory test confirmation to hand (blood lactates can be determined before the transfer to hospital, but cyanides can only be assayed subsequently and, even then, on condition that a venous sample has been taken using a heparinized tube at the site where

the poisoning occurred before the antidote has been administered). This product is still marketed in France and the UK under the name Kelocyanor®.

However, this product could be of some limited interest in the context of catastrophic terrorist attacks if cyanide poisoning has been confirmed and after the immediately available stocks of hydroxocobalamin have been exhausted.

The antidote currently used in France and which received a marketing authorization for the United States, Japan, and Europe in 2006 and 2007 is hydroxocobalamin, which is marketed under the name of Cyanokit® (Figure 16.3).

Hydroxocobalamin acts in acute cyanide poisoning by replacing a hydroxyl group (OH^-) by a cyano group (CN-), thus producing essentially non-toxic cyanocobalamin, which is excreted entirely in the urine. This antidote takes the form of a freeze-dried (lyophilized) product that is used intravenously after being reconstituted – 5 g of the freeze-dried product is supplied in a single glass vial, which corresponds to the 70-mg/kg dose of hydroxocobalamin required for an adult (Fortin, 2011). Five grams of hydroxocobalamin can bind 100 mg of cyanide. The efficacy of hydroxocobalamin has been demonstrated under various different experimental and clinical conditions (Megarbane *et al*., 2003; Fortin *et al*., 2004a; Fortin *et al*., 2006).

Its rapidity of action and virtually complete innocuousness apart from red discoloration of the mucosae and urine make it the preferred drug for use in acute cyanide poisoning and it should be infused without delay when confronted by suspected cyanide poisoning at the site of the poisoning (Fortin *et al*., 2004b). If cardiac arrest or hypotension persist after 5 g of hydroxocobalamin has been administered, a second 5 g should be administered without delay, as our study tends to indicate that cardiac arrest is indicative of serious cyanide poisoning (Fortin *et al*., 2006). In a context of cardiac arrest or cardiorespiratory insufficiency, administering a dose of 10 grams of hydroxocobalamin from the outset appears to improve the prognosis. On the contrary, although administering a dose below these necessary dosages may produce a return to a normal cardiovascular state, it also carries a risk of permanent neurological sequelae (Baud *et al*., 2011).

Hydroxocobalamin should preferably be administered by the intravenous route. If it is difficult to achieve a puncture, the intraosseous route using intraosseous devices offers a rapid alternative solution. Recently, the rapid intraosseous administration of hydroxocobalamin in a child with both burns and poisoning made it possible to re-establish a compromised cardiovascular and neurological situation (Fortin, Capellier, *et al*., 2009) (Table 16.4).

16.5 Conclusion

Fires with enclosed-space smoke inhalation remain the situation in which acute cyanide poisoning most often occurs. However, the risk of its occurring is by no means negligible for the armed forces: not in a context involving gas warfare, but rather as a result of contamination by cyanide salts, storage of cyanide salts abandoned at disused industrial sites, contamination of sources of water used in military encampments during ground operations, or during fires on battle ships or in naval battles (Fortin, Locatelli, *et al*., 2011).

The usual signs of cyanide poisoning are well known, but the cardiac disorders resulting from poisoning must be investigated at an early stage by means of an electrocardiogram. They may require treatment with an antidote and not permit any delay before administration. This is why we recommend that drug supplies of hydroxocobalamin should be carried by ground and air combat units and onboard naval units.

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CHAPTER 17
Respiratory effects of cyanide

A. Eisenkraft A. Falk and Y. Bentur

At a Glance

- Respiratory responses to cyanide poisoning are mediated by peripheral chemosensors (mainly the carotid body) and central respiratory control centers in the medulla oblongata.
- Initial respiratory responses caused by cyanide are hyperpnea and tachypnea.
- In severe cyanide poisoning, there is a rapid progression to bradypnea, respiratory insufficiency, apnea, and death.
- Pulmonary edema may be present. Cyanosis is usually not seen until very late in cyanide poisoning, if at all, because venous blood is hyper-oxygenated due to inability of oxygen utilization by the cells.
- Supportive measures include ensuring a patent airway and ventilation with 100% oxygen.
- Specific cyanide antidotes have the potential to rapidly restore normal respirations. Currently, hydroxocobalamin is preferentially recommended.
- Anecdotal case reports have noted both efficacy or lack of efficacy of hyperbaric oxygen (HBO) and its potential role in severe cyanide poisoning remains unclear.

17.1 Background

Cyanide ion is dissociated from a variety of compounds such as hydrogen cyanide, cyanide salts, and cyanogen and cyanogen halides. It can also be metabolically released from nitrile compounds (e.g., acetonitrile), and can be liberated as a gaseous combustion product (Ballantyne *et al.*, 2007; Ballantyne & Salem, 2008; Baskin *et al.*, 2008). Regardless of the source of cyanide, it forms a stable complex with ferric iron in the cytochrome oxidase enzyme thereby inhibiting the electron transport chain and cellular respiration. This leads to the loss of aerobic energy production resulting in a cascade of adverse biochemical events (Ballantyne *et al.*, 2007; Ballantyne & Salem, 2008; Baskin *et al.*, 2008). Cyanide is one of the most potent poisons known, with lethal doses as low as 200 ppm during five minutes inhalation of hydrogen cyanide, and 2.9 mg/kg after oral consumption of potassium and sodium cyanide salts in humans (Sax & Lewis, 1989).

In humans, cyanide is absorbed by all routes, inhalation, ingestion, intact skin and eyes, and can cause clinical poisoning (Baud *et al.*, 1991; Geller *et al.*, 2006; Lasch & El Shawa, 1981; Sahin, 2011; Suchard *et al.*, 1998; Blanc *et al.*, 1985; Dodds & McKnight, 1985). Cyanide is a serious threat and can be used as a chemical warfare agent, in chemical terrorism and homicide due to its potent toxicity availability and ease of handling and dispersing (Ballantyne *et al.*, 2007; Ballantyne & Salem, 2008; Baskin *et al.*, 2008).

The main targets of cyanide are the central nervous system (CNS) and the cardiovascular system, as they have high oxygen demand and are most vulnerable to the loss of aerobic energy production. Respiratory and arterial blood pressure changes are the first manifestations of acute cyanide poisoning, and respiratory depression is a major cause of death. Respiratory symptoms were also reported in chronic cyanide exposure. Early recognition and prompt adequate treatment of cyanide-induced respiratory impairment is crucial for patient survival.

Toxicology of Cyanides and Cyanogens: Experimental, Applied and Clinical Aspects, First Edition.
Edited by Alan H. Hall, Gary E. Isom and Gary A. Rockwood.

The purpose of this chapter is to review the respiratory effects of cyanide; its mechanisms of actions, clinical manifestations, management and consequences.

17.2 Mechanisms of the respiratory effects of cyanide

17.2.1 General

At the cellular level, the shift to the less efficient anaerobic metabolism causes ATP depletion, mitochondrial dysfunction, oxidative stress, and programmed cell death. In humans and other mammals it results in a metabolic crisis including changes in arterial blood gases, lactic acidosis, CNS effects, apnea, cardiovascular dysfunction, coma, seizures, and death.

The initial cyanide-induced hyperventilation reflects a compensatory attempt to the inhibition of cellular respiration and decline in aerobic metabolism. This ventilatory response to hypoxia is the result of a chemoreflex system which monitors also blood CO_2 levels and pH to control their homeostasis and adapt to aerobic energy depletion by affecting respiration frequency and depth (Teppema and Dahan, 2010). The main peripheral chemoreceptors are the carotid bodies in the carotid artery bifurcations (Gonzalez *et al*., 1994; López-Barneo *et al*., 2008) and, to a lesser extent, the aortic body (Serani & Zapata, 1981). Signals from these sensors are relayed to the CNS, where they modulate the activity of the medullary neural centers which generate and govern the rhythmic respiratory movements. As will be described below, respiration may increase, decrease, stop, or change motor patterns depending on the intensity of chemical stimuli and the resulting pattern of neuronal network activity (Smith *et al*., 2009). In addition to peripheral chemoreceptors, some of the medullary centers have intrinsic oxygen/cyanide sensing capacity which takes part in regulation of the respiratory response (Teppema & Dahan, 2010). The following sections will review the main pathways and mechanisms in the chemoreflex respiratory responses to cyanide (Figure 17.1), their relevance to the pathophysiological effects of cyanide exposure, and the pathophysiology of hypoxia in general.

17.2.2 The carotid body

The carotid body (glomus) is a small (few millimeters) highly vascular chemosensory organ composed of two types of cells. Type I cells (glomus type I, chief), are of neural crest origin, rich in secretory granules, and have chemosensory ability. Type II cells (glomus type II, sustentacular), are of glial origin, envelope type I cells and are considered as supporting cells. The carotid body contains also unmyelinated nerve endings and blood capillaries (Gonzalez *et al*., 1994). These nerve endings are terminals of the carotid sinus nerve. This is an afferent nerve whose cell bodies form the petrosal ganglion which relays to the CNS via the glossopharyngeal nerve (Gonzalez *et al*., 1994), which in turn projects to the medulla at the nucleus tractus solitarius (Donoghue *et al*., 1985). Upon stimulation, type I cells secrete neurotransmitters and neuropeptides (Gonzalez *et al*., 1994; Nurse, 2010). Studies in mixed cultures of type I and petrosal neurons and intact carotid body sinus nerve preparations have shown that both acetylcholine and ATP are the main neurotransmitters responsible for the type I-petrosal synaptic transmission in hypoxia (Zhang *et al*., 2000), while dopamine and other secreted molecules may be more important as modulators of cellular interactions in the carotid body (Nurse, 2010). The chemoreceptor cells respond to changes in the partial pressures of arterial O_2 and CO_2, arterial pH, and serum glucose concentration (López-Barneo *et al*., 2008; Nurse, 2010). The molecular mechanism of oxygen sensing is still an open issue. The heme proteins heme oxygenase-2, NADPH oxidase, and NO synthase, mitochondrial cytochromes, and mitochondrial membrane complexes were proposed to participate in this process (López-Barneo *et al*., 2008; Teppema & Dahan, 2010). These molecules are sensitive to the availability of oxygen, and they were shown to modulate type I cell activity. The graded action of multiple molecular sensors with different affinities for oxygen was suggested by Prabhakar as a mechanism allowing the carotid body to respond to a wide range of partial oxygen pressures (Prabhakar, 2006). It is currently believed that oxygen sensing is based on inhibition of several potassium channels and blockade of K^+ outward currents in hypoxia (Wyatt & Buckler, 2004; Williams *et al*., 2004; Prabhakar, 2006), leading to membrane depolarization, influx of Ca^{2+}, and release of neurotransmitters (Prabhakar & Overholt, 2000). The association between cellular metabolism and K^+ channel inhibition during hypoxia may be through AMP-activated protein kinase which is very sensitive to the ATP/ADP ratio, thus serving as an indicator of metabolic stress (Wyatt *et al*., 2007).

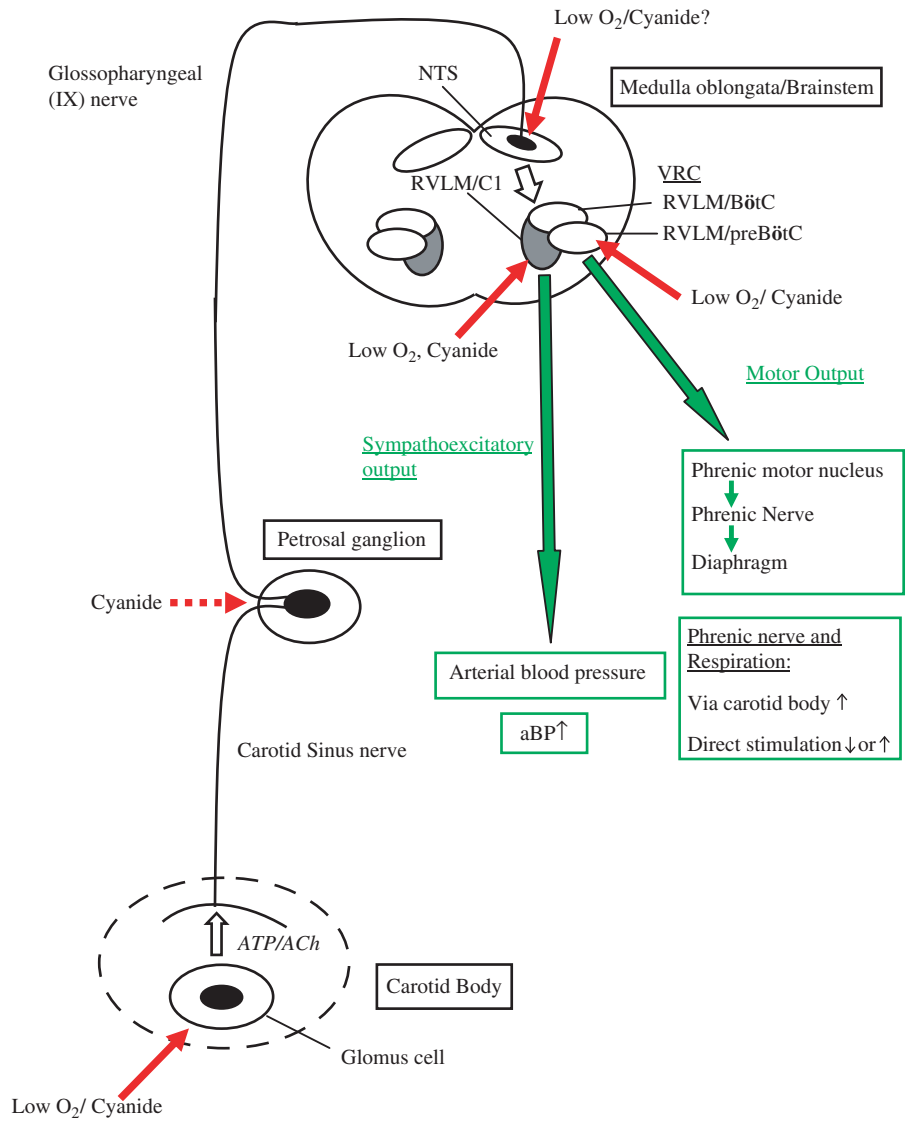

Figure 17.1 Sensory and motor pathways in the chemoreflex response to hypoxia or cyanide. The figure depicts schematically the main sensory and motor pathways, both peripheral and central, involved in the respiratory and cardiovascular response system to hypoxia or cyanide. The main peripheral chemoreceptors for blood oxygen, CO_2 and pH are the glomus cells of the carotid bodies, which can also be activated by cyanide. Endogenous oxygen and cyanide sensing capacity exists in the petrosal ganglion, the nucleus tractus solitarius (NTS), pre-Bötzinger complex (preBötC) and C1 neurons at rostral ventrolateral medulla (RVLM). The expiratory Bötzinger complex (rostral ventrolateral medulla /BötC) and inspiratory pre-Bötzinger complex are the main respiratory rhythm generators, while the C1 nucleus generated the sympathoexcitatory response to hypoxia or cyanide. Direct stimulation of C1 neurons by cyanide stimulates C1 neurons and increases arterial blood pressure. Direct stimulation of the preBötC by cyanide increases phrenic nerve output and induces gasping, while application of cyanide to other respiratory brainstem regions depresses phrenic nerve activity and respiration (Solomon, 2000). The scheme is adapted from Gourine, 2005: fig. 7.

The role of the carotid body and the carotid sinus nerve in the respiratory response to cyanide was established by the loss of the hyperventilatory response to NaCN after cryogenic destruction of carotid body type I cells in rabbits (Verna *et al*., 1975), and restoration of the response of regenerated rabbit carotid sinus nerve to direct stimulation with NaCN only if the regenerated nerve had connections to carotid chemoreceptors (Ponte & Sadler, 1989). Intravenous administration of NaCN to anaesthetized, paralyzed, and mechanically ventilated cats invoked carotid sinus nerve discharge. The responses to NaCN were dose-dependent, enhanced by hypoxia, attenuated by hyperoxia and not affected by P_{CO2} (Mulligan & Lahiri, 1981). Studies on dissociated type I cells showed that they can be electrically excited by NaCN (Biscoe & Duchen, 1989).

Further studies were undertaken to assess the roles of the peripheral chemoreceptors in the response to cyanide. In cats, bilateral carotid denervation did not abolish the respiratory response to cyanide, but attenuated it with shifting of its threshold to a higher dose. This residual responsiveness to cyanide was further decreased by bilateral aortic nerve sectioning, revealing a lesser but significant role of the arterial bodies in the chemoreflex response (Serani & Zapata, 1981). Unilateral carotid denervation did not affect the response to cyanide and allowed to measure the carotid sinus nerve output at the sectioned nerve together with respiratory response. The combined measurements showed that following a threshold, carotid nerve activity resulted in a rise in tidal volume. After a phase of increase in tidal volume correlated with neural activity, the correlation was lost due to gasps occurring at the highest activity levels (Serani & Zapata, 1981). A similar shift in dose-response of respiratory activity to cyanide was observed in carotid sinus denervated rats compared with intact animals (Cardenas & Zapata, 1983). The residual responsiveness to cyanide in carotid-denervated rats was further decreased by bilateral vagotomy, indicating response mediated by extracarotid chemoreceptors (Cardenas & Zapata, 1983). Pretreatment of carotid-intact rats with the NMDA receptor antagonist MK801 markedly attenuated the hyperventilation induced by intravenous NaCN, with a similar shift of the response threshold to a higher NaCN dose (Ohtake *et al*., 1998). This showed that the hyperventilatory response to cyanide via peripheral chemosensing requires functional glutamatergic neurotransmission, and was attributed to direct CNS stimulation by cyanide (Ohtake *et al*., 1998).

17.2.3 The nucleus tractus solitarius

The nucleus tractus solitarius is the terminus of chemoreceptor and baroreceptor afferents, and is thought to integrate and relay sensory signals to other centers at the pons and medulla oblongata (Speyer & Gourine, 2009). Pharmacologic blocking of transmission at the nucleus tractus solitarius by microinjection of glutamate receptor antagonists (Vardhan *et al*., 1993; Mizusawa *et al*., 1994) or opioid μ-receptor agonists (Zhang *et al*., 2011), as well as severing with kainic or domoic acids (Housley & Sinclair, 1998; Cheng *et al*., 2002) impaired or abolished the chemoreflex responses to hypoxia or cyanide similarly to carotid denervation. The nucleus tractus solitarius was shown to have intrinsic sensing capacity for hypoxia (Pascual *et al*., 2002).

17.2.4 Brainstem respiratory control centers

The CNS respiratory control centers, that mediate the respiratory and arterial blood pressure responses to hypoxia, are located at the rostral ventrolateral region of the medulla oblongata of the brainstem. These control centers respond to cyanide and hypoxia either by signals received from the peripheral carotid body chemoreceptors or by direct chemical sensing. Haxhiu *et al*. (1993) studied the effects of NaCN on the respiratory activity in cats after intrathecal or direct application to the ventral medullary surface. Electric respiratory muscles activity was stimulated after intrathecal injection of 10–100 μg of NaCN, but suppressed by direct application of the same amounts of NaCN to the ventral medullary surface. Arterial blood pressure and heart rate increased after both intrathecal and medullary application of NaCN, demonstrating a link of respiratory and sympathetic responses to cyanide.

Studies on the neural activity of the ventral medullary region assessed the function of this region and its carotid and vagal afferents on the respiratory response to cyanide and hypoxia. Gozal and colleagues employed optical imaging to study the neural activity of ventral medullary surface in anaesthetized and mechanically

ventilated cats. The response intensity was dependent on the degree of hypoxia (Gozal *et al*., 1993). The response of ventral medullary surface neural activity to mild hypoxia (12% oxygen in breathing) was enhanced by carotid body denervation but reduced when vagotomy was added (Gozal *et al*., 1994). In order to study the effect of a stronger hypoxic stimulus, they compared the response of ventral medullary surface activity to intravenous NaCN in intact or carotid body-denervated cats. In intact cats, intravenous injections of NaCN at 0.5 to 40 μg/kg doses increased ventilation, decreased the ventral medullary surface activity at the probed location, and both effects were dependent on the dose of NaCN. Carotid denervation abolished the responses to all NaCN doses except for a weak and delayed inhibition of neural activity at 40 μg/kg NaCN. In these experiments, bilateral vagotomy did not exert additional effects to NaCN (Carroll *et al*., 1996). These results corroborated the notion that medullary surface activity on respiration was mainly inhibitory, and carotid input in hypoxia or cyanide counteracted this inhibitory effect. At high doses of cyanide, a carotid independent, possibly direct, effect on medullary activity was observed.

The direct effects of cyanide-induced hypoxia on ventral medullary activity, motor respiratory, and sympathetic nerve outputs were studied by vertebral artery injection of NaCN (1, 10, and 20 μg) in anaesthetized and artificially ventilated cats (Mitra *et al*., 1992). NaCN caused a dose-dependent reduction in phrenic nerve activity down to apnea after 20 μg NaCN, decrease in blood pressure, and a dose-dependent increase in sympathetic nerve output. In a later study, the same investigators mapped the cyanide sensitive sites in the cat ventral medullary surface by locating the phrenic and sympathetic responses to multiple microinjection sites of cyanide. They were able to demonstrate separate phrenic and sympathetic responses to different injection sites. These studies showed that direct action of cyanide in the ventral medullary region suppressed respiration and was mediated by intrinsic neuronal cyanide and oxygen sensing.

The rostral ventrolateral region of the medulla oblongata contains neuronal centers that control the respiratory activity and arterial blood pressure and their response to hypoxia. The main rostral ventrolateral medullary regions involved are the C1 and the pre-Bötzinger complex or preBötC (Figure 17.1) (Teppema & Dahan, 2010). The C1 region neurons regulate the sympathetic response of arterial blood pressure (Sun *et al*., 1992). The C1 neurons are adrenergic, excitable by hypoxia and cyanide, and have intrinsic oxygen-sensing capacity (Sun & Reis, 1994). This is supported by their ability to respond to hypoxia or cyanide in carotid-denervated rats or direct microinjection of cyanide into the C1 region (Sun *et al*., 1992; Sun & Reis, 1994). The brainstem respiratory centers comprise an array of nuclei collectively known as the ventral respiratory group which work in coordination to generate rhythmic signals for inspiratory and expiratory movement, and can be modulated according to physiological demand (Smith *et al*., 2009). The preBötC is the main pacemaker of inspiratory muscle movement (Feldman & Del Negro, 2006; Smith *et al*., 2009). *In vivo* and *in vitro* studies with microinjection of NaCN into isolated preBötC neurons showed that the preBötC has an intrinsic capacity for oxygen sensing and response to direct challenges of cyanide and hypoxia. Microinjection of NaCN into specific sites of the preBötC in anaesthetized cats resulted in an excitatory response of phrenic nerve output, with firing patterns that differed from regular breathing in timing, frequency and amplitude (Solomon *et al*., 2000). As glutamatergic transmission is involved in oxygen sensing and responses to hypoxia and cyanide, its role in the preBötC response to cyanide was studied. Microinjection of NaCN together with IV administration of an ionotropic glutamate receptor antagonist did not abolish the response but modified cyanide-induced phrenic nerve output pattern, indicating a modulatory role of glutamatergic transmission in the response (Solomon, 2005).

The electrophysiological response to cyanide was also studied in cultures of dissociated rat ventrolateral medullary neurons which included cells from the C1 and preBötC regions. Analysis of individual neurons revealed two types of cells, one showing an excitatory response and another with a depressed response to NaCN (Mazza *et al*., 2000). The responses of the excitable cells to NaCN and hypoxia were dependent on the expression and activity of heme oxygenase-2. The depressible cells did not express heme oxygenase-2 nor required it for responding to cyanide or hypoxia (D'Agostino *et al*., 2009). Since heme oxygenase-2 was implicated as an oxygen sensor in carotid body type I cells (Williams *et al*., 2004), this work demonstrated a common oxygen sensing mechanism in medullary and carotid body chemoreceptor cells.

17.2.5 The respiratory control in cyanide poisoning – the paradigm of hypoxia

The course of the respiratory effects of cyanide poisoning is similar to that of moderate or severe hypoxia. As not all aspects of the impact of cyanide poisoning on respiratory control have been covered in the literature, it may be helpful to discuss the relevant mechanisms in light of common insights, and pointing out unexplored issues.

The response to hypoxia is characterized by a biphasic physiological course consisting of an initial stimulatory phase, followed by a depression phase. Later on, the course can be that of recovery, or apnea and death, depending on time to oxygenation and the use of ventilation, severity of exposure, and underlying medical conditions (Vizek *et al*., 1987; Neubauer *et al*., 1990; Richter *et al*., 1991; Ramirez *et al*., 1998; Thoby-Brisson & Ramirez, 2000). The initial stimulatory phase is caused by the respiratory control centers. It appears in response to signals from the carotid body chemoreceptors and is a compensatory response to decreased oxygen supply, and parallel to the early hyperventilation observed in cyanide poisoning. The respiratory depression that follows brain hypoxia, often referred as hypoxic ventilator decline or "roll-off" (Teppema & Dahan, 2010), is an adaptive response of a different type intended to minimize the energy demand and optimize the distribution of oxygen supply (Neubauer *et al*., 1990).

Severe brain hypoxia causes breathing pattern to change from eupnea (normal breathing) to gasping, which is regarded as a "self-resuscitation" mechanism that may either regain breathing or end in death (St. John, 1996). Studies using brainstem slices containing the preBötC region showed that the neural network activity patterns change during apnea to gasp-like firing patterns resulting from reconfiguring of network activity (Lieske *et al*., 2000; Thoby-Brisson & Ramirez, 2000). The microinjection experiments of NaCN into the cat preBötC (Solomon *et al*., 2000), and the *in vitro* exposure of rat brainstem and spinal cord preparations and medullary slices to NaCN (Greer & Carter, 1995) indicated that cyanide exposure may affect medullary activity and efferent nerve firing patterns by modulation of network activity rather than gross metabolic impairment. The induction of "gasp-like" phrenic nerve firing by microinjection of NaCN into some preBötC neurons was dose-dependent. Injection of NaCN at the 1 mM level induced transient and repetitive patterns, while lower doses were ineffective, and higher doses (2–20 mM) caused irreversible apnea (Solomon *et al*., 2000). In the *in vitro* experiments of Greer and Carter, exposure of their preparations to high concentrations of NaCN resulted in modified activity that was sustained for several hours.

The *in vitro* response of the preBötC to severe hypoxia was found to involve several types of neurons with different activation properties. It includes inspiratory pacemakers who fire in spontaneous bursts which are sustained during hypoxia, while non-pacemaker neurons are silenced (Thoby-Brisson & Ramirez, 2000). The bursting of the pacemaker neurons depends on persistent sodium ion currents, which are enhanced by NaCN (Rybak *et al*., 2003, 2008; Koizumi & Smith, 2008). The pacemaker activity of the preBötC is the dominant respiratory rhythmic activity during gasping (St. John, 1996; Smith *et al*., 2009). This is different from eupnea, where the rhythmic activity is generated by a concerted and timed network activity which involves all the medullary respiratory centers, including the preBötC, and extramedullary regions, mainly the pons. Stepwise severing of pontine and medullary connections modifies neural respiratory patterns down to unmasking of the preBötC, which result in gasping activity pattern (Smith *et al*., 2009).

It is well established that the initial drive for hyperventilatory response results from the carotid body; the site responsible for the depressive phase is still not characterized and only some of the factors and mediators implicated in hypoxic respiratory depression have been identified (Teppema & Dahan, 2010). The studies in anaesthetized animals and isolated *in vitro* systems show that the biphasic pattern of the respiratory response is an intrinsic property of neural networks, but may be modulated in the whole organism by external factors. The respiratory depression phase of the hypoxic response was divided by Neubauer *et al*. (1990) into three types, differing in the degree of hypoxia and mechanism of respiratory depression: type I – depression in mild hypoxia, caused by transient changes in brainstem blood flow, regulated by P_{CO2} (Ainslie & Duffin, 2009); type II – in moderate hypoxia, which is related to increased activity of inhibitory modulators rather than metabolic impairment (Richter *et al*., 1999); and type III – resulting from metabolic impairment and toxic products of severe oxygen deficiency. Inhibitory neuromodulators identified in

hypoxic respiratory depression include GABA (Melton *et al.*, 1990; Richter *et al.*, 1999; Tabata *et al.*, 2001), 5-HT (Richter *et al.*, 1999), and endogenous opioids (Neubauer *et al.*, 1990). The biochemical brainstem manifestations of metabolic derangement associated with type III hypoxic depression were lactic acidosis, ATP depletion (Neubauer *et al.*, 1988; Lamanna *et al.*, 1996), and increased extracellular potassium ion levels (Melton *et al.*, 1991). These coincided with gasping activity, but the data did not support a causal role in gasping. Animal data on the effects of variable cyanide toxic loads indicate cardiovascular and respiratory effects related to type III depression early or in mild cyanide exposure, while lactic acidosis and other markers of metabolic crisis related to type III depression, are apparent in progressive or severe poisoning (Brierley *et al.*, 1976, 1977; Purser *et al.*, 1984). We are not aware of studies on neuromodulators related to type II depression in the brainstem of cyanide-poisoned animals. The detailed characterization of the respiratory decline in cyanide poisoning is important as many of the patients may present at this stage.

Not all paradigms of response to hypoxia may share common features with the response to cyanides. In the case of chronic intermittent hypoxia, animals exposed for few days to repeated cycles of hypoxia and normoxia exhibited facilitated carotid body-dependent responses to hypoxia as an adaptation mechanism to the short hypoxic episodes (Teppema & Dahan, 2010). Cats exposed to chronic intermittent hypoxia showed facilitated carotid body responses to hypoxia but not to IV NaCN (Rey *et al.*, 2004). This difference between NaCN and hypoxia may result from quantitative or species specific differences in response characteristics. This was suggested by the occurrence of a facilitated response to IV NaCN in rats exposed to chronic intermittent hypoxia with shorter cycles of oxygen depletion (Peng & Prabhakar, 2004). As the chronic intermittent hypoxia is a paradigm of respiratory control disorders like obstructive sleep apnea (Neubauer, 2001), it is reasonable to hypothesize that modified regulatory responses underlie the adverse consequences of chronic intermittent exposure to cyanides. A possible mechanism involved in chronic intermittent hypoxia is the production of radical oxygen species, which may cause adverse effects through oxidative stress when in excess (Del Rio *et al.*, 2010).

In summary, the physiological pathways in the respiratory response to cyanides involves an initial respiratory augmentation through a chemoreflex mechanism from chemoreceptors at the carotid body to the respiratory control centers in the brainstem. This is followed by a respiratory decline which may end in apnea and involves mainly central mechanisms which are not well characterized. Studies on hypoxia due to oxygen deprivation, which has common features with the respiratory response to cyanide, have shown a clear differentiation between protective regulatory responses and effects of medullary metabolic insufficiency on the hypoxic ventilatory decline. The consequences of metabolic insufficiency may have a greater role in the pathophysiology of cyanide poisoning. Respiratory medullary neurons were shown to have intrinsic oxygen sensing capacity, and the role of this is not yet clear. Recent studies suggest that the direct effects of cyanide on the CNS may be more complex than simple inhibitory activity.

17.3 Clinical manifestations and animal studies

17.3.1 Acute exposure

Tachypnea and hyperventilation are the early respiratory signs of acute cyanide poisoning. They reflect an initial compensatory response to the decline in aerobic energy production. As poisoning progresses, hypoventilation and apnea ensue due to respiratory muscle fatigue and central respiratory depression. Hypoventilation progressing to apnea is considered a major cause of death (Ballantyne *et al.*, 2007; Lawson-Smith *et al.*, 2011; Borron, Baud, Megarbane, *et al.*, 2007; Baud *et al.*, 2002). The decline in cellular oxygen utilization results in increased oxygen content of venous blood, reduced arterial-venous measured oxygen saturation or pO_2 difference, and lack of cyanosis (Johnson & Mellors, 1988). Cyanosis can develop late in the course of poisoning and is usually accompanied by tachycardia and circulatory collapse (Ballantyne *et al.*, 2007). Noncardiogenic pulmonary edema can complicate the course of acute cyanide poisoning; cytotoxic hypoxia can lead to left ventricular failure, increased pulmonary venous pressure, and pulmonary edema in severe cases (Graham *et al.*, 1997; Matsuoka *et al.*, 2009). The type and severity of the clinical manifestations at presentation can vary according to the extent of exposure (dose and duration), time elapsed from exposure, and pre-morbid medical condition of

the victim. Early presentation or low level exposure can manifest with tachypnea while massive exposure, late presentation or pre-existing cardiovascular and pulmonary disorders can manifest with respiratory depression or apnea, as well as impaired consciousness and sometimes cardiovascular dysfunction, requiring cardiopulmonary resuscitation (Ballantyne *et al.*, 2007; Borron *et al.*, 2007). The metabolic derangements that were reported together with the respiratory manifestations include lactic acidosis, high anion gap, and increased lactate/pyruvate ratio (Ballantyne et *al.*, 2007; Graham *et al.*, 1997; Baud *et al.*, 2002). Hypopnea and apnea can contribute to the rise in plasma lactate as they aggravate the cytotoxic hypoxia (Baud *et al.*, 2002). Acidemia was found to increase the penetration of HCN into the brain in rats, which further enhances brain hypoxia and resultant respiratory depression. The increased permeability of the CNS to cyanide may be caused by modulation of blood pH by altering Pa_{CO2} and not related to decreased ionization and higher lipophilicity of HCN, as the blood pH in both respiratory acidosis (pH: 7.07 ± 0.01) or alkalosis (pH: 7.58 ± 0.01) is lower than the pKa of HCN (8.99) (Djerad *et al.*, 2001).

The most frequent manifestations that accompany the respiratory impairment are cardiovascular and neurological, which depend on the severity and clinical phase of poisoning. Due to the rapid course of poisoning, most patients present at an advanced phase, where respiratory arrest is associated with impaired consciousness or coma and hypotension. When poisoning progresses, cardiac arrest may develop with grave prognosis. This was illustrated in a series of 14 cases of cyanide poisoning recorded in Paris between the years 1988 and 2003 (Borron *et al.*, 2007). All patients in this study were treated with hydroxocobalamin and 71% survived. The five patients with the most severe neurological impairment (Glasgow Coma Score < 8) had respiratory arrest at presentation. Four of them had cardiac arrest before hydroxocobalamin could be given. Three of them died and one remained with neurological sequelae (this patient received hydroxocobalamin 12 hours post exposure). Patients with moderate or no neurological impairment (Glasgow Coma Scores of 12 and 15, respectively) had mild or no respiratory manifestations.

In another illustrative example, two groups of children were poisoned, one after consumption of apricot kernels and the other after eating a sweet made of such kernels at a birthday party (Lasch & El Shawa, 1981). In the first group, most of the children were tachypneic; the two most tachypneic children (30 to 40 breaths/minute) were also hypotensive (75 mm/Hg). Another child had coma, respiratory depression, hypotonia, convulsions, and cyanosis and died within 10 minutes after admission. Manifestations recorded in the other five patients were vomiting, crying, headaches, weakness, unsteadiness, confusion, and fainting. Treatment included 100% oxygen, gastric lavage, inhalation of amyl nitrite, and intravenous sodium thiosulfate and sodium nitrite. The seven children recovered. In the second group ($n = 16$), poisoning began within minutes after exposure. The overall signs and symptoms were similar to the former case. Two of the children were comatose and died rapidly after arrival at the hospital. Thirteen of the children recovered after treatment. Another child died during the course of treatment, probably due to a combination of cyanide poisoning and side effects of amyl nitrite. Initially he seemed to respond well to the treatment, but after two hours his condition worsened. He complained of headaches, became drowsy, and developed severe hypotension (60/20 mmHg) and tachycardia (140 beats/min). After additional administration of sodium thiosulfate and sodium nitrite he developed respiratory depression and severe cyanosis, and died within minutes. Although no blood cyanide levels were available it was probable that the child was exposed to high levels of cyanide, as he ate a large amount of the sweet and many of his stomach washings had the typical bitter almond odor.

Several animal studies investigated the respiratory, cardiovascular, and neurological effects and their relations in controlled settings. Two animal studies, in rats (Brierley *et al.*, 1976) and rhesus macaques (Brierley *et al.*, 1977) investigated whether cyanide-induced brain damage and hypoxia are due to inhibition of brain cellular respiration, lack of oxygen delivery caused by inhibition of cardiac cellular respiration, or both. The studies addressed the question by investigating the physiological, biochemical, EEG, and brain histopathology employing slow intravenous infusion of cyanide, in order to avert overdose and apnea. In both species, the start of infusion caused hyperventilation, with increase in arterial pO_2, decrease in arterial pCO_2 and increase in blood pH. Fast infusion rates resulted in severe poisoning, apneas that required the discontinuation of the infusion and sometimes mechanical ventilation and more cases of cardiac arrest and death. Slower infusion rates resulted in no apneas and more moderate

physiological effects. The detailed monitoring of biochemical parameters in the macaque study identified the onset of metabolic stress where blood pH began to fall and blood lactate to rise. This occurred in the fast-infused macaques earlier (within 10–34 minutes of infusion) than in the slow-infused macaques (at 50–80 minutes). The latter group (5 of 11 animals) developed apneas shortly after the start of infusion and required mechanical ventilation. In the macaques, the EEG and brain histopathological changes were minor and white matter changes were detected in four animals. In the rats, more severe effects like epileptic seizures or myoclonus occurred during infusions. These, as well as the histopathological brain changes were concordant with the severity of the poisoning. The authors concluded that the EEG and brain changes during and after cyanide infusions were ischemic, resulting from the respiratory and circulatory effects. This conclusion was strengthened by previous studies in animals and humans, showing that hyperventilation-induced decrease in arterial pCO2 led to constriction of cerebral arterioles, temporary ischemia, and slowing of EEG patterns (Meyer & Gotoh, 1960). In cats receiving NaCN infusions with or without additional blood pressure reduction, white matter damage was more severe in the former. The severity of brain damage was correlated with hypotension, lower blood flow in white matter, and hypoxia and not with acidosis or total cyanide dose, suggesting that the pathology was a result of tissue hypoxia and brain circulatory disturbances (Funata *et al*., 1984).

A study on the impairment of escape ability caused by sublethal inhalation of fire gases was done in cynomolgus macaques (*Macacca fascicularis*) (Purser *et al*., 1984). Four animals were exposed to HCN vapors, and three to gaseous pyrolysis products of polyacrylonitrile (PAN) containing HCN as the major toxic component. Each animal was exposed to a specified concentration of HCN for 30 minutes, with continuous monitoring of respiratory minute volume. ECG and EEG recordings were performed before, during, and after termination of exposure. Escape ability was defined as the time to incapacitation (semiconscious state with loss of muscular tone). The time course of physiological effects was similar in PAN and HCN atmosphere inhalation. Exposure immediately resulted in an abrupt rise in respiratory minute volume, with a concomitant rise in ECG T-wave amplitude and drop in heart rate, followed shortly by an increase in the slow delta wave of EEG. Within a few minutes, respiratory minute volume decreased, the ECG change reversed, heart rate increased, and the EEG slow wave started to decrease. As exposure stopped, a gradual recovery of all parameters was seen, and full consciousness was regained within minutes. Exposure to HCN vapors caused several episodes of convulsions and apnea which required resuscitation in two animals, but only one episode of convulsion was observed in the PAN group. The dose-response relationships showed an inverse linear relation of HCN dose and time to incapacitation ($R = 0.89$, $p < 0.01$ for PAN; $R = 0.96$, $p < 0.05$ for HCN). In PAN-exposed animals, the shortest observed time to incapacitation was two minutes at 196 ppm, and the longest was 30 minutes at 87 ppm of HCN. In the HCN vapor-exposed animals, the shortest time to incapacitation was 8 minutes at 156 ppm and the longest was 18 minutes at 100 ppm of HCN. For comparison, the estimated human median lethal concentrations for 30 minute exposure to HCN vapors were 190 and 630 ppm (210 and 688 mg/m^3, respectively; Ballantyne *et al*., 2007). This study shows that in fires or mass poisoning events, exposure to relatively low levels of cyanides may have respiratory and cardiovascular effects which have neurological impact that hinders escape. The studies discussed above stress that tachypnea and hyperpnea, are the earliest responses to inhibition of cellular respiration, and may be considered as ominous signs for imminent cardiovascular and neurological collapse.

17.3.2 Subchronic/repeated exposure

Repeated or chronic exposure to cyanide may occur in the following circumstances: (i) Occupational; usually industrial, due to inadequate use of occupational hygiene measures and by all routes of exposure, (ii) Consumption of cyanogenic plants (e.g., amygdalin in almond seeds, unprocessed cassava) and tobacco smoking (chronic low level exposure coupled with either a defective rhodanese enzyme or protein-calorie malnutrition with decreased sulfur-containing amino acids intake), and (iii) Treatment with IV sodium nitroprusside (especially the use of moderate to high doses or in the presence of renal impairment). Chronic exposure to cyanide can result in insidious onset of neurologic disorders (e.g., tobacco amblyopia, tropical ataxic neuropathy and Leber hereditary optic neuropathy) (Hall and Rumack, 1986; Ballantyne *et al*. 2007; Baskin *et al*.

2008). Respiratory effects were also reported in studies done in industrial settings (El Ghawabi *et al.*, 1975; Blanc *et al.*, 1985).

In an Egyptian study (El Ghawabi *et al.*, 1975), the prevalence of cyanide-related symptoms in 36 workers employed in three electroplating factories using cyanides was compared with 20 unexposed age- and socioeconomically matched controls. Airborne cyanide concentrations in the three factories ranged between 5.9 and 12.4 ppm (the time-weighted average exposure level allowed for cyanide in Egypt was 10 ppm). A positive correlation was found between daily urinary thiocyanate excretion by the workers and cyanide airborne concentrations at the factories (correlation coefficients not reported). Levels were much higher than controls, suggesting systemic absorption of cyanide in the exposed workers. The most frequent signs and symptoms were neurological, including headaches, weakness, impaired taste and smell, and giddiness (56–81% in workers vs. 15–30% in controls, p not reported). Effort dyspnea was also more frequent in cyanide-exposed workers (44% and 10% in workers and controls, respectively), as well as vomiting (44% in workers vs. 5% in controls) and angina (9% in workers vs. 0% in controls).

A U.S. study (Blanc *et al.*, 1985) surveyed cyanide-related effects in 36 ex-workers from a silver extraction factory that was closed because of a cyanide-related worker death and poor industrial hygiene. Exposure occurred by inhalation, unprotected skin contact, and ingestion. The mean time of employment was 11 ± 10.4 months. Workers were grouped into exposure levels based on exposure history for dose-response analysis. Highly exposed individuals were defined as those reporting at least 10 episodes of exposure per month. These included handling of powdered cyanide, direct contact with cyanide-containing liquids, and eating and drinking in the work areas. The HCN concentration measured in the factory one day after closing was 15 ppm. This level may be considered as a minimal estimate of airborne exposure level. The most prevalent signs and symptoms included headaches, dizziness, nausea/vomiting, and almond or bitter taste (67–72% during active work and 4–12% after factory closing). Respiratory signs and symptoms were also frequently recorded and included epistaxis, dyspnea, cough (39% during active work and 5–13% a month after closing), sore throat, chest pain, hemoptysis, nasal congestion, and wheezing (19–31% during active work and 4–7% a month after closing). These signs and symptoms are also characteristic of acute cyanide exposure and showed a marked trend of dose-response relationship. Headaches, dizziness, nausea or vomiting, and syncope were frequently reported together with dyspnea and analyzed as a symptom complex with a significant dose trend ($p < 0.01$).

The effect of chronic ingestion of cyanide was studied in rabbits fed for 10 months on standard feed mixed with 702 ppm of KCN and compared with control animals fed with a cyanide-free diet (Okolie & Osagie, 1999, 2000). In cyanide-exposed animals, weight gain was reduced relative to controls, and damage to liver, kidneys, and lungs was evident by tissue and serum enzyme studies and histopathology. Lung damage in cyanide-exposed rabbits was evident by a decrease in lung alkaline phosphatase and focal areas of edema and necrosis in histopathological examination. In another study, one group of rabbits was given cyanide-spiked feed (400 ppm/day for 4 weeks) and another received, in addition, daily oral doses of the antioxidant vitamins A, C, and E. In both cyanide-fed groups, the levels of the radical-scavenging enzymes superoxide dismutase and catalase, as well as alkaline phosphatase in lung, liver, and kidneys were decreased compared with controls. In the antioxidant vitamin-treated group the enzymes' decreases were lower and the histopathological changes in liver and kidneys were less severe than in untreated cyanide-exposed animals, with lungs appearing normal (Okolie & Iroanya, 2003). This protective effect of antioxidant vitamins indicates a role for oxidative stress in the tissue pathology of chronic cyanide exposure. The marked vulnerability of the lungs, kidneys, and liver may reflect their high exposure to systemic toxicants in general, and may not be particular to cyanide.

17.4 Management of cyanide poisoning and its respiratory effects

17.4.1 General

Cyanide binds rapidly and avidly to mitochondrial cytochrome oxidase and it can result in grave clinical outcome if not promptly treated. Therefore, management of cyanide poisoning should be based on quick termination of exposure, oxygenation, ventilation and other measures of supportive care. Specific

antidotes should be rapidly administered in order to detoxify cyanide and restore the activity of the inhibited cytochrome oxidase enzyme (Ballantyne & Salem, 2008; Baskin *et al*., 2008). Termination of exposure should include removal from exposure site, removal of contaminated clothing and thorough decontamination if required. It is of utmost importance that emergency rescue and evacuation teams be protected with adequate personal protection gear, including respirators.

17.4.2 Diagnosis of cyanide poisoning

The diagnosis of cyanide poisoning is challenging. The clinical manifestations are not specific, clinical expertise is required, and direct laboratory confirmation is not available in real time (Hall *et al*., 2007; Baud, 2007). Early manifestations include dyspnea, tachypnea, hyperpnea, tachycardia, chest pain, headache, nausea, vomiting, weakness, confusion, and dizziness. The early respiratory manifestations are of paramount importance. The ultimate proof for cyanide intoxication is a blood cyanide level of 19.2 – 39 μmol /l (0.5 – 1 mg/l) or higher (Baud *et al*., 1991), but this assay is not readily available due to technical and logistic difficulties and cannot be used for making clinical decisions. A useful laboratory surrogate is blood lactate, which was found to be proportional to blood cyanide level in cyanide-poisoned patients, with a threshold of 72 mg/dl (8 nmol/l), equivalent to the toxic threshold level of 1 mg/l cyanide (Baud *et al*., 2002). The finding of metabolic acidosis in fire victims is suggestive of exposure to cyanide. Another useful marker is narrow arterial-mixed venous P_{O2} difference and "arterialization" of retinal veins, both suggest impaired oxygen utilization (Johnson & Mellors, 1988). An important issue is the identification of cyanide poisoning in fires, where the victims may be exposed to carbon monoxide either alone or together with cyanide (Baud *et al*., 1991; Baud, 2007). Cyanide exposure is suspected in patients with impaired consciousness and soot around their nose and mouth, indicating smoke inhalation (Lawson-Smith *et al*., 2011). The occurrence and course of respiratory manifestations are important in discriminating between carbon monoxide and cyanide poisoning: in the former case, the respiratory manifestations are less frequent and usually late to appear, contrary to the early respiratory acceleration and later respiratory depression and arrest, which are consistent markers in cyanide poisoning (Baud, 2007).

17.4.3 Supportive treatment

The initial supportive treatment must include administration of 100% oxygen, assisted ventilation in cases of impaired consciousness and respiratory distress, hemodynamic support consisting of IV fluids and vasopressors, and correction of acidosis by IV sodium bicarbonate. At the same time supportive treatment is administered, antidotal therapy should also be given. In no case should antidotes replace oxygenation and ventilation, yet they should not be withheld until laboratory identification of cyanide is provided.

Oxygen administration (humidified 100% oxygen) is practiced as an initial and adjunct to antidotal therapy, as it was shown to potentiate the effectiveness of other therapies but not to be protective when given alone (Way, 1984; Ballantyne & Salem, 2008). In animal studies, oxygen therapy was shown to enhance dramatically the protection and metabolic recovery after cyanide poisoning when given together with sodium nitrite and sodium thiosulfate, but not when given with sodium nitrite alone, and only to a minor degree when given with sodium thiosulfate alone (Isom & Way, 1974). In one human study, breathing 30–40% oxygen for 10 minutes prevented the hyperventilatory response, induced by intravenous injection of a sublethal dose of sodium cyanide (0.1 mg/kg body weight), in a subject breathing room air (Cope, 1961). In dogs, breathing of 100% oxygen, starting 10 minutes before cyanide administration increased the lethal dose of cyanide from 2.4 ± 0.2 mg/kg to 3.2 ± 0.4 mg/kg (Ivankovich *et al*., 1980).

17.4.4 Antidotes

The main cyanide antidotes in use are the Cyanide Antidote Kit® (Lilly), hydroxocobalamin and to a lesser extent dicobalt-ethylenediamine tetraacetic acid (dicobalt Edetate) and 4-dimethylaminophenol (4DMAP) (Hall *et al*., 2007). The standard Cyanide Antidote Kit® contains amyl nitrite, sodium nitrite, and sodium thiosulfate. The nitrites form methemoglobin which attracts cyanide due to its higher affinity compared with cytochrome oxidase. They may have some additional beneficial effects besides cyanide sequestration. Their use can be associated with serious side effects such an unpredictable high levels of methemoglobin, hypotension, and hemolysis. Sodium thiosulfate activates rhodanese which detoxifies cyanide by forming thiocyanate, a renally excreted compound

(Ballantyne & Salem, 2008; Baskin *et al.*, 2008; Geller *et al.*, 2006). A novel detoxifying antidote is hydroxocobalamin which competes with cytochrome oxidase on cyanide to form cyanocobalamin which is readily cleared by the kidneys. Animal studies and uncontrolled human data suggest it is superior to the Cyanide Antidote Kit due to better efficacy and less adverse effects (it does not cause methemoglobinemia, hypotension, or hemolysis). Some clinicians recommend its use together with sodium thiosulfate. Hydroxocobalamin has long been used in France for cyanide poisoning (especially in fire victims) and was approved by the FDA in 2006 for this indication (Ballantyne *et al.*, 2007; Ballantyne & Salem, 2008; Baskin *et al.*, 2008; Geller *et al.*, 2006; Baud, 2007; Borron, Baud, Megarbane, *et al.*, 2007). Recovery depends on the severity of exposure, time to treatment and its adequacy, and co-existing cardiovascular and pulmonary diseases. If administered promptly and appropriately, recovery rate can be high and without sequelae (Ballantyne *et al.*, 2007; Ballantyne & Salem, 2008; Baskin *et al.*, 2008; Geller *et al.*, 2006; Borron, Baud, Megarbane, *et al.*, 2007).

17.4.5 Respiratory beneficial effects of cyanide antidotes

If promptly treated, respiratory recovery in cyanide poisoning is fast but its relation to survival depends on additional factors. In a study of beagle dogs poisoned with intravenous KCN (4mg/kg), the anaesthetized animals manifested a short hyperventilatory gasp, developed apnea within 1–2 minutes, and were treated with 4DMAP or dicobalt Edetate one minute after administration of KCN. Respiratory arrest was immediately relieved by both compounds, and all animals survived. In dogs treated four minutes after KCN exposure, survival rates were lower (4/7 after 4DMAP and 1/4 after dicobalt Edetate). In the surviving animals respiratory arrest was immediately relieved (Klimmek *et al.*, 1979). In this study, the animals did not receive ventilatory assistance. The higher efficacy of 4DMAP versus dicobalt Edetate in the delayed treatment group was attributed to sufficient cyanide sequestration by the 4DMAP-formed methemoglobin, and the metabolic inhibitory effects of dicobalt Edetate, as manifested by higher and more persistent lactic acidosis (Klimmek *et al.*, 1979). In another study with beagle dogs, the efficacy of hydroxocobalamin together with assisted ventilation and 100% oxygen was evaluated (Borron *et al.*, 2006). The dogs were dosed by infusion of KCN for three minutes until reaching apnea, and then were treated by vehicle or 75 mg/kg or 150 mg/kg hydroxocobalamin I.V. and simultaneous mechanical ventilation (for 15 minutes). The earliest improvement in hydroxocobalamin-treated dogs was cardiovascular recovery: a hydroxocobalamin dose-dependent rise in arterial blood pressure began within 1–3 minutes of treatment, peaking at 150–180% of baseline and remaining higher than controls until the end of the experiment. Heart rate was significantly lower than in controls. Spontaneous respiration was regained after ventilation in all treatments, with respiratory minute volume rising to a peak followed by a decline and stabilization. In hydroxocobalamin-treated animals, minute volume peaked earlier and reached higher levels than in vehicle-treated animals, whose minute volume continued to decline. The resolution of lactic acidosis was faster in the hydroxocobalamin-treated group than in vehicle-treated animals. Ten out of 17 animals in the vehicle group were euthanized within 4 hours, while four additional animals were euthanized in 2–4 days due to severe neurologic sequelae. In the 75 mg/kg hydroxocobalamin group, 15 of 19 dogs survived (89% survival) while in the 150 mg/kg group, 18/19 animals survived (95% survival). All surviving animals in both groups had no sequelae.

The studies described above illustrate that recovery was dependent mainly on the time to treatment, type of treatment (with respiratory support or without), type of antidote, and extent of cardiovascular recovery. Respiratory recovery is fast, and seems to be a contributory factor, together with cardiovascular recovery, in the outcome of treatment.

17.4.6 Respiratory and related adverse effects of cyanide antidotes

Sodium nitrite can cause excessive methemoglobinemia which reduces oxygen delivery and aggravates cyanide-induced oxygen utilization insufficiency. Two reported fatal cases of cyanide-poisoned children were attributed to excessive methemoglobinemia caused by sodium nitrite overdose (Berlin, 1970; Lasch & El Shawa, 1981). Other adverse effects of nitrite administration, either amyl nitrite inhalation or IV sodium nitrite, are hypotension and hemolysis, especially in patients with Glucose-6-phosphate dehydrogenase deficiency (Lavon & Bentur, 2010). In fire gases inhalation,

sodium nitrite may be extremely deleterious if given in poisoning where carbon monoxide, a potent carboxyhemoglobin producer, is involved together with cyanide (Baud, 2007).

Treatment with dicobalt Edetate, was associated with pulmonary, laryngeal, and facial edema in a case of a worker with severe cyanide poisoning (Dodds & McKnight, 1985). In general, cobalt salts are known to cause hypotension, gastrointestinal disorders, and respiratory depression (Litovitz *et al*., 1983).

Dogs administered IV 3000 mg/kg sodium thiosulfate alone developed metabolic acidosis, hypoxemia, hypernatremia, ECG abnormalities, and blood pressure changes. Intravenous administration of 500–4000 mg/kg sodium thiosulfate caused hypotension in both dogs and rabbits. These doses exceed the recommended adult dose of sodium thiosulfate in the Cyanide Antidote Kit (179 mg/kg for a 70 kg man) (Hall *et al*., 2007). IV administration of sodium thiosulfate to dogs at 150–180 mg/kg did not cause blood pressure change (Ivankovich *et al*., 1980).

Hydroxocobalamin does not cause hypotension, does not compromise the blood oxygen-carrying capacity, and is well tolerated even at high doses. Its safety and efficacy were evident in pure cyanide poisoning (Borron, Baud, Megarbane, *et al*., 2007) and also in smoke inhalation patients suspected of cyanide exposure, and subsequently confirmed (Borron, Baud, Barriot, *et al*., 2007).

17.4.7 Hyperbaric oxygen

Hyperbaric oxygen (HBO) treatment was reported in a few cases of cyanide poisoning with mixed results, and variable recommendations exist in the literature (Litovitz *et al*., 1983; Gabb & Robin, 1987; Lawson-Smith *et al*., 2011, 2012). Animal studies on the use of HBO for cyanide poisoning also showed ambiguous results (Way *et al*., 1972; Litovitz *et al*., 1983; Gabb & Robin, 1987). A patient suffering from coma and cardiovascular shock following a suicide attempt with cyanide, received antidotal and supportive treatment, including cardiovascular resuscitation. Due to sustained coma despite the above mentioned measures, HBO treatment was used, but found to be ineffective in saving the patient's life (Litovitz *et al*., 1983). A known case was that of a burglar breaking into an electroplating factory, hiding from the police in a cyanide-containing vat. He was severely poisoned but successfully treated with amyl nitrite, thiosulfate, and HBO. This comatose and convulsing patient was treated at three atmospheres for 30 minutes and along with his recovery the pressure was reduced gradually down to one atmosphere for about one hour (Trapp, 1970). However, two articles reported success in two cases of severe poisonings. Both patients attempted suicide by ingesting cyanide-containing solutions, were unconscious at presentation, unresponsive to a standard treatment protocol which included 100% oxygen and IV cobalt Edetate, sodium nitrite, and sodium thiosulfate, but recovered following HBO treatment (Davis & Ewer, 1988; Goodhart, 1994). The authors of both reports suggested considering HBO for severe victims that do not respond to standard therapy. HBO is known to facilitate the dissociation of carbon monoxide (CO) from cytochrome a3, and was shown in a study of cyanide-exposed rats to significantly increase the concentration of cyanide in the blood, relative to controls. However, this effect could not be shown in a clinical study that compared the effect of HBO therapy on blood cyanide and lactate levels between patients exposed to fire gases, cyanide and CO, and CO only exposed controls. Some lowering of cyanide and lactate blood levels were observed in the former patients (Lawson-Smith *et al*., 2010). In another publication, the same authors recommend HBO therapy for severe cases of smoke inhalation presumably because they are poisoned by both cyanide and CO and because HBO is commonly used in CO poisoning (Lawson-Smith *et al*., 2011). The mechanism of HBO in cyanide poisoning is not known, and these authors suggested that HBO, and not normobaric oxygen, increases the bioavailability of nitrous oxide (NO), which was shown to attenuate the inhibition of cytochrome a3 by cyanide (Lawson-Smith *et al*., 2011). The drawbacks for HBO in cyanide poisoning are the limited availability of HBO facilities, risk of transferring severely poisoned patients, efficacy of supportive and cyanide antidotal treatments, and the limited ability to treat critically ill patients in the chambers.

In summary, the management of cyanide poisoning is composed of early removal from exposure, and timely administration of intensive supportive treatment and specific antidotes. The main issues are oxygenation (generally with normobaric 100% oxygen), ventilation, and antidotes that detoxify cyanide, restore the activity of the inhibited cytochrome a3 and reinstate aerobic energy production. Restoration of normal respiration is the first stage in recovery from cyanide poisoning as

long as cardiac arrest has not occurred. Hydroxocobalamin, with or without sodium thiosulfate, is considered the drug of choice for cyanide poisoning due to its excellent safety and efficiency profiles.

17.5 Conclusion

Cyanide in its various forms inhibits cytochrome oxidase and interferes with the electron transport chain and aerobic energy production. The respiratory and cardiovascular systems are the first to react to cyanide.

The immediate responses to acute cyanide exposure are hyperventilation and a drop in arterial blood pressure, followed by respiratory distress and apnea if not treated. This course of events, which is also characteristic in severe hypoxia, reflects adaptive regulatory responses intended to protect the brain and heart from the energy shortage.

The respiratory responses to cyanide involve peripheral chemoreceptors (mainly the carotid body), and respiratory control centers in the medulla oblongata which respond to signals from peripheral chemoreceptors and directly to cyanide. Not all mechanisms underlying the respiratory effects of cyanide are well defined, for example, the respiratory decline and apnea, pathophysiology of chronic or intermittent exposure to cyanide, and the role of oxygen (including HBO) in the treatment of cyanide poisoning.

The diagnosis of cyanide poisoning is based on possible history of exposure to cyanide compounds, suggestive clinical manifestations, and direct laboratory tests (e.g., lactic metabolic acidosis). Blood cyanide levels are not readily available and in no case should treatment be withheld until laboratory confirmation is obtained.

The management of cyanide poisoning is early removal from exposure, prompt intensive respiratory support and timely administration of specific antidotes, mainly hydroxocobalamin.

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CHAPTER 18
The analysis of cyanide in biological samples

Brian A. Logue and Brendan L. Mitchell

At a Glance

- Analysis of cyanide in biological matrices is important for determination of human cyanide exposure, experimental studies of cyanide poisoning and therapeutic agents, and monitoring patients administered cyanogenic medications (i.e., nitroprusside).
- Cyanide's short *in vivo* half-life, instability in specimens under typical storage conditions, and significant endogenous cyanide concentrations in biological matrices present analytical challenges.
- For most analytical techniques, accurate cyanide measurement directly from biological matrices is not possible. The cyanide must be removed from the matrix before analysis can be done.
- A variety of analytical techniques have been developed for measuring cyanide, including: spectroscopy, gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis, and electrochemical methods.
- Sensors (portable units using biological or chemical components to elicit a selective analytical response to cyanide) may be employed for cyanide detection.
- Some analytical techniques measure cyanide metabolites rather than the cyanide itself.

18.1 Introduction

The analysis of cyanide from biological matrices is important for a variety of reasons, including the determination of cyanide exposure for humans, experimental studies of cyanide toxicity or therapeutics, and monitoring cyanide concentrations for individuals receiving cyanogenic drugs. Although many techniques are available, analysis is fraught with difficult challenges, including the short half-life of cyanide *in vivo* (Sousa *et al*., 2004; Hartung, 1982; Ansell & Lewis, 1970), the instability of cyanide concentrations under typical storage conditions (Pettigrew & Fell, 1973; Boxer & Rickards, 1952a; Seto, 1995; Lindsay *et al*., 2004; Seto, 2002; Calafat & Stanfill, 2002; Lundquist *et al*., 1987; Vesey & Wilson, 1978; Ballantyne, 1977), and significant endogenous concentrations of cyanide in biological matrices (Table 18.1). Considering the problematic nature of cyanide analysis from biological samples, the most appropriate biological matrix, storage conditions, sample preparation, and analysis techniques must be carefully chosen to ensure accurate determination of cyanide concentrations.

18.2 Biological matrices

For humans, analysis of cyanide has been attempted from blood (Felby, 2009; Sadeg & Belhadj-Tahar, 2009; Gambaro *et al*., 2007; Fasco *et al*., 2007; Lindsay & O'Hare, 2006; Frison *et al*., 2006; Dumas *et al*., 2005; Lv *et al*., 2005; Dirikolu *et al*., 2003; Tracqui *et al*., 2002; Meiser *et al*., 2000), tissue (postmortem) (Westley & Westley, 1989; Suzuki *et al*., 1982; Ballantyne, 1975; Tomoda & Hashimoto, 1991; Bogusz, 1976), urine (Sano *et al*., 1989b, 1989a; Suzuki *et al*., 1982; Hassan *et al*., 2009; Imanari *et al*., 1982; Funazo *et al*., 1981), saliva (Tsuge *et al*., 2000; Paul & Smith, 2006; Hassan *et al*., 2009; Xu *et al*., 2008), and expired air (Kaur *et al*., 1987; Stamyr *et al*., 2008; Raju & Gupta, 1989; Lundquist *et al*., 1988; Boxer & Rickards, 1952b). Although traces

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Table 18.1 Endogenous concentrations of cyanide in common biological matrices.

Biological matrix	Human non-smokers		Human smokers		Mice	
	Mean[a]	Ref	Mean[a]	Ref	Mean[a]	Ref
Whole Blood (μg/l)	23.66 ± 27.82 (155)	Lundquist *et al*., 1985, 1987; Ballantyne, 1977; Chinaka *et al*., 1998; Clark *et al*., 1981; Chandra *et al*., 1980; Toida *et al*., 1984; Sano *et al*., 1989b, 1992; Symington *et al*., 1978; Tsuge *et al*., 2000; Hasuike *et al*., 2004	59.80 ± 49.40 (142)	Lundquist *et al*., 1985, 1987; Ballantyne, 1977; Chinaka *et al*., 1998; Clark *et al*., 1981; Chandra *et al*., 1980; Toida *et al*., 1984; Sano *et al*., 1989b, 1992; Symington *et al*., 1978; Tsuge *et al*., 2000; Hasuike *et al*., 2004	22.88 ± 28.08 (28)	McMahon and Birnbaum, 1990; Morgan, Isom and Way, 1979; Doherty, Smith and Ferm, 1982; Smith and Kruszyna, 1974; Chanas, Wang and Ghanayem, 2003; El Hadri, Chanas and Ghanayem 2005; Chan *et al*., 2010; Wang, Chanas and Ghanayem, 2002
Urine (μg/l)	8.84 ± 0.52 (27)	Chandra *et al*., 1980; Sano *et al*., 1989a, 1989b	15.34 ± 1.30 (28)	Chandra *et al*., 1980; Sano *et al*., 1989a, 1989b	NA[b]	
Saliva (μg/l))	107.12 ± 106.34 (30)	Tsuge *et al*., 2000; Paul & Smith, 2006	17.16 ± 13.52 (20)	Tsuge *et al*., 2000	NA	
Expired Air (ppb)	3.54 ± 3.91 (346)[c]	Spanel *et al*., 2007a, 2007b; Schmidt *et al*., 2011; Stamyr *et al*., 2009; Wang *et al*., 2008; Cap *et al*., 2008; Kushch *et al*., 2008	1.60 ± 1.50 (81)	Kushch *et al*., 2008	NA	
Tissue (nmol CN/g tissue)						
Liver	NA		NA		1.56 ± 1.00 (9)	McMahon & Birnbaum, 1990; Chanas *et al*., 2003; Wang *et al*., 2002
Kidney	NA		NA		1.25 ± 0.64 (8)	
Brain	NA		NA		0.78 ± 0.40 (9)	
Heart	NA		NA		NA	
Spleen	NA		NA		NA	
Stomach contents	NA		NA		NA	

[a] The number in parenthesis represents the number of subjects evaluated.

[b] NA = not available.

[c] Includes subjects from experiments that did not differentiate between smokers and non-smokers.

of cyanide have been found in urine, saliva, and expired air, correlation of cyanide toxicity and *systemic* cyanide concentrations with the cyanide concentrations found in these matrices is difficult. Therefore, blood (living and postmortem) and tissues (postmortem) have been the preferred biological samples for cyanide analysis, with blood being the most versatile biological sample. Although this is the case, urine, saliva, or expired air may be more appropriate, depending on the necessary ease of obtaining the sample, the analytical method employed, and the goal of the investigation. For animals, saliva and urine samples can be difficult to obtain, especially from small animals. Therefore, blood and tissues are generally more amenable to cyanide analysis.

There are a number of difficulties in measuring cyanide concentrations from tissues that an analyst must recognize. Just as endogenous concentrations of cyanide are variable between species, rhodanese and sulfur donor concentrations are as well, causing the interspecies rate of cyanide metabolism to be inconsistent (Baskin *et al*., 2004). Also, tissue concentrations of free cyanide are generally low, with

significant concentrations of bound cyanide. Therefore, the possibility of extracting bound cyanide should be considered prior to conducting a specific cyanide analysis technique.

18.3 Sample storage

One of the major problems associated with analysis of cyanide is its instability in biological matrices, which can lead to highly variable and inaccurate results (Pettigrew & Fell, 1973; Boxer & Rickards, 1952a). It has been found that the amount of cyanide within a biological sample can vary by up to 66% in 14 days, depending on the storage temperature (Seto, 1995, 2002; Lindsay *et al*., 2004; Calafat & Stanfill, 2002; Lundquist *et al*., 1987; Vesey & Wilson, 1978; Ballantyne, 1977). Additionally, variable cyanide concentrations must be considered when interpreting results for postmortem cyanide analysis (Ansell & Lewis, 1970; Ballantyne, 1975; Curry, 1976; Bright *et al*., 1990; Chikasue *et al*., 1988). Therefore, whenever possible the analysis of cyanide from biological samples should be completed immediately to ensure an accurate measurement of cyanide concentrations.

Although immediate analysis of cyanide is the best approach to determining accurate cyanide concentrations, this is not possible in some cases. Therefore, steps should be taken to minimize cyanide loss. Because HCN is volatile, high pH (> 10.5), air-tight vials, and preserving agents have been used to minimize evaporative loss. Although low temperature storage may reduce loss due to evaporation and metabolism, there are many discrepancies in the literature on this subject (Pettigrew & Fell, 1973; Ballantyne, 1977; Lundquist *et al*., 1985; Egekeze & Oehme, 1979; Groff *et al*., 1985). Adjusting the pH of biological samples reduces the loss of HCN by converting it to the cyanide ion (CN^-), but because CN^- is nucleophilic, this can lead to a loss of free cyanide in biological samples. Generally, nucleophilic losses are reduced by sequestering the cyanide using chemical agents to complex the cyanide (e.g., hydroxocobalamin) (Lundquist *et al*., 1985; Lundquist & Sörbo, 1989; Houeto *et al*., 1995).

When storing biological samples for cyanide analysis, loss of cyanide through volatilization or nucleophilic reactions is not the only issue that must be considered. Artifactual formation of cyanide may also occur (Seto, 2002; Lundquist *et al*., 1987; Vesey & Wilson, 1978; Ballantyne, 1977). Oxyhemoglobin (Seto, 1995), thiocyanate oxidase (Seto, 2002; Vesey & Wilson, 1978), and white blood cells (Lundquist *et al*., 1987) may oxidize thiocyanate to cyanide and cause an increase in cyanide concentrations in biological samples during storage. Microorganisms may also be responsible for cyanide production, although low temperature storage helps to eliminate their growth (Seto, 2002). Several authors have suggested techniques to prevent artifactual cyanide formation. For example, Seto *et al*. (1995) and Sano *et al*. (1992) demonstrated artificial formation of cyanide from thiocyanate and showed that ascorbic acid and methanol, respectively, were successful at preventing cyanide formation.

18.4 Sample preparation

Because of the highly complex chemical nature of biological samples, the analysis of cyanide *directly* from this type of matrix is extremely difficult. Therefore, sample preparation techniques are generally necessary to separate cyanide from the biological matrix of interest. By far, the most popular sample preparation technique is microdiffusion/distillation (Pettigrew & Fell, 1973; Lundquist *et al*., 1987; Sano *et al*., 1989b; Dirikolu *et al*., 2003; Tracqui *et al*., 2002; Maseda *et al*., 1989; Rodkey & Collison, 1977; Hughes *et al*., 2003; Conway, 1950; Aldridge, 1944). This technique is carried out in a two chamber apparatus with an outer "sample" chamber and an inner "capture" chamber (Figure 18.1).

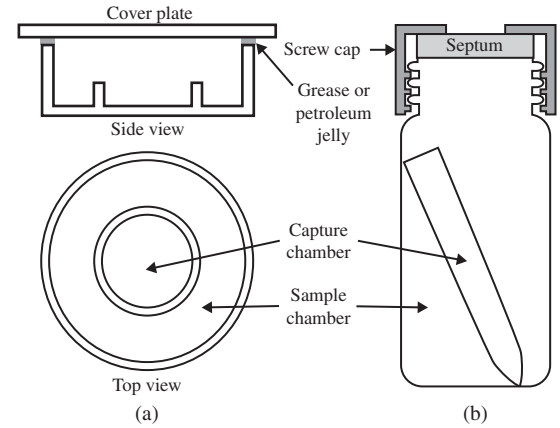

Figure 18.1 Microdiffusion apparatuses for the preparation of biological samples for cyanide analysis. (a) Classical Conway microdiffusion apparatus (Conway, 1950). (b) A modern microdiffusion apparatus (Tracqui et al., 2002) typically used prior to chromatography.

The biological sample is added to the sample chamber and it is acidified, normally with a mineral acid (e.g., H_2SO_4 or H_3PO_4). Cyanide gas (HCN) is transferred to a capture solution via diffusion and is "trapped" in the capture solution. This solution is subsequently removed from the microdiffusion apparatus and analyzed. This elegant sample preparation technique is possible because of the aqueous equilibrium between CN^- and HCN ($pK_a = 9.21$ at 25°C) (Izatt *et al.*, 1962). The addition of acid forces the acid-base equilibrium to favor HCN(aq), which is subsequently liberated from solution as HCN(g). The capture solution is normally an alkaline solution (e.g., aqueous NaOH or KOH) that favors the nonvolatile cyanide ion. Buffered hydroxocobalamin (Laforge *et al.*, 1994; Cruz-Landeira *et al.*, 2000) and methemoglobin (Tomoda & Hashimoto, 1991) solutions have also been used to capture cyanide.

Microdiffusion not only removes the cyanide from complex biological matrices, it can serve to concentrate the cyanide for analysis as well. This is done by using a small volume of capture solution relative to the volume of the sample. Microdiffusion can be used prior to any analytical technique and allows the analysis of cyanide by techniques that would not normally be well-suited for biological samples. For example, most simple spectroscopic methods that would not perform adequately with direct cyanide analysis in biological fluids have been used following microdiffusion.

Other cyanide sample preparation steps have been used in conjunction with microdiffusion (Sano *et al.*, 1989b; Dirikolu *et al.*, 2003; Tracqui *et al.*, 2002; Maseda *et al.*, 1989) or to circumvent microdiffusion altogether (Calafat & Stanfill, 2002; Gambaro *et al.*, 2007; Frison *et al.*, 2006; Dumas *et al.*, 2005; Ishii *et al.*, 1998; Seto *et al.*, 1993; Zamecnik & Tam, 1987; McAuley & Reive, 1983; Darr *et al.*, 1980; Yoshida *et al.*, 1989; Murphy *et al.*, 2006; Deussing, 2010; Asselborn & Wennig, 2000; Kage *et al.*, 1991). Depending on the goals and constraints of the analysis, investigators have used solid-phase microextraction (Frison *et al.*, 2006; Takekawa *et al.*, 1998), liquid-phase microextraction (Paul & Smith, 2006; Kage *et al.*, 1991, 1994, 1996; Chen *et al.*, 1991), liquid-phase extraction (Paul & Smith, 2006; Kage *et al.*, 1994), and headspace extraction following acidification, both with (Lundquist *et al.*, 1987; Egekeze & Oehme, 1979; Boxer & Rickards, 1951; Johnson & Williams, 1985) and without (Calafat & Stanfill, 2002; Gambaro *et al.*, 2007; Dumas *et al.*, 2005; Seto *et al.*, 1993; Deussing, 2010; Asselborn & Wennig, 2000) aeration, for the analysis of cyanide from biological matrices. Also, specific chemical pretreatment steps have been used for the analysis of cyanide by a particular technique of interest. For example, because the direct analysis of cyanide by fluorescence is not possible, 2,3-naphthalenedialdehyde (NDA) and 2-aminoethanesulfonic acid (taurine) have been used to create a fluorescent NDA-CN complex for fluorescence analysis (Sano *et al.*, 1989b; Felscher & Wulfmeyer, 1998; De Montigny *et al.*, 1987) (Figure 18.2). Both the sample preparation and chemical pretreatment steps necessary for the analysis of cyanide by individual analytical techniques will be discussed in more depth below.

18.5 Spectroscopy

Spectroscopic methods, mainly spectrophotometry and fluorometry, have been applied for decades as the staple analytical technique for the evaluation of samples containing cyanide. A large number of early spectroscopic methods relied on the König reaction (Figure 18.3) in order to generate a spectrophotometrically active product (Lundquist *et al.*, 1987; Dirikolu *et al.*, 2003; Lundquist & Sörbo, 1989; Kaur *et al.*, 1987; Baar, 1966; Aldridge, 1945; Epstein, 1947; Nagashima, 1984; Parmar *et al.*, 2010). For example, Lundquist *et al.* (1987) used the König reaction for the spectroscopic analysis of cyanide concentrations in blood. Blood

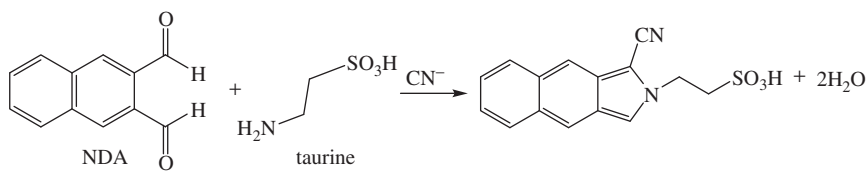

Figure 18.2 Reaction of cyanide with 2,3-naphthalenedialdehyde (NDA) and taurine to produce a highly fluorescent 1-cyano benzoisoindole product.

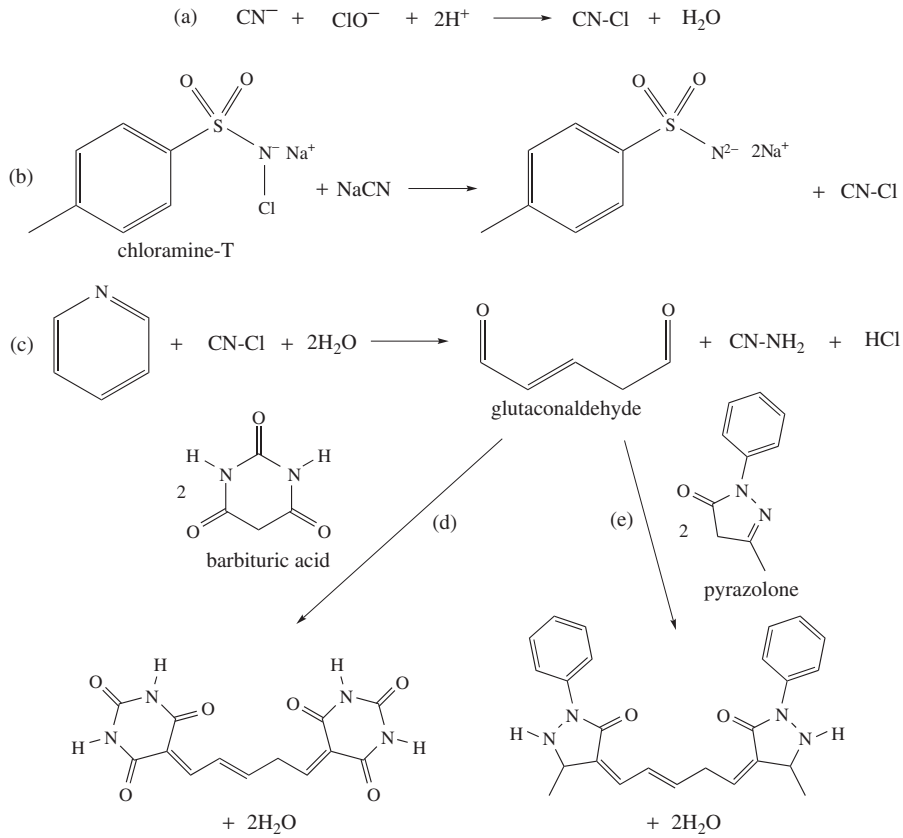

Figure 18.3 König reaction in which cyanogen chloride (CN-Cl) is synthesized by reaction of CN- and hypochlorite (a) or chloramine-T (b). CN-Cl is then converted to glutaconaldehyde by reaction with pyridine (c) and finally converted to a chromogen by reaction with barbituric acid (d) or pyrazolone (e).

samples were prepared using microdiffusion, the captured CN^- was subjected to the König reaction, and the resulting solution was analyzed fluorometrically (Figure 18.3a, c, and d). This method produced a detection limit of 0.13 µg/l, a coefficient-of-variation (CV) of 4.4%, and a linear range of 0.13–13 µg/l. Dirikolu *et al*. (2003) applied a modified König reaction scheme to the analysis of cyanide in horse blood. In this method, the cyanide ion was converted to cyanogen chloride (CN-Cl) by tosylchloramide (chloramine-T) (Figure 18.3b) and reacted with pyridine and barbituric acid (Figure 18.3c and d) to produce a red-blue dye that was analyzed spectrophotometrically. The limit of detection (LOD) for this method was 2 µg/l and the linear range was from 2 to 300 µg/l with no CV reported. Baar (1966) described a method for the determination of cyanide in whole blood using the König reaction with pyrazolone instead of barbituric acid (Figure 18.3b, c, and e) in which a blue dye was produced and analyzed spectrophotometrically after conversion of cyanide to glutaconaldehyde through a multistep reaction.

A number of reactions other than the König reaction have been used for spectroscopic analysis of cyanide. Guilbault and Kramer (1965) detected cyanide in solution by creating fluorescent quinone derivatives and Ganjeloo *et al*. (1980) adapted the method for biological fluids by reacting *p*-benzoquinone with cyanide in DMSO in order to generate a green fluorescent product (Figure 18.4). This classical method boasts a large linear range (200 to 50,000 µg/l), good precision (5% CV), but a poor detection limit of 200 µg/l. Morgan and Way (1980) adapted a fluorescent method developed by Takanashi *et al*. (1970) for the determination of cyanide in blood, where pyridoxal is catalytically

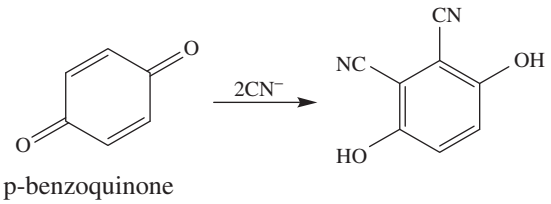

Figure 18.4 General reaction scheme for the conversion of p-benzoquinone in the presence of CN^-, resulting in a green fluorescent product.

converted by cyanide to the fluorescently active product, 4-pyridoxolactone (Ohishi & Fukui, 1968; Morales *et al*., 1997) (Figure 18.5a). Takanashi *et al*. (1970) found that the conversion of pyridoxal by cyanide should occur at pH 7.5 with the product analyzed at pH 10. While no LODs, CVs, or linear ranges were given for these original methods, Suzuki *et al*. (1982) modified the methods by using pyridoxal-5′-phosphate (Figure 18.5b) to analyze cyanide in human blood, stomach contents, and urine, resulting in an LOD of 1.59 μg/l and a linear range of 1.59–95.12 μg/l (no CV was reported).

More recently, the non-fluorescent precursors, NDA and taurine, have been used to generate a strongly fluorescent 1-cyano-2-benzoisoindole product upon interaction with cyanide (De Montigny *et al*., 1987) (Figure 18.2). Felscher and Wulfmeyer (1998) used this NDA reaction after microdiffusion to produce a method for cyanide analysis that showed good linearity (dynamic range between 2 and 1000 μg/l), sensitivity (LOD of 2 μg/l for cyanide in whole blood) and precision (<8% CV). Sano *et al*. (1989b) also utilized the NDA reaction after microdiffusion for the analysis of cyanide in blood and urine to produce an analytical method with an LOD of 0.78 μg/l, a CV of 5%, and a linear range of 0.78–260 μg/l.

Although spectrophotometric and fluorescence methods dominate the spectroscopic analysis of cyanide, other techniques, including chemiluminescence and atomic absorption, have been proposed. Lv *et al*. (2005) described a microchip chemiluminescence flow injection method for cyanide detection from whole blood. Blood samples were delivered to a reaction cell and exposed to a luminol solution, which produced a chemiluminescence reaction that was monitored to determine cyanide concentrations. The method produced an LOD of 5.98 μg/l, a linear range of 13–1300 μg/l, and a CV of 1.9% for cyanide analysis from whole blood. Atomic absorption (AA) spectroscopy has also been used to indirectly analyze CN^-, using CN-metal complexes (Danchik & Boltz, 1970; Manahan & Kunkel, 1973; Tsougas & Kevatsis, 1979). Danchik and Boltz (1970) described a method in which cyanide concentrations were determined by measurement of iron in a dicyano-bis(1,10-phenanthroline)-Fe(II) complex. They also described a method in which cyanide was determined by analysis of silver in the centrifugal precipitation of AgCN. The linear range was 60 to 5000 μg/l for Fe complexation and from 300 to 2500 μg/l for Ag precipitation. The limits of detection were 60 and 30 μg/l and the precision of the analyses were 2.2 and 1.5% CV for the Fe and Ag method, respectively. As indicated by the examples, AA methods are plagued by high LODs, an issue that has yet to be overcome for this spectroscopic technique.

Overall, spectroscopic methods generally involve relatively simple and inexpensive instrumentation but require complex reactions schemes in order to analyze cyanide from biological matrices. In addition, most techniques require microdiffusion or some other generally time consuming sample preparation technique to allow for adequate accuracy, precision, and LODs. Therefore, other sample analysis techniques have been developed as alternatives for cyanide analysis.

18.6 Gas chromatography

More recently, gas chromatography (GC) has become a popular technique for cyanide analysis because of its sensitivity, multiple detection options and the ease of cyanide determination. Prior to GC analysis, headspace (HS) sample preparation is typically performed (Calafat & Stanfill, 2002; Felby, 2009; Gambaro *et al*., 2007; Dumas *et al*., 2005; McAuley & Reive, 1983; Darr *et al*., 1980; Yoshida *et al*., 1989; Asselborn & Wennig, 2000; Nota *et al*., 1982; Cardeal *et al*., 1993, 1995; Tsukamoto *et al*., 1994; Zhang *et al*., 2005). HS is similar to microdiffusion in that cyanide is converted to HCN by the addition of a strong acid and the HCN(g) collects in the headspace of a sealed vial. The liberated HCN is collected and analyzed by GC with any number of detectors. Common detectors for cyanide analysis include the nitrogen-phosphorus detector (NPD) (Calafat & Stanfill, 2002; Gambaro *et al*., 2007; Levin *et al*., 1990; Funazo *et al*., 1981, 1982; Dong *et al*., 2008; Watanabe-Suzuki

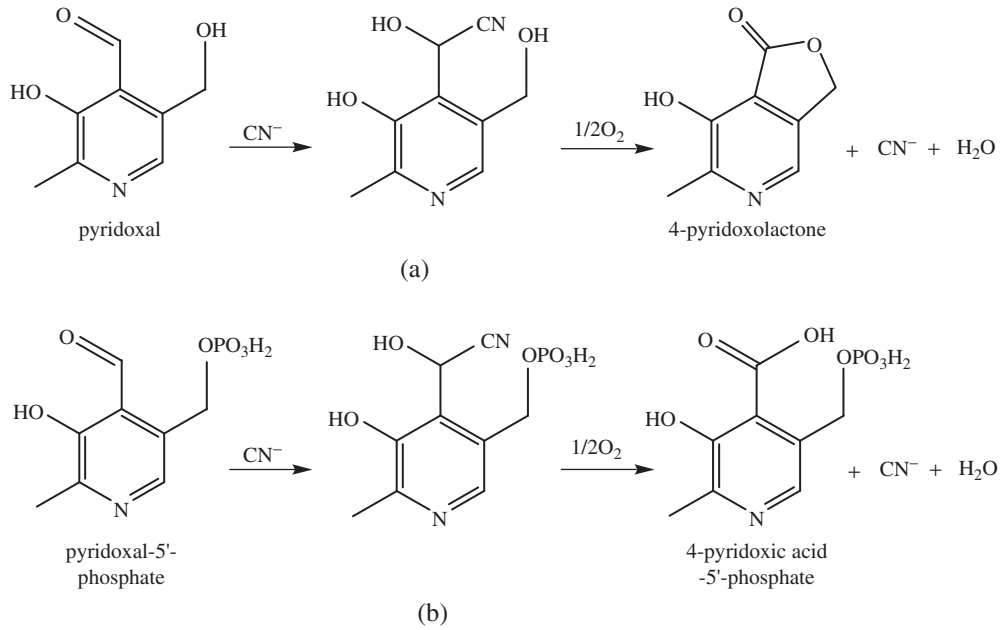

Figure 18.5 General reaction schemes for the conversion of pyridoxal in the presence of CN^- to the fluorescent product or 4-pyridoxolactone (Ohishi & Fukui, 1968; Morales et al., 1997) (a) and pyridoxal-5′-phosphate in the presence of CN- to the fluorophore, 4-pyridoxic acid-5′-phosphate (b).

et al., 2002), the electron capture detector (ECD) (Felby, 2009; Odoul *et al*., 1994; Shiono *et al*., 1991b; Maseda *et al*., 1990; Zhang *et al*., 2005; Chen *et al*., 1994; Valentour *et al*., 1974; Fouillet *et al*., 1992), and the mass spectrometry (MS) detector (Frison *et al*., 2006; Dumas *et al*., 2005; Meiser *et al*., 2000; Kage *et al*., 1996; Murphy *et al*., 2006; Thomson & Anderson, 1980). The NPD is used for cyanide analysis because it provides good sensitivity and selectivity by detecting nitrogen and phosphorus containing compounds. The ECD is used because it provides good sensitivity and stability, the latter of which the NPD lacks. When using the ECD for cyanide analysis derivatization is necessary to take advantage of the detector's strengths. Advantages of the MS detector include excellent selectivity, sensitivity, analyte identification, and the ability to distinguish between isotopes of cyanide. The ability of MS to detect cyanide based on mass allows isotope dilution (ID) (Murphy *et al*., 2006; Dumas *et al*., 2005) to be implemented, which can significantly increase the precision and accuracy of cyanide analysis.

McAuley and Reive (1983) and Zamecnik and Tam (1987) were able to analyze cyanide in the blood from fire victims with headspace extraction and GC-NPD, after liberation of HCN(g) using octanol and glacial acetic acid. McAuley and Reive (1983) produced a linear range of 500–5000 µg/l a precision of 3.4% CV and an LOD of 50 µg/l. Using acetonitrile as an internal standard, Zamecnik and Tam (1987) produced a method with better intra-assay precision (1.31% CV), a linear range of 250–1500 µg/l, and an LOD of 50 µg/l. Murphy *et al*. (2006) developed a HS-GC-MS method for the analysis of cyanide in blood using $K^{13}C^{15}N$ as an internal standard. The LOD reported for the method was 3.2 µg/l with a precision of <4.4% CV and a linear range of 10 – 3085 µg/l.

Cryogenic trapping, where volatile analytes are trapped in a small area usually at the head of the GC column, can produce better peak shapes, and decreased detection limits for HCN analysis (Ishii *et al*., 1998; Watanabe-Suzuki *et al*., 2002). Ishii and colleagues (1988) developed a cryogenic oven-trapping HS-GC-NPD method for the analysis of cyanide in whole blood. Phosphoric acid, ascorbic acid, and Na_2SO_4 were added to the blood and HCN was trapped in the injection port at −30°C. The method produced an LOD of 2 µg/l with a linear range of 25–1000 µg/l and acceptable precision (<11.8% CV for QC standards).

Solid-phase microextraction (SPME) has also been combined with HS and GC to offer increased extraction efficiencies and decreased detection limits. Takekawa and colleagues (1998) developed a HS-SPME GC-NPD technique with a carbowax divinylbenzene-coated fiber for the extraction of cyanide from whole blood samples. This method provided a LOD of 20 μg/l, a precision of <9.2% CV, and a linear range of 40–4000 μg/l. Frison *et al*. (2006) developed a method which utilizes HS-SPME along with GC-MS for the analysis of cyanide in blood. SPME was carried out with a 75 μm carboxen polydimethylsiloxane fiber and acetonitrile-d_3 was used as an internal standard. The assay produced good linearity (50–10,000 μg/l) with a precision of <8% CV and an LOD of 6 μg/l in water. The true LOD could not be established in blood because of endogenous levels of cyanide present in the matrix.

Because HS analysis with cryogenic trapping can be time consuming, the conversion of HCN to CN-Cl by reaction with chloramine-T (Maseda *et al*., 1989; Asselborn & Wennig, 2000; Thomson & Anderson, 1980) has been used as an alternative method for cyanide analysis (Figure 18.3b). The production of CN-Cl also allows detection by ECD. Maseda *et al*. (1989) produced a GC-ECD method for the analysis of blood with an LOD of 50 μg/l, a precision of <3.9% CV, and a linear range of 100–5000 μg/l using this technique.

When it is necessary to analyze cyanide and thiocyanate simultaneously, HS analysis cannot be used because of the low vapor pressure of thiocyanate. Therefore, derivatization with pentafluorobenzyl bromide (PFB-Br) with subsequent GC analysis has been performed for the simultaneous analysis of these two analytes (Paul & Smith, 2006; Kage *et al*., 1991, 1994, 1996; Chen *et al*., 1991, 1994) (Figure 18.6). Kage *et al*. (1994) described a method for the analysis of cyanide and thiocyanate in blood by GC-MS and GC-ECD after a simple alkylation with PFB-Br and simultaneous extraction into ethyl acetate. The method produced an LOD of 260 μg/l and a linear range of 520–26,000 μg/l, with a precision of <10% CV. Paul and Smith (2006) applied a modified PFB-Br method to simultaneously analyze cyanide and thiocyanate in human saliva, which produced an LOD of 26 μg/l, a linear range of 26–2600 μg/l, and a precision of 11.6% CV. Bhandari *et al.* (2012) also utilized PFB-Br for simultaneous analysis of cyanide and thiocyanate, but utilized chemical ionization to increase selectivity and linear dynamic range. This method produced a CV of 9%, an LOD of 26 μg/l, and an extremely large linear range of 260–520000 μg/l.

Analytical methods for the detection and quantification of cyanide by GC have been extensively developed and reported throughout the literature. GC methods are advantageous for cyanide analysis because of the volatility of HCN, which correlates directly with the strength of GC analysis. A significant disadvantage of HCN analysis is its low molecular weight, which may cause difficulties in the chromatographic separation of HCN from other small molecular weight compounds of the sample. Therefore, although GC analysis of cyanide provides many advantages, high-performance liquid chromatographic methods have also been developed as alternative chromatographic methods for the analysis of cyanide.

18.7 High-performance liquid chromatography

The analysis of cyanide by most high-performance liquid chromatographic (HPLC) methods requires modification of cyanide through a chemical reaction to produce a compound that is detectable and has acceptable chromatographic properties, with ion chromatography being the exception. For example, one of the most elegant HPLC methods is based on the reaction

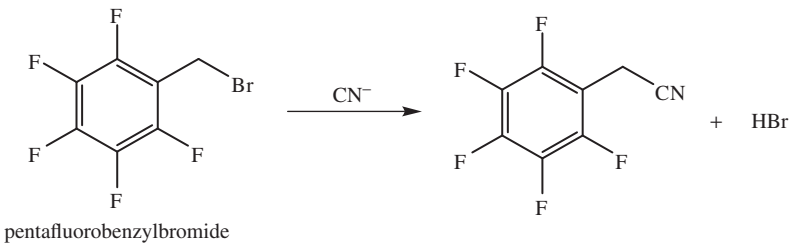

Figure 18.6 Reaction of cyanide with pentafluorobenzylbromide (PFB-Br) to produce PFB-CN.

of cyanide with NDA and taurine in order to generate a complex for fluorescence detection (Figure 18.2). An advantage of the NDA reaction is that the desired fluorescence is only produced when NDA and an amine are in the presence of cyanide. Sano *et al*. (1989a) initially applied this method to human urine, and later applied the same method to red cells and whole blood (Sano *et al*., 1992) with LODs of 0.78 and 2.6 μg/l in urine and blood, respectively. Linearity and precision were not reported in blood or urine, but were evaluated in water with a linear range of 0.0364–52 μg/l and a CV of <2.6%. Tracqui *et al*. (2002) developed an HPLC-MS method to analyze cyanide in blood using the NDA reaction after microdiffusion (Figure 18.1b) with $K^{13}C^{15}N$ as an internal standard. The method produced a good LOD of 5 μg/l, a linear range of 15–3000 μg/l, and a precision of <8.9% CV. More recently, analysis of cyanide by HPLC-MS-MS after reaction with NDA and taurine has produced analytical methods with excellent specificity and sensitivity. Lacroix *et al*. (2011) developed an HPLC-MS-MS method with online extraction for the determination of cyanide in blood using the NDA reaction. The method produced an LOD of 10 μg/l, a linear range between 26–2600 μg/l, and a precision of <2.6% CV. Mottier *et al*. (2010) developed an HPLC-MS-MS method for the analysis of hydrogen cyanide in cigarette smoke using the NDA reaction with a linear range between 2.4–331 μg/l, an LOD of 0.5 μg/l, and a CV of <10%. Bhandari *et al*. (2014) also used NDA to analyze cyanide in a method to simultaneously analyze both cyanide and thiocyanate, producing an LOD of 0.26 μg/l, a CV of 8%, and a linear range of 2.6–1300 μg/l for cyanide. The König reaction has also been used for HPLC analysis of cyanide. For example, Toida *et al*. (1984) developed a method for the analysis of cyanide in blood plasma and red blood cells by HPLC with fluorometric detection of König reaction products (Figure 18.3b, c, d). The method produced an LOD of 0.52 μg/l with a linear range of 1.3–260 μg/l (no CV was reported).

Ion chromatography (IC) is another liquid chromatographic technique which has been used for the analysis of cyanide in biological fluids (Chinaka *et al*., 1998; Zhang *et al*., 2011). Only a few methods have been developed using this technique because of high limits of detection and time consuming analysis. Chinaka *et al*. (1998) developed an IC method for the analysis of cyanide in blood. Cyanide was initially extracted from blood with methanol and water, reacted with NDA and taurine and the product was detected fluorometrically following IC separation with an LOD of 0.0988 μg/l, a linear range of 0.0988–200 μg/l, and a CV of <1.5%. Zhang *et al*. (2011) developed a method for the analysis of cyanide in cigarette smoke by IC with pulsed amperometric detection (PAD) and produced a linear range of 14.7–2450 μg/l, an LOD of 3 μg/l, and a precision of <5.2% CV.

HPLC methods for the detection of cyanide in biological fluids have been developed with some success. Liquid chromatographic methods can provide very consistent separations, excellent LODs, and good reproducibility. Although several excellent HPLC methods have been produced, the technique also has several shortcomings. Cyanide generally cannot be analyzed directly, so reaction schemes must be used in order to produce a chemical species that is amenable to liquid chromatographic methods. Furthermore, the sample preparation and analysis procedures can be time consuming and isotope dilution can be problematic for HPLC-MS because of significant mass bleed (Logue *et al*., 2005; Bogusz, 1997; Youso *et al*., 2010; Mottram & Evershed, 2003) interfering with analysis.

18.8 Capillary electrophoresis

Capillary electrophoresis (CE) has seldom been used for the analysis of cyanide (Copper & Collins, 2004; Jermak *et al*., 2006; Papezova & Glatz, 2006; Meng *et al*., 2009). However, it does provide another method to separate components of complex biological mixtures for the analysis of cyanide. Meng *et al*. (2009) described a method for the simultaneous derivatization and extraction of free cyanide in biological samples by headspace single-drop microextraction (SDME) prior to separation by CE with UV detection. This method produced an estimated LOD of 0.26 μg/l, a linear range of 2.6 – 520 μg/l, and a precision of <5.6% CV. Jermak *et al*. (2006) applied SDME with CE for the analysis of free cyanide from human urine. The method provided a linear range of 6.3–520 μg/l, an LOD of 2.0 μg/l, and a precision of <6.8% CV. Papezova and Glatz (2006) described a CE method by which cyanide was converted to thiocyanate (SCN^-) through an enzymatic reaction with rhodanese. The SCN^- was then detected spectrophotometrically after CE separation to produce

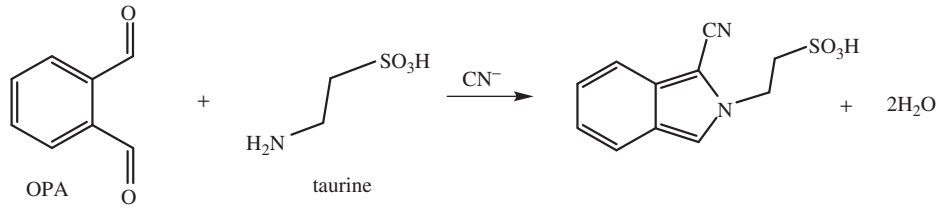

Figure 18.7 Reaction of cyanide with OPA and taurine to produce a fluorescent 1-cyano benzoisoindole product.

an LOD of 78 μg/l, a linear range of 390–13,000 μg/l, and a precision of <3.2% CV. Copper and Collins (2004) described a method for the fluorometric detection of cyanide after CE separation using the fluorescent derivatizing agent *o*-phthaladehyde (OPA) (Figure 18.7) to produce an LOD of 9.3 μg/l, a precision of 3.0% CV and a linear range of 250–2000 μg/l in water.

Overall, CE is advantageous compared to chromatography because it provides increased resolution, less waste generation, and simpler equipment, but is disadvantageous because it typically results in higher LODs, variable migration times between sample runs, and the solubility of the analyte may be low in the electrolyte. In addition, cyanide cannot be analyzed directly by CE without time consuming sample preparation techniques like SDME or reaction schemes that convert cyanide into a CE amenable compound.

18.9 Electrochemical methods

Electrochemical methods have been used sparingly for the analysis of cyanide in biological fluids (Westley & Westley, 1989; McAnalley *et al*., 1979; Stamyr *et al*., 2008; Egekeze & Oehme, 1979; Nascimento *et al*., 1998). These methods mainly involve the cyanide selective electrode. Because this electrode is not amenable to direct use in biological fluids, sample preparation must be performed. Using electrochemical methods for the analysis of cyanide is further complicated by interferences caused by sulfide, halogens, thiocyanate, thiosulfate (Rocklin & Johnson, 1983), and the requirement of high pHs (Egekeze & Oehme, 1979) in order to produce reliable, accurate results. McAnalley *et al*. (1979) described a method for the quantitative analysis of cyanide in blood using ion-selective electrodes and Conway microdiffusion. The method produced an LOD of 100 μg/l with a precision of <10% CV in blood, and a linear range of 10–10000 μg/l in water. Egekeze and Oehme (1979) described a method for the analysis of cyanide in biological materials using a cyanide selective electrode. After liberating HCN from biological samples with acid, sulfide was eliminated by bubbling HCN(g) through a lead acetate ($Pb(OAc)_2$) solution and cyanide was collected in 0.1 M NaOH and analyzed as CN^-. The method produced an LOD of 26 μg/l, a linear range of 26–13000 μg/l, and a precision of <4.8% CV.

Direct electrochemical analysis of cyanide from biological fluids has been attempted with mixed results (Lundquist & Sörbo, 1989; Stamyr *et al*., 2008; Nascimento *et al*., 1998). Westley and Westley (1989) described a method for the voltammetric determination of cyanide in biological samples with an LOD of 52 μg/l and a linear range of $52 - 2.6 \times 10^7 \mu g/l$ with no measure of precision reported. The method was designed to detect CN^- with a silver rotating disk electrode at 90 mV. Nascimento *et al*. (1998) described a method for the analysis of cyanide in whole blood, plasma, and urine using an automated polarographic detection flow system with an LOD of 7.4 μg/l and a precision of <3.4% CV. Stamyr *et al*. (2008) described rapid analysis of cyanide in exhaled air from humans for a time course analysis of HCN using an electrochemical detector without absolute quantitation of cyanide. Although cyanide wasn't quantified, the results supported the use of breath HCN as an indicator of intoxication.

Overall, electrochemical methods for cyanide produce adequate sensitivity and large working ranges. The benefits of using electrochemical methods for cyanide analysis are high accuracy, sensitivity, and analysis speeds, all of which are important in diagnosing cyanide exposure. The major drawbacks of electrochemical methods are their poor selectivity and reliability.

18.10 Sensors

Sensors are typically portable instruments that use biological or chemical components to elicit a selective analytical response to a specific analyte. Various

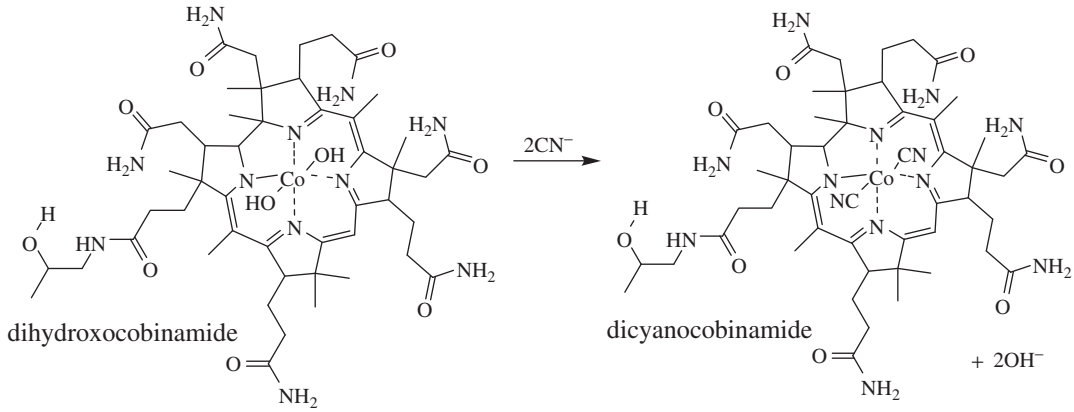

Figure 18.8 General reaction scheme for the conversion of dihydroxocobinamide into dicyanocobinamide by reaction with CN-.

sensors have been proposed for the detection of cyanide (Lindsay & O'Hare, 2006; Tatsuma & Oyama, 1996; Shan *et al*., 2003; Mak, Law, *et al*., 2005; Mak, Yanese, *et al*., 2005b; Ketterer & Keusgen, 2010; Suiping *et al*., 2010; Ma *et al*., 2011; Tian *et al*., 2013; Jackson *et al*., 2014). The sensors proposed have been based on electrochemistry, biological interactions, spectrophotometry, fluorometry, or a combination of the techniques. Also, most sensors do not directly analyze cyanide from biological samples but generally take advantage of microdiffusion to liberate HCN from complex biological samples. Shan *et al*. (2003) described a biosensor for the detection of cyanide where an amperometric biosensor was developed based on immobilization of polyphenol oxidase (PPO). Cyanide was detected based on its inhibitory action on the PPO electrode. This biosensor showed an excellent LOD of 2.6 ng/l, a linear range of 52–1300 ng/l, and a precision of 3% CV. Tatsuma and Oyama (1996) described a simple biosensor for the detection of cyanide, based on a pyrolytic graphite (PG) electrode with absorbed horseradish peroxidase (HRP), which produced an LOD of 5.2 µg/l and a linear range of 260–26,000 µg/l (precision was not reported). Mak *et al*. (2005) described a biosensor for the analysis of cyanide in fish based on the conversion of cyanide into formate and ammonia using cyanide hydrolase with an LOD of 190 µg/l and a linear range of 780–7800 µg/l (precision was not reported). Lindsay and O'Hare (2006) developed an amperometric sensor using a gold electrode plated with Nafion to detect cyanide in albumin and blood constituents without sample pretreatment. The method produced an LOD of 0.104 µg/l, and a linear range of 0.104–31.72 µg/l (precision was not reported). Blackledge *et al*. proposed a method for the detection of cyanide in blood by measuring cobinamide spectrophotometrically where dihydroxocobinamide was converted to dicyanobinamide in the presence of cyanide (Figure 18.8), inducing a shift in the absorbance. This method was modified by Ma *et al*. (2011) to develop a spectrophotometric sensor for the detection of cyanide in blood. After microdiffusion, HCN was absorbed onto filter paper impregnated with borate-buffered (pH 9.0) hydroxoaquocobinamide. The absorbance of cobinamide at 583 nm was monitored with a photodiode and related to cyanide concentration. The method produced an LOD of 13 µg/l, a precision of 1.09% CV, and a linear range of 2600–26,000 µg/l. The advantage of this method was the rapid detection of cyanide, which was achieved in approximately two minutes. A similar chemical reaction with cobinamide has also been used as a sensor platform. The Dasgupta lab produced two technologies that could be categorized as sensors using the core reaction of cyanide with cobinamide and spectrophotometric detection. The Ma *et al*. (2011) sensor produced an LOD of 13 µg/l and a CV of 1% (no linear range was reported). The Tian *et al*. (2013) sensor reported a number of LODs of 26–130 µg/l for volumes of blood from 20–100 µL, a CV of 10%, and a linear range of 13–5200 µg/l. Jackson *et al*. (2014) produced the first fluorometric sensor for the analysis of cyanide from blood using the NDA reaction (Figure 18.2) and microdiffusion to prepare the sample. The goal of the sensor was rapid diagnosis of cyanide exposure. The sensor produce an LOD of 20 µg/l, a linear range of 81–5200 µg/l, and a CV of 12%.

Sensors for the analysis of cyanide have provided good sensitivity, portability, ease of use, and rapid analysis. Therefore, sensors are well-suited to be used by first responders or medical personnel needing quick confirmation of cyanide exposure. The major drawbacks of sensor methods are their limited use in complex biological matrices, the lack of precision data for most sensors (indicating that reproducibility might be poor), and degradation of the sensor components, especially for biosensors.

18.11 Cyanide metabolites

In some situations, the analysis of the metabolites of cyanide may be a viable alternative to the direct cyanide analysis. The main reason for using a metabolite in place of direct cyanide analysis is the short *in vivo* half-life of cyanide and the difficulties in storing biological samples for cyanide analysis. The metabolites of cyanide exposure are generally longer-lived *in vivo* (Sousa *et al*., 2004; Pettigrew & Fell, 1972) and more stable than cyanide under normal storage conditions (Logue *et al*., 2005; Lundquist *et al*., 1995). Although multiple issues must be addressed when considering using a cyanide metabolite thiocyanate and 2-amino-2-thiazoline-4-carboxylic acid (ATCA), the main metabolites of cyanide exposure, have been determined in urine, saliva, tissue, and blood (Pettigrew & Fell, 1973; Tsuge *et al*., 2000; Hasuike *et al*., 2004; Hassan *et al*., 2009; Xu *et al*., 2008; Dong *et al*., 2008; Logue *et al*., 2005, 2009; Lundquist *et al*., 1995; Ershad *et al*., 2009; Patel *et al*., 2009; Demkowska *et al*., 2008; Mori *et al*., 2008; Mazloum-Ardakani *et al*., 2008; Shokrollahi *et al*., 2008; Minarowski *et al*., 2008; Baskin *et al*., 2006). Another metabolite of cyanide exposure, cyanocobalamin, has been determined from plasma (Astier & Baud, 1995; Chatzimichalakis *et al*., 2004; Butte *et al*., 1982). The analysis of cyanocobalamin is complicated by the fact that hydroxocobalamin, the precursor of cyanocobalamin, is a treatment for cyanide exposure (Houeto *et al*., 1995; Borron *et al*., 2006; Hall *et al*., 2007). Cyanide-protein adducts have also been found in blood (Fasco *et al*., 2007, 2011; Youso *et al*., 2010) and the correlation of some of these markers to cyanide exposure has been examined (Maseda *et al*., 1989; Logue *et al*., 2005, 2009; Youso *et al*., 2010; Baskin *et al*., 2006; Liu & Yun, 1993; Wood & Cooley, 1956; Cardozo & Edelman, 1952; Petrikovics *et al*., 2011, 2012). For a more detailed review of the issues concerning the analysis of cyanide metabolites from biological fluids, there are a number of excellent reviews on the subject (Lindsay *et al*., 2004; Baskin *et al*., 2004; Mak *et al*., 2005a; ATSDR, 2004; Valdes & Diaz-Garcia, 2004; Troup & Ballantyne, 1987; Bark & Higson, 1963; Logue *et al*., 2010).

18.12 Insights on cyanide analysis

The accurate analysis of cyanide from biological matrices is fraught with many difficulties mainly stemming from the instability of cyanide in biological matrices (*in vivo* and *in vitro*). Therefore, no matter the analytical technique, it is important to immediately analyze cyanide from biological samples. If this cannot be accomplished, immediate isotope dilution or the addition of preservatives should be explored depending on the analytical technique used. For most analytical techniques, accurate determination of cyanide concentrations *directly* from biological matrices is not possible. Therefore, sample preparation, normally involving acidification and sampling (e.g., headspace) or capture (e.g., microdiffusion) of HCN(g), is necessary. Once cyanide has been removed from interfering biological matrix components, a number of analytical techniques perform very well for the analysis of cyanide. That being said, internal standardization can be extremely important to ensure acceptable precision from biological matrices. This is best done using isotope dilution early in the analysis process in conjunction with mass spectrometric detection, which has led to the popularity of the HS-GC-MS technique to determine cyanide concentrations from biological samples. Of course, it is imperative that standard analysis protocols are implemented (e.g., the use of quality control standardization) when performing the final analysis of cyanide concentrations.

Overall, there are many pitfalls to consider when analyzing cyanide from biological matrices, but accurate analysis can be accomplished if care is taken at each step of the analysis.

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CHAPTER 19

Postmortem pathological and biochemical diagnosis of cyanide poisoning

Daniel Lugassy and Lewis Nelson

At a Glance

- Reported minimally fatal doses are 0.2 g KCN and 0.3 g NaCN; 1–5 mg/kg in children. HCN airborne concentrations > 100 ppm are considered potentially life-threatening.
- Persons in a number of occupations can be exposed to cyanide and these should be recognized as risk factors, especially given the paucity of reliable autopsy findings to raise suspicion of the diagnosis.
- A significant cyanide poisoning component should be suspected in all enclosed-space fire smoke inhalation fatalities. Confirmatory laboratory testing for cyanide should be done and such cases not simply recorded as "carbon monoxide poisoning" or "smoke inhalation."
- There are no pathognomonic autopsy findings for cyanide poisoning.
- Prosectors performing autopsies on suspected cyanide poisoning victims are at risk for cyanide exposure and clinical signs/symptoms of cyanide poisoning. Proper personal protective equipment including respirators and training in proper wear and use should be provided. The stomach should only be opened in a laboratory hood.

19.1 Introduction

Cyanide is a potent toxin than can cause rapid death. Historically this poison has been involved in many high profile fatalities, both as a homicidal and suicidal agent. This chapter examines antemortem and postmortem features, biochemical analysis, and the safe approach to examining of the cyanide poisoned victim.

19.2 Cyanide pathology and antemortem presentation

Cyanide inhibits multiple enzymes, most importantly mitochondrial cytochrome oxidase (a-a3 position), causing an arrest of oxidative phosphorylation. This disrupts the ability of electrons to bind to their final acceptor, oxygen, at the terminal end of the electron transport chain. Despite adequate oxygenation of the blood, oxygen cannot be utilized by the tissue, and ATP cannot be produced. Cellular hypoxia occurs, with a shift to anaerobic metabolism, and development of a metabolic acidosis with an increased lactic acid production. Lactate values of >10 mmol/l were indicative of CN concentration in fire victims compared to a non-fire control group (Baud *et al.*, 1991).

Reported minimally fatal doses are 0.2 g KCN, and 0.3 g for NaCN, or 1.2–5 mg/kg in children (Rhee *et al.*, 2011). Hydrogen cyanide concentration that exceed 100 ppm is considered life threatening. When determining the cause of death it is obviously important to gather as much information regarding the peri-mortem and antemortem clinical history. Acute cyanide poisoning manifests with rapid onset neurological findings such as headache, anxiety, agitation, confusion, lethargy, seizures, and coma. Vital sign abnormalities include initial tachypnea followed by bradypnea, and initial hypertension and bradycardia followed by hypotension

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with a reflex tachycardia. Hypotension and bradycardia are generally the pre-terminal event. The rate of onset of toxicity is related to the route of exposure. Inhalation of gaseous hydrogen cyanide results in nearly immediate collapse, while ingestion of a cyanide salt may not result in clinical effects for 20 minutes.

19.3 Exposures

19.3.1 Occupational

The occupation and/or the recent actions by the deceased may provide critical clues to uncover possible cyanide poisoning. Cyanide is readily available to chemists, jewelers, exterminators, and photographers, among other professionals. The industries of electroplating, metallic luster/hardening processing, dyeing/printing, salmon poaching, and paper/textile manufacturing use cyanide in their routine operations (Rhee *et al*., 2011; Gill *et al*., 2004; Fernando & Busuttil, 1991). It is important to recognize such occupations as risk factors for potential cyanide toxicity given the paucity of reliable autopsy findings to raise suspicion for the diagnosis (Gill *et al*., 2004). "Cymag," magnesium cyanide salt is used for pest control and is also used for salmon poaching. For this use it is put into water in small amounts and the salmon are stunned and float to top, but humans who eat these poached fish do not develop cyanide toxicity (Fernando & Busuttil, 1991).

While there are occupations with ready access to cyanide, a quick Internet search uncovers how easily anyone can purchase cyanide for "professional" reasons.

19.3.2 Homicide/suicide

Cyanide is commonly used in cases of suicidal or homicidal ingestions. The book, *Final Exit*, by Derek Humphrey and the Hemlock Society details several methods on how to commit suicide including the use of cyanide (Cina *et al*., 1994). Cyanide was implicated in the death of Rasputin in 1919, the mass cult suicide/murder of over 900 people in "Jonestown" Guyana in 1978, and the courtroom suicide of Michael Marin, a millionaire trader who ingested a cyanide pill moments after a guilty verdict returned in his arson trial. In fact a teen was found responsible for placing potassium cyanide in a beverage that lead to the death of his 17-year-old best friend, remarkably the cyanide was purchased online using his mother's credit card and without any background check or inquiry from the distributor as to his intended use of the product. This case illustrates the lack of regulation regarding Internet cyanide purchases.

19.3.3 Combustion

Hydrogen cyanide gas is responsible for causing cyanide toxicity via inhalation of fire smoke due to incomplete combustion of organic nitrogen products. This is particularly important in fires involving wool, plastics, nylon, and polyurethane found in automobiles, carpets, home furniture, and appliances. Hydrogen cyanide poisoning in a house fire may impede one's ability to escape even before other toxic effects of the fire take place, such as hypoxia and carbon monoxide (McAllister *et al*., 2008). Finally, *in vivo* metabolism of nitroprusside, nitriles (e.g., acetonitrile, acrylonitrile), and cyanogenic glycosides can produce cyanide (Desharnais *et al*., 2012).

19.4 Autopsy features

There are several "classic" postmortem findings of cyanide related deaths, including bright pink lividity, bitter almond odor, oral/esophageal lesions, and gastric hemorrhage (Gill *et al*., 2004). However, none of these are either pathognomonic or very sensitive for the diagnosis of cyanide poisoning. In fact published findings in postmortem case reports and experimental studies of animal toxicity demonstrate no reliable autopsy findings that consistently suggest cyanide toxicity. In one of the earliest investigations of postmortem cyanide, nearly half of the cases had no unusual relevant pathological findings at autopsy (Sunshine & Finkle, 1964).

Pink livor mortis or also known more simply as pink lividity has been found in several cases of acute cyanide related deaths (Nolte & Dasgupta, 1996). It is speculated the arrest of cellular respiration produces a bright pink appearance to the skin and organs postmortem due to the inability of tissues to extract oxygen. For this reason, peri-mortem venous blood may have a bright red arterial appearance and significantly elevated measured oxygen content. Pink lividity, sometimes described as lilac or purple lividity, is neither sensitive nor specific for cyanide poisoning, and may occur from refrigeration and putrefaction (Gill *et al*., 2004; Fernando & Busuttil, 1991). In a controlled study of cyanide poisoned pigs, no pathognomonic signs of pathological or histopathological changes were observed, and there was

a lack of pink lividity in the cyanide group (Fernando & Busuttil, 1991; Ballantyne, 1975; Ballantyne *et al*., 1974). Anecdotal descriptions of cyanide necropsies include pink lividity to differentiate from the "cherry red" carbon monoxide finding, but there has been no evidence to demonstrate this is a reliable finding.

While theoretically it makes sense that cyanide will inhibit the normal pathway of oxygen utilization leaving venous blood to look "arterial or red." This finding may also be due to incorrect crossover finding in cases of carbon monoxide poisoning where just over 50 years ago carbogen was used to resuscitate CO-poisoned victims. Carbogen is a mix of carbon dioxide and oxygen. The mixture went through several formulations, early on it was composed of 10% carbon dioxide and 90% oxygen, and later it was universally accepted to be 5%/95% carbon dioxide/oxygen. With some animal evidence to support it, carbogen increased the clearance of carbon monoxide compared to oxygen alone, the carbon dioxide also theoretically stimulated ventilation (Douglas *et al*., 1961). High concentration of oxygen and carbon dioxide induced vasodilation likely contributed to the cherry-red finding of blood and tissues in carbon monoxide necropsies.

Cyanide itself and the bodies of those who succumb to this toxin often emit a characteristic odor of bitter almonds (Padwell, 1997). While this scent in gastric contents or biological material during autopsy may be helpful in identifying possible cyanide deaths, many cannot detect this odor. Even when cyanide poisoning is suspected this autopsy finding is not reportedly consistently (Fernando & Busuttil, 1991). The ability to perceive this smell is a sex-linked genetic trait which about 40% to 60% of the population is unable to detect (Gill *et al*., 2004; Cina *et al*., 1994). The odor threshold is estimated to be 1–5 ppm for hydrogen cyanide (Padmakumar, 2010). But the odor of putrefaction may prevent detection of this subtle almond scent (Fernando & Busuttil, 1991). Furthermore, modern day air-flow protection systems used in morgues may dampen the ability to detect this odor (Gill *et al*., 2004).

Autopsies of patients with cyanide ingestion often reveal esophageal injury and diffuse gastric mucosal hemorrhage (Fernando & Busuttil, 1991; Nolte & Dasgupta, 1996). It appears that these findings are a result of the direct corrosive effects of cyanide salts. These findings do not appear to occur in case of cyanide injection or inhalation and are not necessarily found in every case of cyanide ingestion (Abeyasinghe *et al*., 2011). The lack of corrosive effect may be limited by the amount of cyanide, time of autopsy after death, and the presence of gastric contents (i.e., food) at the time of cyanide ingestion (Gill *et al*., 2004).

In most cases of acute cyanide toxicity, death is so rapid that there are often minimal or no neuropathological findings. In the rare instances where the patient survives more than 24 hours, diffuse hypoxic and ischemic changes in the brain are present, predominantly in the basal ganglia (Riudavets *et al*., 2005). A case report demonstrated pseudolaminar necrosis in the cerebral cortex of a young man who was poisoned with cyanide and died four days after the exposure. This finding had only been recognized in one other case of cyanide toxicity on a magnetic resonance image (Riudavets *et al*., 2005).

It should be clear that the color of the skin, mucous membranes, or organs as well as the smell emanating from the body during autopsy is neither sensitive nor specific for cyanide toxicity. The tragic events that occurred in Bhopal, India in 1984 demonstrate the confusion that color, smell, and even cyanide laboratory analysis can have on determining the cause of death at autopsy. Nearly 4000 people were killed immediately and tens of thousands more suffered immense morbidity and increased mortality when occurred when methyl-isocyanate gas leaked from a pesticide plant (Broughton, 2005). Examining the details of this event sheds some light on the often incorrect and presumptuous postmortem findings of cyanide toxicity. There has been much confusion and controversy over the role cyanide had in this disaster.

While it is true that if methyl-isocyanate is exposed to high temperatures such as those exceeding 300°C it will degrade to hydrogen cyanide, there was clear evidence that the tank that held the methyl-isocyante did not reach this level (Broughton, 2005). The cherry-red color of the blood and viscera of several victims were immediately presumed to be caused by cyanide toxicity (Broughton, 2005). Published reports of the first few autopsies after the disaster indicated that medical examiners had noticed some of the victims had "bright pink blood or cherry-red blood" and even reports of "an odor of bitter almonds." This led many to believe cyanide was the main culprit of toxicity in this event. Contradictory to this finding in the same report it was noted a "purple red" color of the blood was noted (Nemery, 1987).

The fact that some patients were given sodium thiosulfate, a treatment for cyanide toxicity also caused confusion. Sodium thiosulfate was given to patients many hours or days later and despite some reports of improved symptoms, if this were truly a case of cyanide exposure, this antidote would be required in the first few minutes of exposure to aid in survival.

The little that was known about MIC toxicity, the massive chaos, and tragedy in the immediate hours and days after the disaster contributed to the uncertainty of the exact cause of human toxicity. It also seems that the deceitful actions regarding the information released by the owners of this chemical plant, Union Carbide Corporation added to the confusion. Despite the presence of elevated thiocyanate concentrations in the urine of victims proposing cyanide toxicity, there has been no convincing analytical data to conclude the presence of cyanide toxicity from methyl-isocyanate exposure (Salmon, 1986).

19.5 Biochemical analysis

The approach to biochemical analysis in cyanide fatalities follows a similar approach to that used in other autopsies. The concentration of cyanide at the time of death will be related to several factors including route of exposure, magnitude of exposure, type of cyanide exposure, time of death, administration of antidotes, and time of collecting and performing postmortem analysis.

Blood and serum samples from the femoral artery and veins are procured as well as cardiac blood. Lungs, brain, spleen, liver, kidneys, and other visceral organs are removed for homogenate preparation as well as histological investigation. The highest concentrations of cyanide postmortem are found in whole blood when compared to cerebrospinal fluid, plasma, or serum cyanide concentrations. Homogenates of the spleen because of its sequestrations of red blood cells can also demonstrate elevated postmortem cyanide values (Gill *et al*., 2004; Ballantyne *et al*., 1974). This is because HCN is not ionized allowing it to rapidly diffuse throughout the body and into erythrocytes presumably bound primarily to methemoglobin (Gambaro *et al*., 2007).

Screening for cyanide during an autopsy often begins with the Cyantesmo test paper. This test detects hydrocyanic acid and cyanides in aqueous solutions, which means blood and tissue homogenates can be tested. This paper is pale green and turns blue in the presence of hydrocyanic acid. It has a sensitivity of 0.2 μg/ml (Gill *et al*., 2004). Normal whole blood cyanide < 0.25 μg/ml, elevated but not causing death 0.25 μg/ml to 2–3 μg/ml, and death consistent with values above 3 μg/ml (Gambaro *et al*., 2007). Average endogenous cyanide in blood is 0.059 μg/ml for non-smokers and 0.123 μg/ml for smokers (Desharnais *et al*., 2012). In cases of suicide from cyanide, whole blood cyanide concentrations have ranged from 1 to 53 μg/ml (average 12.4 μg/ml) (Desharnais *et al*., 2012). The wide range of these values demonstrates the difficulty in correlating clinical symptoms to laboratory cyanide values.

Preliminary cyanide presence is determined by classic spectrophotometric, and then confirmed via gas chromatographic, methods (Gambaro *et al*., 2007). Head space GC-MS method of postmortem cyanide blood values have been described as a faster, sensitive method using isotopically labeled cyanide as an internal standard (Desharnais *et al*., 2012). Other advantages of the HS-GC method include easier sample preparation, quicker results, and a lack of need to extraction or derivations steps. Most importantly the HS-GC method appears to be more sensitive with a limit of detection of 0.02–0.05 μg/ml compared to the 0.2 μg/ml using the spectrophotometric method. The linear dynamic range of the HS GC-MS method is 0.07–50.00 μg/ml (Desharnais *et al*., 2012; Gambaro *et al*., 2007).

The greatest challenge to determining if cyanide was the cause of death is that blood cyanide concentrations may be significantly decreased after death if the body is not preserved properly and in a timely manner. Following death, blood and organ cyanide concentrations fall as biotransformation to several metabolites including thiocyanate, 2-aminothiazoline-4-carboxylic acid, and formic acid occurs (McAllister *et al*., 2008). Administration of cyanide antidotes given antemortem must be considered when interpreting postmortem results as they may decrease cyanide concentrations and increase metabolites, depending on the antidote (Gill *et al*., 2004; McAllister *et al*., 2008). Cyanide biotransformation postmortem is dependent on four criteria; initial sample concentration at time of death, length of time sample remains in cadaver after death, length of time sample remains in storage before analysis, and preservation conditions such as temperature and additives (McAllister *et al*., 2008).

Obviously procuring the body, obtaining samples as soon as possible after death is the goal of each medical examiner, but there are several steps that can improve the stability of cyanide analysis, including blood samples. Refrigeration of the body and samples collected is an important factor to improve stability of cyanide in blood. Elevated temperature causes cyanide values to drop precipitously. While there is only minimal statistical significance between samples stored at refrigeration temperature of 4°C, or freezer temperature of −20°C Desharnais *et al*., 2012; Chikasue *et al*., 1988). The most stable temperature for blood cyanide preservation is −20°C, which is advised by most experts (McAllister *et al*., 2008).

Of the preservatives used to stabilize cyanide concentrations in analytical samples, sodium fluoride is most commonly used. Sodium fluoride blocks enzymatic activity to eliminate or reduce biological activity that leads to the production of cyanide producing microorganisms and bacteria. Sodium fluoride impedes putrefaction and inhibits glycolysis (Chan *et al*., 1989; McAllister *et al*., 2011). Addition of a 2% sodium fluoride solution to whole blood samples has little effect on the measured cyanide concentrations after 9–11 days of storage and appears to significantly stabilize cyanide concentrations when stored for 25–30 days (McAllister *et al*., 2011). Untreated samples had an average increase of cyanide concentrations by 35% (McAllister *et al*., 2011).

While less of a concern some reports have demonstrated that postmortem unpreserved blood specimens may yield abnormally high postmortem cyanide concentrations, as high as 150 μg/ml (Lokan *et al*., 1987). Visceral organ fluid analysis can sometimes demonstrate elevated levels of cyanide from putrefactive changes that occur postmortem (Paul & Kumar, 1992). Speculated causes of falsely elevated cyanide concentrations include putrefaction and cyanogenic producing microbes such as pseudomonas aeruginosa (Lokan *et al*., 1987).

While blood is the most reliable source of individual analysis for cyanide, other organs systems tested may uncover the mechanism of toxicity by comparing values. While it may not correlate well with the cause of death, in cases of cyanide ingestion, due to first pass metabolism liver concentrations will likely be higher than lung concentrations. In cases of inhalational cyanide poisoning the opposite may be observed. Cyanide ingestions also may liberate hydrogen cyanide gas that victims may inhale before death. If the poison is inhaled, the lung will show a higher hydrocyanic acid content than the stomach contents. Concentrations were higher than gray or white matter in a sheep model of cyanide toxicity. This is important because cyanide can be produced in the decomposition human at the surface of the brain, but less likely to occur in deeper white matter (Ballantyne, 1975).

In fire victims there are other factors associated with blood cyanide concentrations in postmortem evaluation, including the length of time the deceased was exposed to the fire, and severity of carbon monoxide toxicity (Moriya & Hashimoto, 2001). In the United States, smoke inhalation accounts for up to 10,000 deaths per year. In a study of 285 fire deaths in Poland, 59% were found to have elevated cyanide concentrations ranging from 0.5 to 78.2 mg/l with a mean of 16.83 mg/l. Cyanide was also detected in 50% of fire survivors with a range of 0.3 to 20.1 mg/l, and a mean of 4.0 mg/l (Grabowska *et al*., 2012). In one series of fire deaths, the average blood cyanide concentrations was 1.2 μg/ml (Desharnais *et al*., 2012). It is controversial, but values above 1 μg/ml in fire victims are considered lethal (Desharnais *et al*., 2012; Silverman *et al*., 1988).

Cyanide blood concentrations vary greatly in fire victims, with values that are often well above what is considered lethal (Shiono *et al*., 1991). It would be difficult to interpret these blood cyanide concentrations in isolation without the additional clinical history of fire exposure, and concurrent carbon monoxide values (Shiono *et al*., 1991). These people are often concurrently found to have elevated concentrations of carbon monoxide. There does not appear to be a predictable correlation between CoHb and cyanide values (Moriya & Hashimoto, 2001). However, in a study of fire victims, none had lethal cyanide values without also having lethal carbon monoxide concentrations. Carbon monoxide concentrations correlated with cyanide concentrations, though the relationship was not significant (Grabowska *et al*., 2012; Shiono *et al*., 1991).

The importance of cyanide toxicity in fire victims has been an area of research and controversy. It is clear that many fire victims are concurrently poisoned with carboxyhemoglobin and cyanide, but what is uncertain is what role each poison has in the ultimate cause of death. It should be noted that polymer industry has continued to flourish worldwide and most fire victims and nearly all fatalities will have elevated concentrations of cyanide.

One investigation examining cyanide toxicity in fire victims suggested that concurrent cyanide toxicity in addition to carboxyhemoglobin poisoning in fire fatalities was rare. Within a group of 433 fire fatalities, 364 had postmortem cyanide testing. While 85 of the 364 patients had no detectable cyanide values, the rest had an average cyanide concentration of 1 mg/l and 31 victims had what was considered lethal ((>3 mg/l) cyanide concentrations. Despite these findings, the authors of this study concluded that postmortem testing and empiric treatment for cyanide toxicity was rarely necessary (Barillo *et al*., 1994). This was in contrast to a publication released at approximately the same time that reported that concurrent cyanide toxicity in fire victims was clinically significant, finding lethal (>1 mg/l) cyanide concentrations in 12 of 14 victims who also had lethal carboxyhemoglobin (>60%) values and in 14 of 20 victims with sublethal carboxyhemoglobin values. These authors advocated for the empiric testing of and treatment of cyanide in cases of fire exposure and carboxyhemoglobin toxicity (Silverman *et al*., 1988).

Much of the controversy is a result of the varying opinions as to what is considered a "lethal" cyanide concentration. The most commonly accepted fatal values for carboxyhemoglobin are 60% in non-fire situations, while >50% in smoke inhalation injury in a fire. Cyanide concentrations in fire victims from 1 to 3 mg/l are often considered fatal, which seems like a small range but covers quite a large spectrum of clinical affects. Although it seems intuitive it is also unclear if cyanide toxicity compounds carboxyhemoglobin poisoning or is just a marker of more severe smoke inhalation exposure in fire victims. In a review of carboxyhemoglobin and cyanide values in several infamous fires, including the Happy Land Social Club, Manchester Aircraft Fire, and Glasgow Area Fire Deaths it is clear that fire fatalities are multi-factorial and the correlation between COHb and CN toxicity is unclear. In fact in some situations CN may actually antagonize the uptake of CO decreasing COHb values (Alarie, 2002). It is quite possible that many fire deaths are ascribed to carbon monoxide even with COHb values below 50%. It is possible some of these cases were concluded to be carbon monoxide deaths without CN testing.

It is possible that some deaths in fire victims may be attributed to carbon monoxide toxicity or other fire related effects without even testing for cyanide. To date there are no specific studies that have examined the potential complication in determining the cause of death in a fire victim. But there is one study that assessed the agreement between medical examiners and medical toxicologists in determining the cause of death in poisoning victims. Agreement was lower than expected and peri-mortem fire deaths were particularly increased in their disagreement rate. The multiple potential fatal exposures in fire victim such as carbon monoxide, smoke inhalation, thermal injury, and hydrogen cyanide makes it difficult to determine the actual cause of death. Ultimately it is a matter of opinion when all of the postmortem information is gathered. While it may seem trivial, disagreements or inconclusive cause of death determinations can have significant implications in areas such as public health policy, epidemiology, and medical-legal litigation (Manini *et al*., 2011).

Falsely lowered cyanide concentrations may occur in fire victims treated with antidotes such as sodium nitrite/thiosulfate with cyanide transformed into thiocyanate (Rhee *et al*., 2011). Thiocyanate is a major component of cyanide metabolism by rhodonase, and therefore can be used as a marker of cyanide exposure. But blood thiocyanate can fluctuate greatly with dietary intake, including cyanogenic glycosides in plants. Thiocyanate appears to be elevated in victims where cyanide death is delayed. In patients with acute cyanide toxicity there may not be enough time to sufficiently metabolize cyanide to thiocyanate, likely explaining why fire related descendants have relatively low concentrations of thiocyanate (McAllister *et al*., 2008). In fire victims, left ventricular blood cyanide concentrations are statistically significantly higher than those of right ventricular blood, often twice as high, and this may provide evidence that the cause of death was from cyanide (Shiono *et al*., 1991).

19.6 Risk to autopsy staff

The postmortem evaluation performed by autopsy prosectors poses an occupational hazard in victims poisoned by cyanide. For example, significant amounts of hydrogen cyanide gas can be liberated from stomach contents when examining the viscera. Anecdotal and published reports describe prosectors developing symptoms of nausea, lightheadedness, dizziness, and mucosal

irritation (Nolte & Dasgupta, 1996). Several cases have demonstrated elevated cyanide concentrations in individuals performing autopsies on cyanide victims (Cina *et al*., 1994; Nolte & Dasgupta, 1996). Stomach contents can liberate large amounts of hydrogen cyanide gas (Nolte & Dasgupta, 1996). The "classic" bitter almond smell may not be detected by autopsy staff as described earlier. While there are no specific guidelines on how to perform an autopsy when there has been suspicion of cyanide poisoning there are several published reports or advisable methods to prevent exposure to the prosector (Nolte & Dasgupta, 1996).

During the postmortem evaluation in a negative pressure room with large air exchange (supply rate of 500 cubic feet per minute and an exhaust rate of 941 cubic feet per minute) and a biosafety hood of a descendent who intentionally ingested potassium cyanide, a pathologist and two technicians had no detectable elevations in blood cyanide before or shortly after they performed the autopsy. Despite this two of them developed minor symptoms of light headedness, headache, and throat burning (Nolte & Dasgupta, 1996).

Others have advocated for prosectors to wear a full face respirator to protect from the liberation of hydrogen cyanide gas during the autopsy, which simple surgical masks often worn will not. Regardless of the specific measures taken, the room should be well ventilated, staff should rotate/take breaks to limit exposure to contents and cadaver, and be mindful of symptoms or warning signs of toxicity. Stomach and gastrointestinal contents should be removed intact and then dissected carefully under a well-ventilated hood (Forrest *et al*., 1992). It may also be prudent to have present during autopsy a staff member who is clearly able to detect the "bitter almond" smell to heed warning upon opening the viscera.

In summary, while there are several features considered classic findings of cyanide poisoning on autopsy, such as pink lividity, bitter almond smell, and esophageal-gastric injury, none of them are sensitive or specific. Fire victims are often concurrently poisoned with carbon monoxide and cyanide. Collect blood samples as soon as possible after death and place in an air tight seal vessel, with sodium fluoride preservative, stored at −20°C. Always consider the safety and potential exposure of cyanide to staff performing autopsy.

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CHAPTER 20

Medicolegal and forensic factors in cyanide poisoning

Jorn Chi-Chung Yu and Ashraf Mozayani

At a Glance

- A case of death from cyanide poisoning is usually confirmed by such forensic factors as crime scene presence of cyanide, presence of a "bitter almonds" odor, presence of livor mortis and/or gastric chemical injury, and elevated cyanide concentrations in post-mortem blood.
- However, blood cyanide can either decrease or increase in autopsy specimens during storage (depending on such factors as storage duration, temperature, and presence of cyanogenic bacteria in samples).
- Cyanide concentrations in post-mortem blood samples are relatively unstable.
- The forensic confirmation of cyanide poisoning death cases requires an approach involving crime scene investigators, pathologists, and toxicologists.
- While not currently mainstream forensic practice, analysis of post-mortem biological samples for cyanide biomarkers such as cyanide-protein adducts or concentrations of the cyanide metabolite ACTA may in future prove to be useful.

20.1 Introduction

In criminal investigation, cyanide poisoning commonly refers to the cause of death in a homicidal, accidental, or suicidal case where victims are exposed to cyanide (CN^- or HCN). The exposure may occur as a result of ingestion of cyanide salts (CN^-) or hydrogen cyanide (HCN) liquid, or inhalation of HCN gas. In the case of ingestion, 200–300 mg of cyanide salts ingested by an adult is likely to be fatal without treatment, although the death may be delayed for at least one hour (Vale, 2007). In the case of inhalation of HCN gas, symptoms may occur within seconds and death can occur within minutes. The high volatility of HCN gas reduces its threat in an outdoor environment, but it can be highly lethal in a confined space. Occasionally, HCN gas is an important factor in many deaths caused by smoke inhalation (Eckstein & Maniscalco, 2006). Firefighters and other first responders to a fire scene are at high risk for serious injury or death from exposure to HCN gas.

Although accidental and criminal cases of cyanide poisoning were considered rare (Pasi *et al.*, 1985; Musshoff *et al.*, 2002), the acute toxicity and lethality of cyanide make it a potentially powerful weapon for criminals and terrorists. For example, HCN has been used as a chemical agent in gas chambers and as a weapon of war (Baskin, 2001). Cyanides have also been used in assassinations and mass suicides, such as the Jonestown massacre in 1978 (Thompson *et al.*, 1987). The possible use of cyanides by terrorists as a weapon of mass destruction is always a concern in any security issues (Eckstein, 2008). In a recent political scandal, a British businessman, Neil Heywood, might have been murdered in China by the use of a cyanide compound (Branigan, 2012). According to media reports, the cause of death of Heywood was initially reported as excessive alcohol consumption and his body was

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cremated without a proper autopsy. Therefore, critical physical evidence for forensic analysis may have been lost. When forensic evidence is not properly collected and preserved, it is hard to prevent the accidental and criminal cyanide poisoning cases from going undetected or unconfirmed in a criminal investigation. To detect and confirm a cyanide poisoning case, the teamwork of crime scene investigators, forensic pathologists, and forensic toxicologists is required.

20.2 Forensic practice for the investigation of cyanide poisoning

20.2.1 Crime scene investigation

Source of cyanide

For a cyanide poisoning case, the presence of cyanide at the crime scene and the source of cyanide should be investigated during the crime scene search. Cyanide exposures most commonly occur from inhalation of hydrogen cyanide (HCN) or direct ingestion of cyanide salts. In ambient environments, cyanide salts usually appear as white crystalline powders, while HCN is present as a colorless (or pale blue) liquid or as a gas with a bitter almond-like odor (Lv *et al.*, 2005). Therefore, the source of gas or liquid HCN and solid cyanide salts in the crime scene should be properly documented, collected, and preserved for laboratory testing. Many other commonly used products in our society, such as plastics, polyurethane, various types of foam, synthetic fibers, and pesticides may be the source of cyanide when those materials are thermally degraded (Jellinek & Takada, 1977). Cyanide salts or its derivatives have also been used in electroplating, pharmaceutical, cosmetic, digital electronics, conventional photographic developing, and metallurgy industries. Plants, fruits and nuts such as almonds, fruit pits, sweet potato, bamboo shoot, linseed, lima beans, and millet, may also contain cyanogenic glycosides, which can be the source of cyanide in human metabolism. The metabolite of nitroprusside, acetonitrile, and acrylonitrile can also produce cyanide in the body (Dumas *et al.*, 2005). Other non-toxic sources of cyanide, such as cooking potassium ferrocyanide to produce potassium cyanide (KCN), have been reported (Musshoff *et al.*, 2011). Therefore, unknown powders or substances present at the scene should be properly collected and preserved for future testing.

Examination of the body at the crime scene

Diagnostic signs at a crime scene, such as a bitter almond odor emanating from the victim, and the presence of pink lividity during the postmortem examination, may steer the investigation toward acute cyanide poisoning. The salt form of cyanide is often present at the scene in a case involving the ingestion of cyanide. Deposition or residues of cyanide salts could be found in the areas near the victim's nose or mouth. A quick test of pH value for basicity on the surface of a victim's hand and mouth could often demonstrate a high likelihood for acute cyanide poisoning. Note that cyanide is a base. The pKa of its conjugate acid, HCN, is about 9.21. When it is present in aqueous solution, a pH higher than neutral value (7.0) is expected. These visible solid powders should be collected and preserved for further forensic testing. In cases where no visible substances are observed at the scene of the death, a conclusion of cyanide poisoning may require a test for the presence of cyanide in the victim's body.

When the body undergoes decomposition, no diagnostic signs of cyanide poisoning can be observed. The presence of cyanide powders at the crime scene or the place of employment of the victim may suggest the involvement of cyanide in a death. In the case that no cyanide powder or HCN gas can be discovered at the crime scene, the detection of the involvement of cyanide in a death will be most likely the responsibility of medical examiners and toxicologists.

20.2.2 Medical examination

Exposure to HCN gas or cyanide salts can cause chemical asphyxia (DiMaio & DiMaio, 2001). Cyanide produces cellular hypoxia by combining with the ferric iron atom of intracellular cytochrome oxidase. Inhalation of high concentrations of HCN gas could produce almost immediate collapse and death (Bismuth *et al.*, 2004). In the case of ingestion of cyanide salts, the stomach may show signs of corrosion by the mucosa appearing hemorrhagic. If there was vomiting of ingested substance, these corrosion burns might be observed in the area near the mouth. Microscopic epithelial morphological changes have been described in the gastroesophageal mucosa with cyanide ingestion (Fernando & Busuttil, 1991). The distinct smell of bitter almonds emitted from the mouth and stomach may be detected during the autopsy. Livor mortis may be bright pink. This bright red discoloration is caused from

the inhibition of the cytochrome oxidase by cyanide, which prevents utilization of circulating oxyhemoglobin (Way, 1984).

20.2.3 Toxicological analysis

In forensic toxicology, the common laboratory test in a cyanide poisoning case is to measure cyanide concentration in the autopsy sample. Biological fluids, such as blood or urine can be taken from the subject for toxicological analysis (Ishii *et al.*, 1998). The elevated cyanide concentration in the autopsy samples has been the major forensic evidence to detect a cyanide poisoning case. The toxicological detection of cyanide involves extraction and measurement of HCN from biological extracts (Darr *et al.*, 1980; Shiono *et al.*, 1991). Blood or urine can be collected from the victim for laboratory analysis (Lundquist *et al.*, 1989; McAuley & Reive, 1983). The Cyantesmo color test is a common screening test for cyanide and it is used for the detection of hydrocyanic acid and cyanides in aqueous solutions of blood and tissue homogenates. The pale green color of the test paper turns blue in the presence of hydrocyanic acid. The limit of detection of the test is about 0.2 mg/l HCN after 15 min reaction time (Gill *et al.*, 2004). Following a positive Cyantesmo screening test, gas chromatography (GC) is used to confirm the presence of cyanide. The limit of detection of a GC method is usually at the level of 0.125 mg/l and the limit of quantitation at 0.25 mg/l (Zamecnik & Tam, 1987). Recently, a headspace gas chromatography-mass spectrometry (GC-MS) method has been developed that produced an extended linear dynamic range between 0.07 and 50 mg/l, and a method detection limit of 0.02 mg/l (Desharnais *et al.*, 2012).

The pharmacokinetics and pharmacodynamics of cyanide toxicity have been reviewed (Baskin & Brewer, 1997). The average endogenous levels (normal level) of cyanide in blood samples in healthy individuals have been found to be 0.059 mg/l for non-smokers and 0.123 mg/l for smokers (Baselt & Cravey, 1994). In suicide cases, the blood concentration of cyanide can range from 1 to 53 mg/l. In fire cases, the reported average blood concentration of cyanide is around 1.12 mg/l. Toxic levels of cyanide in blood can range from 0.1 to 2.2 mg/l. Generally speaking, cyanide concentration in blood greater than 1.1 mg/l is considered to be fatal.

20.3 Discussion

20.3.1 Forensic factors associated with cyanide poisoning cases

The forensic factors that are commonly used for the confirmation of a cyanide poisoning case is the combination of physical evidence collected from the crime scene, autopsy, and toxicological testing, that is, the presence of cyanide at the scene, the presence of bitter almond odor, the presence of liver mortis, the presence of gastric burns, and the elevated concentration of cyanide in the blood sample. When all these factors are evaluated and analyzed, the conclusion of cyanide poisoning may be issued with high degrees of confidence.

20.3.2 Cyanide salt and hydrogen cyanide gas

In the investigation of a cyanide poisoning case, the source of cyanide should be confirmed. The solid form of cyanide salt can be preserved for laboratory confirmation. Since hydrogen cyanide gas is present in gaseous form, it cannot be collected from a crime scene, unless a gas container is present at the scene. If hydrogen cyanide is suspected, a sample can be collected in a gas container for laboratory analysis.

20.3.3 The bitter almond odor and livor mortis

In the cases of both ingestion of cyanide salts and inhalation of hydrogen cyanide gas, the diagnostic signs of bright pink color of the blood (cherry red) and livor mortis may be present during the autopsy. However, the odor of bitter almond from the crime scene and the victim may not always be detected in a well-ventilated area. Moreover, the ability to sense this "cyanide smell" may be genetically related. A significant percentage of the population cannot smell it. Livor mortis may be pink for non-pathologic reasons, such as refrigeration and putrefaction. Livor mortis and scent should not be used solely to include or exclude cyanide poisoning.

20.3.4 Gastric burns and cherry red blood

The observation of gastric burns and blood that has a cherry red color are sound forensic factors to confirm acute cyanide poisoning cases. The toxicological test can usually confirm the presence of cyanide from gastric content and blood samples. However, not all cyanide ingestion develops a hemorrhagic gastric mucosa. This

may be a reflection of the amount of cyanide ingested or the amount of food in the stomach at the time of the ingestion. Inflammation is unlikely to be detected if the death occurred rapidly. Unfortunately, these diagnostic signs might not be observed in decomposed bodies.

20.3.5 Elevated cyanide concentration from toxicological samples

Forensic evidence, such as stomach contents and whole blood of the victims, are usually collected and analyzed in order to confirm the cause of death (Laforge *et al.*, 1994). There are several biomarkers for cyanide exposure, such as cyanide (CN^-) itself, thiocyanate (SCN^-), 2-amino-2-thiazoline-4-carboxylic acid (ATCA), and cyanide-protein adducts (Bruckner & Roberts, 2008; Vinnakota *et al.*, 2012). In practical forensic toxicology, the detection of cyanide as a biomarker in toxicological samples is the primary laboratory test for cyanide poisoning cases. Testing SCN^-, ATCA, and cyanide-protein adducts in the toxicological sample as the markers for cyanide poisoning are not yet commonly accepted in the forensic science community.

Due to the relatively short half-life of cyanide (from minutes to hours depending on the matrix), toxicological detection of cyanide to confirm cyanide poisoning may only be feasible within the first few hours following exposure (Ballantyne *et al.*, 1973; Moriya & Hashimoto, 2001; Calafat & Stanfill, 2002). Natural dietary and pulmonary intake of cyanide from the environment provide a nonzero cyanide background level in the body. Smoke inhalation in fires greatly increases background cyanide levels. Cyanide could be detected in autopsy samples from people who die of natural causes unrelated to fires or intoxications. These "normal" (or endogenous) postmortem cyanide concentrations have ranged from 0.2 to 0.43 mg/l in New York City (Gill *et al.*, 2003). Cyanide concentrations up to 2.2 mg/l have also been reported in chronically exposed factory workers in the electroplating industry (Chandra *et al.*, 1980). Individuals in industries exposed to cyanide on a chronic basis could have a level of 0.232 mg/l for smokers and 0.183 mg/l for non-smokers. One worker, a smoker, had a level of 2.2 mg/l, which would ordinarily be considered a lethal level. The use of cyanide concentration in toxicological samples as a marker for cyanide poisoning may not be reliable (Lokan *et al.*, 1987; Chikasue *et al.*, 1988; Noguchi *et al.*, 1988). Moreover, the volatility and reactivity of cyanide can result in measurements that are highly susceptible to errors introduced during the sample collection and separation steps (Lindsay *et al.*, 2004). Therefore, the detection of the presence of cyanide becomes less feasible when the detection window has passed or the victim's body has been damaged, such as in cases where autopsies are delayed or tissues have been damaged by fire or undergone advanced decomposition.

20.3.6 Alternative biomarkers for cyanide poisoning

Thiocyanate (SCN^-), 2-aminothiazoline-4-carboxylic acid (ATCA), and cyanide-protein adducts in biological fluids and tissues have been reported as alternative biomarkers for cyanide exposure and poisoning (Isom & Baskin, 1997; Baskin *et al.*, 2006). SCN^- is the major cyanide metabolite found in blood (Baskin *et al.*, 2004). However, SCN^- is also a natural metabolite of non-cyanide mediated pathways and thus is not a good marker for cyanide exposure (Ballantyne, 1977). Detoxification of cyanide by cystine to produce ATCA *in vivo* was first reported in the early 1950s by Wood and Cooley (Wood & Cooley, 1956). They found that the pathway producing ATCA represents approximately 20% of cyanide metabolism. The quantity of ATCA produced is directly proportional to the amount of cyanide metabolized, and harvested ATCA is stable for months in the freezer. More details of human metabolism of cyanide and detection of its biomarkers have been reported in a recent review article (Logue *et al.*, 2010). Therefore, ATCA has been considered a promising candidate as a chemically stable biomarker for cyanide exposure (Logue *et al.*, 2005). However, there are two important factors that need to be determined in order to use ATCA as a biomarker for cyanide poisoning. First, there is no knowledge about the production of endogenous ATCA levels in the human body other than cyanide exposure. In other words, other than cyanide, there is no known source that could produce ATCA *in vivo*. Also, insufficient data exist to determine if useful correlations exist between ATCA and cyanide exposure levels (Fasco *et al.*, 2011). Second, there is no study about postmortem reaction of cyanide and cystine. There is no knowledge about whether ATCA concentration in the victim's body can increase from the postmortem diffusion of cyanide in the surrounding environment. These questions should be addressed in the future and more research is needed

in order to use ATCA as a forensic biomarker for the investigation of cyanide poisoning cases. However, the detection of stable biomarkers of cyanide is a promising approach to extend the time window in which cyanide exposure can be reliably assayed in a postmortem examination.

20.3.7 Requirement to confirm a cyanide poisoning case

To detect and confirm a cyanide poisoning case, the teamwork of crime scene investigators, forensic pathologists, and forensic toxicologists is required. Usually, the requirement to confirm a cyanide poisoning case is based on the medical examiner's diagnosis of the totality of the case. The forensic factors associate with cyanide poisoning should be considered, but not all of them need to be collected and tested. Each case is unique in its own way. The collection and analysis of the forensic factors in a cyanide poisoning case may vary from case to case. For example, a 27-year-old male research assistant at a major university had recently been told by his doctor that he was HIV positive. He called his girlfriend at about 11:00 a.m., at which time she became upset and hung up on him. When his girlfriend and several friends arrived at his apartment several hours later, she found the door locked. Her friends forced the door open, and she found the man unresponsive on the floor. Because two suicide notes were present, and a vial of clear liquid was found in the victim's shirt pocket, the case was concluded as a cyanide poisoning. As for the toxicological test, cyanide was also found in the stomach contents and blood. However, cyanide levels were not reported. After all, a systematic strategy to detect and collect all physical evidence is highly recommended for the investigation of a cyanide poisoning case. Hearn and colleagues have outlined a systematic approach for the general process of death investigation (Druid, 2007).

20.4 Conclusion

A cyanide poisoning case is usually confirmed by forensic factors such as the presence of cyanide in the crime scene, the presence of bitter almond odor, the presence of liver mortis, the presence of gastric burns, and the elevated concentration of cyanide in the blood sample. It is important to recognize the limitations of the forensic factors in a cyanide poisoning case. For example, blood concentrations of cyanide can increase or decrease during storage depending on the length of time, the temperature, and the presence of cyanogenic bacteria (Catanese & Labay, 2009). Elevated cyanide concentration in toxicological samples due to postmortem diffusion of cyanide has been reported (Karhunen *et al.*, 1991). Cyanide concentrations in toxicological samples may increase due to the diffusion and absorption of hydrogen cyanide gas in the surrounding environment. Cyanide concentration in blood samples can be relatively unstable, and it is more difficult to establish blood cyanide concentrations and the extent of cyanide exposure (McAllister *et al.*, 2008). However, some studies found no change of cyanide concentration in blood samples over a two-week period for samples stored at 4°C or−20°C (Desharnais *et al.*, 2012). Because the stability of cyanide in postmortem samples is still a controversial topic (McAllister *et al.*, 2011), the use of elevated cyanide concentration in a postmortem sample to confirm cyanide poisoning requires more research within the forensic science community. There is no single test in forensic toxicology that can confirm a cyanide poisoning case. The confirmation of a cyanide poisoning case involves teamwork of the crime scene investigators, pathologists, and toxicologists. An alternative and promising approach that can help to minimize false positive (no cyanide is involved but cyanide is detected) and false negative (cyanide is involved but cyanide is not detected) results in cyanide poisoning cases is to detect stable biomarkers of cyanide rather than cyanide itself (Petrikovics *et al.*, 2011). To prevent false positive and false negative conclusions, the use of alternative biomarkers should be considered for future forensic testing in cyanide poisoning cases. However, more research is still needed before the implementation of the use of alternate biomarkers, possible ATCA, and/or cyanide-protein adducts, for the investigation of cyanide poisoning case.

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CHAPTER 21

Brief overview of mechanisms of cyanide antagonism and cyanide antidotes in current clinical use

Alan H. Hall

At a Glance

- There are 3 general classes of cyanide antidotes in current clinical use in various countries around the world: 1) methemoglobin inducers, 2) sulfur donors, and 3) direct cyanide chelating agents.
- Methemoglobin inducers: amyl nitrite, sodium nitrite, 4-dimethylaminophenol (4-DMAP)
- Sulfur donors: sodium thiosulfate
- Direct cyanide chelating agents: hydroxocobalamin, dicobalt-EDTA (Kelocyanor®)
- There is anecdotal clinical evidence for efficacy of all these antidotes in cyanide poisoning.
- All have more or less severe adverse effects and all have to be administered parenterally (except for amyl nitrite which is administered by inhalation).
- Hydroxocobalamin appears to have the best safety profile, especially in patients with a significant cyanide component to inhalation of enclosed-space fire smoke.

21.1 Introduction

Cyanide is unusual among chemical toxicants in that it has not one, but several specific antidotes (Hall *et al.*, 2009). There are various mechanisms by which medications may have an impact on cyanide poisoning. These include: agents which induce methemoglobinemia (nitrites, 4-DMAP [4-Dimethylaminophenol]); those which can enhance endogenous biodetoxification pathways, mainly by increasing the conversion of cyanide to much less toxic thiocyanate (sulfur donors); and those which directly chelate cyanide (hydroxocobalamin, dicobalt EDTA (Kelocyanor®)) (Hall *et al.*, 2009; Ballantyne *et al.*, 2007). In addition, a large number of agents have been investigated as countermeasures for either specific toxic mechanisms or signs/symptoms (Ballantyne *et al.*, 2007), but as these are considered adjunctive/supportive treatment they will not be further discussed here.

The characteristics and clinical properties of a hypothetical "ideal" cyanide antidote have been proposed (Hall *et al.*, 2009). These are summarized in Table 21.1. Of note, none of the specific cyanide antidotes discussed briefly below and in more detail in the following chapters completely fulfills all of the "ideal" criteria.

Developmental cyanide antidotes that may become available for clinical use over the next several years are discussed in Chapter 24.

21.2 Methemoglobin inducers

21.2.1 Amyl nitrite/sodium nitrite

Amyl nitrite

Amyl nitrite is administered by inhalation, either by breaking a perle in gauze and holding near a

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Table 21.1 Clinical characteristics of an "ideal" cyanide antidote (adapted from Hall *et al*., 2009).

Rapid onset of action.
Would neutralize cyanide without interfering with cellular oxygen utilization or oxygen transport.
Would have safety and efficacy profiles such that it would be suitable for use in both the pre-hospital and hospital settings.
Would be safe for use in smoke inhalation victims who may have carbon monoxide poisoning, other combustion products poisoning, and soot inhalation in addition to cyanide poisoning.
Would not be harmful if inadvertently administered to non-cyanide-poisoned patients.
Easy to administer.

still-breathing patient's nose and mouth or by placing inside the lip of a resuscitation mask for non-breathing patients (Hall *et al*., 2009). It has been the first component of the Cyanide Antidote Kit (CAK) since the 1930s and was originally reported effective in a few anecdotal case reports and dog studies in the late 1880s. It has a rapid onset, but also a short duration of action (Baskin *et al*., 1992). Amyl nitrite was originally intended to be a first aid measure until intravenous access could be established.

The methemoglobin level induced by amyl nitrite usually does not exceed 7% (Meredith *et al*., 1993) and it has generally not been considered to be adequate resuscitation when used alone (Dart & Bogdan, 2004). However, administration of amyl nitrite with or without oxygen was reported to be useful in a small case series of cyanide exposures in an industrial setting (Wurzburg, 1986).

Sodium nitrite

Sodium nitrite has been part of the CAK since the 1930s. It is also part of a recently marketed generic two-component Cyanide Antidote Kit containing only sodium nitrite and sodium thiosulfate (Nithiodote®). Sodium nitrite must be administered intravenously and has almost always been used in combination with sodium thiosulfate (Hall, 2007). Initial animal studies of sodium nitrite as a cyanide antidote were done in the 1930s in both the United States of America and Argentina.

Sodium nitrite is a more potent methemoglobin inducer than amyl nitrite, although it has sometimes been thought to induce methemoglobin concentrations that are too low with too slow an onset (von Clarmann, 1993; Zilker, 2005). It has sometimes been noted that the maximum clinical improvement precedes the peak induced methemoglobin level. However, there are numerous case reports of the efficacy of sodium nitrite combined with sodium thiosulfate in the literature (Hall *et al*., 2009).

Sodium nitrite is a potent vasodilator, particularly when given rapidly intravenously, and such too-rapid administration may result in significant hypotension (Hall, 2007). Rare cases of excessive methemoglobinemia have been reported, especially in children inadvertently administered adult doses (Hall, 2007).

Because it induces methemoglobinemia and therefore reduces oxygen transport, sodium nitrite must be used with caution, if at all, in cases of smoke inhalation where concomitant carbon monoxide poisoning will also result in decreased oxygen transport (Hall *et al*., 2009).

21.2.2 4-DMAP (4-Dimethylaminophenol)

4-DMAP (4-Dimethylaminophenol) has been investigated as a methemoglobin-inducing cyanide antidote (von Clarmann, 1993). 4-DMAP acts by methemoglobin induction, converting the ferrous (Fe^{2+}) of normal hemoglobin to the ferric (Fe^{3+}) valence state. Methemoglobin has a higher affinity for the cyanide ion that does the Fe^{3+} of cyanide-inhibited mitochondrial cytochrome$_c$ oxidase, the terminal enzyme in the electron transport chain (Von Clarmann, 1993; Weger, 1983; Ballantyne *et al*., 2007).

4-DMAP is usually administered intravenously, but it has been suggested for intramuscular (IM) administration in mass cyanide casualty incidents (Hall *et al*., 2009; Zilker, 2005). IM administration has been associated with local pain and necrosis at injection sites (Hall *et al*., 2009; Gracia & Shepherd, 2004; Cummings, 2004).

4-DMAP has also been associated with development of reticulocytosis, nephrotoxicity, and hemolysis (Mégarbane *et al*., 2003). Especially when administered to patients without significant cyanide poisoning, 4-DMAP may result in significant adverse effects. Excessive methemoglobinemia may occur, requiring treatment with toluidine blue or methylene blue (Van Heijst *et al*., 1987).

21.3 Sulfur donors

21.3.1 Sodium thiosulfate

While a number of sulfane sulfur donors have been tested as cyanide antidotes (Ballantyne *et al*., 2007), only sodium thiosulfate is currently in clinical use (Hall *et al*., 2007). The cyanide antidotal mechanism of action of sodium thiosulfate is to provide an exogenous source of sulfane sulfur. Availability of sulfane sulfur constitutes the rate-limiting step for rhodanese, the natural endogenous cyanide detoxifying enzyme present in the liver and other tissues. Increasing the availability of sulfane sulfur, which is present in limited amounts endogenously, increases the biodetoxification of cyanide to much less toxic thiocyanate (SCN^-), which is readily excreted in the urine (Hall & Rumack, 1986).

Most of the available literature on sodium thiosulfate involves its use in combination with other cyanide antidotes (Hall *et al*., 2009). Sodium thiosulfate has been a component of the amyl nitrite/sodium nitrite/sodium thiosulfate Cyanide Antidote Kit (CAK) since the 1930s. It is also included in the recently marketed two-component antidote kit (Nithiodote®) with sodium nitrite. Sodium thiosulfate has also been co-administered with 4-DMAP and hydroxocobalamin (Hall *et al*., 2007).

While sodium thiosulfate has sometimes been advocated to be administered alone to victims of fire smoke inhalation, concerns have been raised about its efficacy in this setting (Hall *et al*., 2007). It has limited penetration into the brain, one of the organs most sensitive to the toxic effects of cyanide (Baskin *et al*., 1992). It also has limited penetration into mitochondria where rhodanese is located (Baskin *et al*., 1992). It has generally been thought that sodium thiosulfate has too slow an onset of action to be efficaciously used alone in acute cyanide poisoning (Hall *et al*., 2007; Ballantyne *et al*., 2007). The limited published data available on administration of sodium thiosulfate alone have been reviewed (Hall *et al*., 2007).

Reported adverse effects of sodium thiosulfate in normal volunteer studies have been: nausea, vomiting, retching, and local adverse effects of injection site pain, irritation, and a burning sensation (Forsyth *et al*., 1993; Osterloh & Hall, 1997; Ivankovich *et al*., 1983).

21.4 Direct cyanide chelating agents

21.4.1 Hydroxocobalamin

Hydroxocobalamin is the natural form of vitamin B12 (vitamin $B12_a$), sometimes described as a vitamin B12 precursor. It is a large heme-like molecule but with a complexed cobalt atom rather than iron (Hall *et al*., 2007). The main mechanism of action of hydroxocobalamin is direct binding of cyanide (1 mole of cyanide per 1 mole of hydroxocobalamin) to form non-toxic cyanocobalamin (vitamin B12) which is excreted in the urine (Hall *et al*., 2009).

An additional mechanism of action appears to be nitric oxide scavenging (Gerth *et al*., 2006). This latter effect may be responsible for the mild, self-limited hypertension seen after hydroxocobalamin administration in normal volunteers (Forsyth *et al*., 1993; Uhl *et al*., 2006). While this has been considered an adverse effect, in fact, in patients with acute cyanide poisoning where hypotension is an ominous clinical sign, an effect that increases blood pressure may instead be therapeutic.

Efficacy of hydroxocobalamin in cyanide poisoning has been shown in studies of fire smoke inhalation victims, as well as in anecdotal case reports and a small case series of "pure" cyanide poisoning (Fortin *et al*., 2005, 2006, 2011; Fortin, Desmettre, *et al*., 2010; Fortin, Waroux, *et al*., 2010; Hall *et al*., 2007, 2009; Borron *et al*., 2004; Borron, Baud, Barriot, *et al*. 2007; Baud, Mégarbane, *et al*., 2007; Weng *et al*., 2004; Espinoza *et al*., 1992).

Adverse effects of hydroxocobalamin have generally been mild. Nearly all patients administered hydroxocobalamin develop a reddish-brown discoloration of the skin, sclera, mucous membranes, and urine (Hall *et al*., 2009; Uhl *et al*., 2006). This seems to be mainly from the color of the antidote itself and clears over several days. Transient, self-limited hypertension in normal volunteers has been noted. Other reported adverse effects in normal volunteers have been pustular/papular rashes, headache, erythema at injection sites, decrease in lymphocyte percentage, nausea, pruritus, chest discomfort, and dysphagia (Uhl *et al*., 2006).

While a few scattered cases of anaphylaxis following IM chronic or sub-chronic administration of other low-dose hydroxocobalamin preparations have been reported, none have been reported following

administration of antidotal hydroxocobalamin doses (Hall *et al*., 2009; Ballantyne *et al*., 2007).

21.4.2 Dicobalt EDTA (Kelocyanor®)

Dicobalt edetate (dicobalt EDTA; Kelocyanor®) is the dicobalt salt of edetic acid. After Kelocyanor® injection, the Co^{2+} complexes with two cyanide anions; next the cobalt salt produces another Co^{2+} which then complexes another two cyanide anions. The cobalt-cyanide complexes are very stable and are eliminated in the urine (OVP, 1987).

No normal volunteer studies or controlled clinical trials have been performed, but anecdotal case reports have suggested efficacy against cyanide poisoning (Marrs, 1993; Hall *et al*., 2009).

Reported adverse effects have included nausea, vomiting, sweating, cardiac anginal chest pain, skin rashes, facial edema, hypertension, hypotension, nervousness, significant tremors, gastrointestinal hemorrhaging, and seizures (Hall *et al*., 2009; OVP, 1987; Marrs, 1993; Peters *et al*., 1982). These have particularly been noted when Kelocyanor® was administered to patients without significant cyanide poisoning (Hall *et al*., 2009; Marrs, 1993; Marrs *et al*., 1985; Pickering, 1985; Hillman *et al*., 1974), but have also occurred in patients with significant cyanide poisoning (Dodds & McKnight, 1985). Free cobalt cations in the antidotal preparation may be responsible for serious cobalt toxicity (Hall *et al*., 2009). Hypertonic glucose administration following Kelocyanor® administration may have a protective effect, although the mechanism is unclear (Hall *et al*., 2009).

21.5 Conclusion

All of the cyanide antidotes in current clinical use have some evidence of efficacy. Based on relative efficacy and safety profiles, hydroxocobalamin seems to be at least as efficacious in human clinical use as the others and currently has the most favorable safety profile.

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CHAPTER 22

Cyanide antidotes in clinical use: 4-dimethylaminophenol (4-DMAP)

Alan H. Hall

At a Glance

- 4-DMAP is a methemoglobin-inducing cyanide antidote.
- It was invented by German researchers to replace the old Cyanide Antidote Kit as it was thought to be safer.
- Unlike the nitrite portions of the old Cyanide Antidote Kit, it does not cause hypotension.
- It can, in rare cases, cause excessive methemoglobinemia.
- It can have other adverse effects.

22.1 Introduction

4-Dimethylaminophenol (4-DMAP) was investigated in Germany as a replacement methemoglobin-inducing cyanide antidote for sodium nitrite which was thought to induce methemoglobin concentrations that were too low with too slow an onset (Von Clarmann, 1993; Zilker, 2005). Studies by Kiese and Weger (1969) of several aminophenols showed 4-DMAP to result in controlled and rapid methemoglobin induction, with an IV dose of 3.25 mg/kg producing 15% methemoglobinemia within 1 minute and 30% methemoglobinemia in 10 minutes (Zilker, 2005).

4-DMAP has the chemical formula $C_6H_{11}ON.HCl$ and has a molecular mass of 173.5. It consists of snow-white crystals as the raw material and is very soluble in water. It must be stored in closed, opaque containers because it rapidly oxidizes in air with a color change to black-brown. Turbid, colored 4-DMAP cannot be used, and so an open ampoule cannot be kept (Von Clarmann, 1993). The half-life of methemoglobin after a single IV injection of 250 mg of 4-DMAP is about 2 hours (Zilker, 2005).

22.2 Mechanism of action

4-DMAP acts by formation of methemoglobin, converting the Fe^{2+} of normal hemoglobin to the ferric (Fe^{3+}) valence state by setting up a catalytic cycle in erythrocytes in which oxygen oxidizes 4-DMAP to *N*,*N*-dimethylquinoneimine, which then oxidizes normal hemoglobin to methemoglobin (Ballantyne *et al.*, 2007; Marrs, 1987). Certain side reactions, including glutathione reaction, terminate the catalytic cycle (Marrs, 1987). Methemoglobin cannot transport oxygen, but does have a higher affinity for the cyanide ion than does the Fe^{3+} of cyanide-inhibited mitochondrial cytochrome$_c$ oxidase, the terminal enzyme in the electron transport chain (Von Clarmann, 1993; Weger, 1983; Ballantyne *et al.*, 2007). 4-DMAP has been shown to rapidly induce methemoglobinemia in several species including humans, mice, dogs, rabbits, and non-human primates (Marrs, 1987). This may or may not be the only or even the main mechanism of action of methemoglobin-inducing compounds, as the role of nitric oxide/nitric oxide synthetase has become increasingly clear in cytochrome oxidase inhibition by cyanide and in removal of cyanide from this enzyme.

Methemoglobin concentrations of 30–50% may be seen within a few minutes of IV 4-DMAP administration

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(Hall *et al.*, 2009; Von Clarmann, 1993). These methemoglobin levels are in the range where illness including cardiovascular collapse or even death can occur (Hall *et al.*, 2009; Bhattacharya *et al.*, 2002; Mégarbane *et al.*, 2003; Cummings, 2004; Van Heijst *et al.*, 1987).

4-DMAP is usually given intravenously, but it has been recommended to be used intramuscularly in mass cyanide casualty situations (actual data are lacking) (Hall *et al.*, 2009; Zilker, 2005). However, IM administration is associated with local pain and necrosis at injection sites (Hall *et al.*, 2009; Gracia & Shepherd, 2004; Cummings, 2004). Pain at the injection site may be delayed in onset for 12 hours and reaches its maximum at about 24 hours (Weger, 1983). A local sterile abscess may develop.

22.3 Experimental data

Paulet and Dassonville (1985) studied the effects of 4-DMAP in anesthetized beagle dogs poisoned with a continuous infusion of 0.1 mg/kg of sodium cyanide solution. The cyanide infusion was continued until the phase of primary apnea and 4-DMAP was administered at a dose of 5–6 mg/kg followed by 300–500 mg/kg of sodium thiosulfate. In the 10 antidote-treated dogs, there were four immediate deaths. Two dogs survived temporarily for 2–3 hours, and four dogs survived definitively (Paulet & Dassonville, 1985).

In dogs poisoned with IV sodium cyanide, administration of 5 mg/kg of 4-DMAP IM resulted in a 100% survival rate compared to 0% survival in dogs receiving no antidote (Vick & Froehlich, 1991). 4-DMAP successfully antagonized cyanide poisoning in rats administered methacrylonitrile (Peter & Bolt, 1985).

Weger (1983) summarized the experimental data on 4-DMAP from Germany. In dogs, more animals were rescued from cyanide poisoning with 4-DMAP than with dicobalt-EDTA or dicobalt histidine, when the antidotes were administered 4 minutes after poisoning. Positive effects of 4-DMAP and 100% oxygen were noted in dogs on cerebral blood flow, peripheral circulation, arterial and venous blood gases, and other parameters during the course of continuous slow cyanide infusion (Weger, 1983).

Marrs (1987) summarized the experimental data on 4-DMAP, noting that in several species (mice, dogs, rabbits, and non-human primates) as well as in humans, 4-DMAP more rapidly produces methemoglobinemia than the standard methemoglobin-former, sodium nitrite. In human volunteers, Klimmek *et al.* (1983) found that an IV dose of 3.25 mg/kg of DMAP produced a methemoglobin concentration of approximately 30% within 5 minutes (n = 3), and the same methemoglobin concentration was reached 50 minutes after an IM dose of 3.5 mg/kg of 4-DMAP (n = 6) and 30 minutes after an oral dose of 900 mg of 4-DMAP (n = 5). 4-DMAP is mainly excreted in the urine as the tris-cysteinyl derivative. Pharmacokinetic data are generally lacking (Von Clarmann, 1993).

22.4 Published clinical data

Kampe *et al.* (2000) reported the case of a man who ingested cyanide powder and beer. He was initially unconscious with dilated reactive pupils and was in respiratory failure and hypotensive, but not cyanotic. 4-DMAP (250 mg) was administered IV followed by an infusion of sodium thiosulfate. The peak methemoglobin level was 9.9% about 2 hours after 4-DMAP administration. The ingested cyanide dose was estimated to be 1250 mg and a measured whole blood cyanide level was 6.9 mg/l. The patient made an uneventful recovery and was discharged from the ICU to a psychiatric ward on the second hospital day (Kampe *et al.*, 2000).

Zilker and Schweizer (1987) reported the case of a young man who ingested about 320 mg of sodium cyanide in a suicide attempt following an argument with his wife. He shortly afterwards became unconscious. The patient was administered 250 mg of 4-DMAP IV followed by sodium thiosulfate and sodium bicarbonate. On arrival at the clinic, the patient was unconscious and his exhaled breath smelled of bitter almonds. He had generalized cyanosis from induced methemoglobinemia with an initial measured methemoglobin level of 15.8%, after which a second dose of 250 mg of 4-DMAP was administered, increasing the methemoglobin level to 37.7%. The patient fully recovered.

Daunderer *et al.* (1974) reported the case of a patient who ingested approximately 10 grams of potassium cyanide. About 45 minutes after the ingestion, the patient was treated with 250 mg of 4-DMAP and 300 mg of dicobalt-EDTA (Kelocyanor®), followed by 60 ml of 10% sodium thiosulfate solution, resulting in

a return to consciousness. The patient was profoundly acidotic (pH = 7.07) prior to antidote administration. The dicobalt-EDTA produced significant hypotension which persisted for 3 hours.

Von Clarmann (1987, pers. comm. at Joint Meeting of the EAPCC.CEC, IPCS and the National Poison Centre in Utrecht, The Netherlands) reported a series of 19 cases of cyanide poisoning treated with 4-DMAP and sometimes other cyanide antidotes and Weger (1983) gave some details on 15 such cases and noted that more than 20 such cases had been treated in the same institution in Munich, Germany. It is not totally clear if these case series overlap. In the Weger (1983) paper, it is reported that therapy with 4-DMAP and sodium thiosulfate was successful if the cyanide-poisoned individual was still breathing or no more than 5 minutes into respiratory arrest (but without cardiac standstill).

Of the cases described by Weger (1983), there were eight ingestions of potassium cyanide treated with 4-DMAP and seven of the eight patients survived. There were two cases of ingestion of >35 bitter almonds treated with 4-DMAP and both survived. There were three patients exposed to cyanide in galvanic baths (two cutaneous and one cutaneous + oral) and all three survived. There was one incident of hydrogen cyanide gas inhalation and one benzyl cyanide inhalation case and both patients survived (Weger, 1983).

In the Von Clarmann (1993) series of 19 patients, 4 of these cases were from other publications (Daunderer *et al*., 1974; Jacobs, 1984; Van Heijst *et al*., 1987). Of the remaining 15 cases, whole blood cyanide concentrations ranged from 0.24 to 70 mg/l (unknown or not measured in 5 cases). 4-DMAP doses given were from 250 to 1250 mg. Another antidote (dicobalt-EDTA; Kelocyanor®) was administered in three cases. There were 9 recoveries and 6 deaths among these 15 patients.

Pediatric dosing: Pediatric doses have not been established based on clinical trials. Adult dosing is generally said to be 3.25 mg/kg body weight (Zilker, 2005), and an empirical pediatric dose might be derived from this.

Special considerations: 4-DMAP is *contraindicated* in patients with glucose-6-phosphate dehydrogenase deficiency because it could induce intravascular hemolysis (Ballantyne *et al*., 2007).

22.5 Adverse/side effects

4-DMAP has been associated with severe toxicity with reticulocytosis, nephrotoxicity, and hemolysis (Mégarbane *et al*., 2003). 4-DMAP has resulted in serious adverse effects, especially when administered to patients without significant cyanide poisoning. A German chemical plant worker exposed to methyl isocyanate was mistakenly thought to have cyanide poisoning and was administered 450 mg of 4-DMAP followed by sodium thiosulfate and sodium bicarbonate. This patient developed a gray-black cyanosis and a methemoglobin level of 86.7% which decreased to 60% after administration of toluidine blue. He also developed cardiorespiratory depression, acute renal failure necessitating dialysis, pulmonary edema which progressed to acute respiratory distress syndrome (ARDS), bone marrow depression with persistent severe anemia, liver failure, septic encephalopathy, and tetraparesis. Recurrent episodes of excessive methemoglobinemia occurred over a period of weeks which were treated with further administration of toluidine blue. Intensive care treatment lasted for 39 days followed by two months of treatment in a rehabilitation clinic. He eventually fully recovered without any neurological or other organ system dysfunction (Kerger *et al*., 2005).

The difficulties with 4-DMAP administration are demonstrated by two cases reported by van Heijst *et al.* (1987). In one case, a cyanide-poisoned patient was administered 1000 mg of 4-DMAP IV in the mistaken belief that a vial contained 50 mg, when in fact a vial contains 250 mg. A methemoglobin level of 70% ensued which was treated with toluidine blue, decreasing the level to 25% over 33 hours. The following day this patient developed pneumonia and jaundice secondary to massive hemolysis, as well as elevated liver function tests. He had apparently ingested 1.5 grams of potassium cyanide.

A second patient who was employed in a galvanizing facility ingested sodium cyanide pellets and was deeply cyanotic on hospital admission. There was profound metabolic acidosis. Sodium nitrite administration only resulted in a methemoglobin level of 3.6%, so 250 mg of 4-DMAP was administered IV. Twenty-five minutes later, the patient developed a deep brown cyanosis, prompting therapy with toluidine blue. The methemoglobin level was 69% before toluidine blue administration, decreasing to 58% and then to

46% after additional toluidine blue. 4-DMAP was again administered resulting in an increase of the methemoglobin level to 52% The initial whole blood cyanide level of 7.5 mg/l had decreased to 0.41 mg/l, but the patient remained deeply comatose, developed ventricular fibrillation, and died (van Heijst *et al*., 1987).

A child who had eaten a few bitter almonds was treated with a 5-fold dose of 4-DMAP because of a calculation error (Weger, 1983). Extreme cyanosis and coma resulted, but treatment with toluidine blue overcame the 80% methemoglobin level and the child survived.

22.6 Conclusions

While limited experimental and clinical data suggest that 4-DMAP is an efficacious cyanide antidote, its safety profile is such that it would not be a good empirical antidote for use in the most common cyanide poisoning scenario – that of enclosed-space fire smoke inhalation where the presumptive diagnosis must currently be made on clinical grounds (Hall *et al*., 2009).

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CHAPTER 23

Cyanide antidotes in clinical use: dicobalt EDTA (Kelocyanor®)

Alan H. Hall

At a Glance

- Kelocyanor was developed in the UK and has been used in other countries including France.
- It is an efficacious cyanide antidote.
- It has various adverse effects which make it undesirable for use in smoke inhalation cases.

23.1 Introduction

Dicobalt edetate (dicobalt EDTA; Kelocyanor®) is the dicobalt salt of edetic acid. A French preparation has the composition of 0.300 grams of dicobalt edetate in 4 grams of water for injection, and is indicated for the antidotal treatment of cyanide poisoning (*Dictionnaire Vidal*, 1987). A UK preparation is supplied as a clear violet solution in 20 ml glass ampoules containing 0.196–0.240 g/100 ml of free cobalt, 1.35–1.65 g/ml of dicobalt edetate, and 4 g of glucose (Marrs, 1993). Kelocyanor® has a three-year shelf life (*Dictionnaire Vidal*, 1987) when stored at 25°C. It should be stored in the dark (Marrs, 1993).

The possible presence of free cobalt cations in the antidotal preparation may be responsible for serious cobalt toxicity when the patient does not have any significant cyanide poisoning (Hall *et al*., 2009). Administration of hypertonic glucose following IV administration of Kelocyanor® may have a protective effect, although the mechanism is unclear (Hall *et al*., 2009).

An early industrial publication suggested that alternate inhalations of amyl nitrite and oxygen might be an adequate first response, followed by what was then believed to be "non-toxic" Kelocyanor® (Davison, 1969). This idea of "non-toxic" Kelocyanor® has not been borne out. Adverse effects of nausea, vomiting, sweating, cardiac anginal chest pain, skin rashes, facial edema, hypertension, hypotension, nervousness, significant tremors, gastrointestinal hemorrhaging, and seizures have occurred (Hall *et al*., 2009; *Dictionnaire Vidal*, 1987).

23.2 Mechanism of action

According to a French package insert, after injection of Kelocyanor®, the Co^{2+} complexes with two cyanide anions; the cobalt salt then produces another Co^{2+} which complexes another two cyanide anions (*Dictionnaire Vidal*, 1987). The cobalt-cyanide complexes are very stable and are eliminated in the urine (*Dictionnaire Vidal*, 1987). Other thoughts are that the cobalt of Kelocyanor® is a formation first of cobaltocyanide ion $(CoCN_6)^{4-}$ and then cobalticyanide ion $(CoCN_6)^{3-}$ which is stable and much less toxic than cyanide (Hall *et al*., 2009; Marrs, 1993; Evans, 1964).

The usual dose of Kelocyanor® is 300–600 mg (1–2 20 ml ampoules) administered IV as rapidly as possible after the onset of acute cyanide poisoning (*Dictionnaire Vidal*, 1987). Each injection should be followed by IV administration of hypertonic glucose (*Dictionnaire Vidal*, 1987; Mégarbane *et al*., 2003).

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A third ampoule may be injected if the blood pressure is stable 5 minutes after the second if there is insufficient clinical improvement (*Dictionnaire Vidal*, 1987). Formal pharmacokinetic studies of Kelocyanor® have not been carried out (Marrs, 1993). It is likely that either free cobalt or Kelocyanor® crosses the blood-brain barrier producing centrally mediated effects (Marrs, 1993). In mice, the cyanide-cobalt complex is excreted in the urine (Frankenberg & Sörbo, 1975).

23.3 Experimental data

Animal studies of the antidotal effects of Kelocyanor® have been summarized by Hall *et al*. (2009) and Marrs (1987, 1993). Studies in France by Paulet and colleagues in several animal species showed that after IV or IM potassium cyanide administration, Kelocyanor® was efficacious for improving survival, even when administered rather late in the classical sequence of events of cyanide poisoning (Paulet & Dassonville, 1985; Paulet, 1984; Paulet *et al*., 1959; Paulet & Le Bars 1959). In mice, Terzic and Milosevic (1963) found Kelocyanor® to have a better therapeutic ratio of effective dose (ED_{95}) than sodium nitrite.

Others experimental animal studies of a variety of cobalt chelators including Kelocyanor® done in the U.K. were published by Evans (1964). These studies showed many cobalt-containing compounds to be efficacious in animal models of cyanide poisoning and that dicobalt edetate was the least toxic of the tested compounds (Evans, 1964).

Marrs *et al*. (1985) performed studies of Kelocyanor® as a cyanide antidote as compared to the methemoglobin-inducing agent, 4-dimethylaminophenol (4-DMAP). In dogs poisoned with potassium cyanide by gavage, 4-DMAP gave better results on survival with all treated animals surviving while only 1 of 4 dogs administered Kelocyanor® survived (Marrs *et al*., 1985).

23.4 Published clinical data

No normal volunteer studies or controlled clinical trials have been carried out (Marrs, 1993). The following is a summary of reported usage in human poisoning cases.

Bain and Knowles (1967) reported the case of a 61-year-old man with occupational cyanide poisoning. He presented comatose and seizing. Kelocyanor® was administered in 2 doses of 150 mg each, 10 minutes apart. Vomiting occurred after each dose. After antidote administration, the patient became coherent and the seizures ceased. The whole blood cyanide level before antidote administration was 5.1 mg/l (Bain & Knowles, 1967).

Bourrelier and Paulet (1971) reported three cases of dermal exposure to molten sodium cyanide. In one of these cases, a 50-year-old man, initially comatose, regained consciousness after administration of 600 mg of Kelocyanor®.

Naughton (1974) reported the case of a 35-year-old man who ingested cyanide and became comatose and tachypneic within 10 minutes. After receiving amyl nitrite by inhalation and IV Kelocyanor®, skin flushing and atrial fibrillation with ventricular extrasystoles developed. The patient regained consciousness and could be extubated 2 $^1/_2$ hours after hospital admission. Measured whole blood cyanide level was 0.6 mg/l (Naughton, 1974). Generally recognized whole blood cyanide toxic level is 1.0 mg/ml and potentially lethal level is 3.0 mg/l (Hall *et al*., 2009).

Hillman *et al*. (1974) reported two cases of sodium cyanide ingestion. The first patient accidentally drank sodium cyanide solution, collapsed 2–3 minutes later, and was cyanotic and in respiratory distress 15 minutes later. On arrival at hospital 55 minutes after ingestion, he was apneic and pulseless. ECG showed a slow idioventricular rhythm. An initial 300 mg dose of Kelocyanor® resulted in development of sinus tachycardia with a blood pressure of 60 mmHg, but no spontaneous respirations, and he was administered two additional doses 10 and 15 minutes after the initial dose, which produced no change in his condition. Three additional doses of Kelocyanor® were administered, and then nine more doses at half-hourly intervals. The patient developed irreversible brain damage and asystolic cardiac arrest 44 hours after hospital admission. The whole blood cyanide level was 550 µg/dl (5.5 mg/l) (Hillman *et al*., 1974).

A second patient ingested a small amount of sodium cyanide and collapsed but did not become unconscious (Hillman *et al*., 1974). After administration of two ampoules (600 mg) of Kelocyanor®, this patient developed sweating, anginal chest pain, ectopic ventricular beats, and intense nausea and vomiting. He later developed a transient red maculo-papular rash over the chest

and arms. These adverse effects were attributed to the Kelocyanor® (Hillman *et al*., 1974).

Wright and Vesey (1986) reported a case of cyanide poisoning from ingestion of potassium gold cyanide salt. Four hours later, the patient presented with polypnea and hypotension. The blood pressure increased to 90 mmHg after treatment with oxygen, colloid infusion, and amyl nitrite inhalation. However, following IV administration of 300 mg of Kelocyanor®, an apparent anaphylactic reaction occurred with periorbital edema. The patient regained consciousness, but died 13 hours after hospital admission (Wright & Vesey, 1986).

Singh *et al*. (1989) reported the case of a 24-year-old patient who was found in a coma with apnea, cardiovascular shock, and metabolic acidosis. Kelocyanor®, 300 mg each of three doses was administered 1–2 minutes apart, and was followed by facial edema and hemodynamic instability. This patient died in a decerebrate condition.

In some cases, combinations of antidotes (e.g., amyl nitrite, sodium thiosulfate, hydroxocobalamin) have been administered and the contribution of Kelocyanor® to clinical efficacy is difficult to determine (Singh *et al*., 1989; Wright & Vesey, 1986; Hillman *et al*., 1974; Naughton, 1974; Bourrelier & Paulet, 1971; Bain & Knowles, 1967; Jerretin, 1963; Yacoub *et al*., 1974; Lutier *et al*., 1971; Jaeger *et al*., 1992; Hoang The Dan *et al*., 1981).

Pediatric dosing: No pediatric doses have been established by clinical trials.

Special considerations: Precaution – Kelocyanor® administration should be reserved for confirmed cyanide poisoning because of it adverse toxic effects (*Dictionnaire Vidal*, 1987). In effect, Kelocyanor® and cyanide are mutually antidotal, and presence of either substance alone in a patient leads to significant toxicity.

23.5 Adverse/side effects

Adverse effects of nausea, vomiting, sweating, cardiac anginal chest pain, skin rashes, facial edema, hypertension, hypotension, nervousness, significant tremors, gastrointestinal hemorrhaging, and seizures have occurred (Hall *et al*., 2009; *Dictionnaire Vidal*, 1987; Marrs, 1993; Peters *et al*., 1982). Such side effects have particularly been noted when Kelocyanor® has been administered to patients not having significant cyanide poisoning (Hall *et al*., 2009; Marrs, 1993; Marrs *et al*., 1985; Pickering, 1985; Hillman *et al*., 1974), but also when serious cyanide poisoning is actually present (Dodds & McKnight, 1985).

Dodds and McKnight (1985) reported the case of an industrial worker who was accidentally immersed in a vat containing 1000 gallons of hot cupric cyanide, resulting in cyanide poisoning with unconsciousness, cyanosis, and irregular respirations. His condition did not improve after IV administration of 300 mg of Kelocyanor®, so an additional 300 mg was administered. Severe facial and laryngeal edema then developed, as well as pulmonary edema. After intubation, mechanical ventilation and other intensive supportive therapy, the patient made a full recovery from both the cyanide poisoning and the adverse effects of the antidote (Dodds & McKnight, 1985).

A review by Marrs *et al*. (1985) noted unpleasant adverse reactions when Kelocyanor® was administered in the absence of significant cyanide poisoning.

23.6 Conclusions

While experimental and clinical data suggest that Kelocyanor® is an efficacious cyanide antidote, its safety profile is such that it would not be a good empirical antidote for use in the most common cyanide poisoning scenario – that of enclosed-space fire smoke inhalation where the presumptive diagnosis must currently be made on clinical grounds (Hall *et al*., 2009).

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CHAPTER 24

Amyl nitrite, sodium nitrite, and sodium thiosulfate

Richard J. Geller

At a Glance

- The combination of amyl nitrite, sodium nitrite, and sodium thiosulfate (the Cyanide Antidote kit [CAK]) was researched in animal models in the early 1930s in the US and Argentina, and was in clinical use from the 1930s to 2012 when the last US manufacturer ceased production.
- At present, amyl nitrite can be purchased separately and a kit containing sodium nitrite and sodium thiosulfate (Nithiodote®) is available.
- Amyl nitrite inhalation alone has been reported to be efficacious in a small group of industrial cyanide production workers exposed to HCN by inhalation.
- Amyl and sodium nitrites are methemoglobin inducers. Methemoglobin has a higher affinity for cyanide than does the ferric (Fe^{3+}) iron of cytochrome oxidase a_3. A second mechanism of action may be nitric oxide (NO) generation.
- Significant adverse effects of sodium nitrite are hypotension from vasodilation with too-rapid IV administration and excessive methemoglobin induction.
- Sodium thiosulfate usually has only minor adverse effects, may sometimes be efficacious when administered alone in acute cyanide poisoning, and is efficacious in preventing cyanide accumulation when co-infused during high-dose nitroprusside treatment.

24.1 History and chemistry

Inhaled amyl nitrite ($C_5-H_{11}-N-O_2$, CAS RN: 110-46-3), intravenous sodium nitrite ($Na-N-O_2$, CAS RN: 7632-00-0) and intravenous sodium thiosulfate ($Na_2-O_3-S_2$, CAS RN: 7772-98-7) have historically been the available antidotes used in the treatment of cyanide poisoning in the United States (see structures in Table 24.1).

In 1888, Pedigo described the use of amyl nitrite to prevent cyanide poisoning in a canine model. Sodium thiosulfate was demonstrated to be useful as a cyanide antidote in 1895 by Lang (Chen & Rose, 1952). Neither of these observations was put to immediate clinical use. In 1929, Mladoveanu and Gheorghiu, using a canine model, prevented death from lethal doses of potassium cyanide by using sodium nitrite (Chen & Rose, 1952).

By 1933, investigators in multiple countries were reporting that a combination of intravenous sodium nitrite plus intravenous sodium thiosulfate was effective in treating cyanide poisoning. Working in Argentina, Hug demonstrated efficacy in treating cyanide poisoning in a rabbit model (Hug, 1933). Working in the Eli Lilly and Company laboratories in Indianapolis, Indiana, Chen, Rose, and Clowes demonstrated in a canine model that inhaled amyl nitrite, intravenous sodium nitrite and intravenous sodium thiosulfate each improved survival in cyanide poisoning (Chen *et al.*, 1933a, 1933b). The investigators found that amyl nitrite and sodium nitrite, when given to dogs as single agents, could each detoxify four minimum lethal doses (MLDs) of sodium cyanide. They also found that sodium thiosulfate detoxified 3 MLDs of sodium cyanide when administered as a single agent. When administered

Toxicology of Cyanides and Cyanogens: Experimental, Applied and Clinical Aspects, First Edition.
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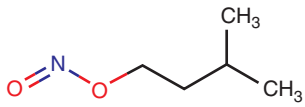
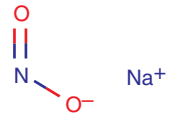
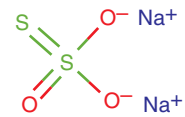
Table 24.1 Chemical structures of cyanide antidotes.

Amyl nitriteRN: 110-46-3	Sodium nitriteRN: 7632-00-0	Sodium thiosulfateRN: 7772-98-7
		

Adapted from ChemIDplus, and used with permission from the Division of Specialized Information Services NLM/NIH

one after another, however, the combination of amyl nitrite and sodium thiosulfate detoxified 10 MLDs of sodium cyanide, and the combination of sodium nitrite followed by sodium thiosulfate detoxified 13 MLDs of sodium cyanide. The synergistic therapeutic effect of nitrite and thiosulfate was thus established in the treatment of cyanide poisoning. Way produced similar results in 1972 working with mice, demonstrating that nitrite increased the median lethal dose of potassium cyanide from 11 mg/kg to 21 mg/kg, and that adding thiosulfate further increased the median lethal dose to 52 mg/kg (Way *et al.*, 1972).

It had previously been established that nitrite compounds were capable of oxidizing the Fe^{2+} (ferrous) atom of hemoglobin to the Fe^{3+} (ferric) state, creating methemoglobinemia. Also, it was known that cyanide had a strong binding affinity for the Fe^{3+} atom, causing severe poisoning by binding to the Fe^{3+} atom of what was then called mitochondrial ferricytochrome oxidase, and inactivating the enzyme. Chen *et al.* credited Hug (1933) and Wendel (1933) with formulating the hypothesis that the mechanism of action of nitrite as a cyanide antidote was as a methemoglobin former, the oxidized Fe^{3+} atom scavenging CN^- from ferricytochrome oxidase to form cyanomethemoglobin. Chen and Rose (1952) advanced the following three equations as evidence that cyanomethemoglobin formation was key:

ferricytochrome oxidase + NaCN = ferricytochrome oxidase cyanide

$NaNO_2$ + hemoglobin = methemoglobin

methemoglobin + NaCN = cyanomethemoglobin

They inferred that ferricytochrome oxidase cyanide + methemoglobin would yield cyanomethemoglobin as well as regenerated ferricytochrome oxidase. This would be, for the next 50 years, the accepted mechanism of action of nitrite therapy. Their work led to the introduction of the Lilly Cyanide Antidote Kit in the 1930s, containing both nitrites and sodium thiosulfate, which was, going forward, the standard treatment for cyanide poisoning in the United States through the end of the 20th century.

24.2 Theoretical bases for use/mechanism of action

No clinical trials in humans have ever assessed the efficacy of either the nitrites or thiosulfate in the treatment of cyanide poisoning. Clinical utility has largely been established in animal models, primarily in dogs. There is, however, a robust medical literature of case reports associating antidotal use with clinical success. These include, in alphabetical order: Bismuth (1993), Chen (case series of 16) (Chen & Rose, 1952, 1956), Cheok (1978), Chin (Chin & Calderon, 2000), Garlich *et al.* (2012), Geller *et al.* (1991), Goodhart (1994), Hall and Rumack (1987), Heintz *et al.* (1990), Lam and Lau (2000; case series of 2), Johnson *et al.* (1989), Nakatani *et al.* (1993), Perrson (1993; case series of 5), Potter (1950; case series of 2), Ruangkanchanasetr *et al.* (1999), Scolnick *et al.* (1993), Turchen *et al.* (1991) and Wurzburg (1996). The case described by Hall *et al.* (1987) is especially compelling, as it describes a man who ingested 1 gram of potassium cyanide in a suicide attempt and who experienced classic, life-threatening, cyanide poisoning including seizures, metabolic acidosis, coma, and apnea. At the conclusion of infusions of sodium nitrite and sodium thiosulfate, he had regained spontaneous respiration, and recovered without sequelae. Potter's (1950) cases are equally compelling reading.

The combination of nitrite plus thiosulfate therapy, administered one after another, is far more effective than either one alone. In addition to the synergism demonstrated in the studies cited above, the use of the two drug combination has been shown to be highly effective in mice (Way *et al.*, 1966; Sheehy & Way,

1968; Frankenberg & Sörbo, 1975), and also in sheep (Burrows & Way, 1977).

24.2.1 Amyl nitrite and sodium nitrite

There are two postulated mechanisms of therapeutic action for the nitrite compounds. The first is as methemoglobin formers. The oxidation of Fe^{2+} atoms to Fe^{3+} atoms in circulating oxidized hemoglobin is thought to provide alternative binding sites for cyanide. This allows cyanide to leave the Fe^{3+} binding sites within enzymes of the electron transport chain, most importantly cytochrome oxidase a_3. The use of inhaled amyl nitrite produces variable methemoglobin concentrations, but can be as high as 6–7% (Jandorf & Bodansky, 1946) (Hall *et al*., 2009).

Intravenous sodium nitrite produces a variable amount of methemoglobinemia, which after the recommended dose can reach as high as 40% in as little as 12 minutes (Van Heijst *et al*., 1987). Following an infusion of 300 mg sodium nitrite, methemoglobin levels averaging 20–30% are created (Mégarbane *et al*., 2003). The optimal level of methemoglobin to treat cyanide poisoning is unknown, although a target level of 20–30% is suggested in the literature (Reade *et al*., 2012). Methemoglobin levels are thus not useful in guiding dosage of the drug, except to aid in avoiding a toxic state of methemoglobinemia.

Induction of methemoglobinemia is unlikely to be the sole mechanism by which the nitrites antagonize cyanide. Several case reports have documented a positive effect in victims who developed no substantive methemoglobin (Johnson *et al*., 1989; Vick & Froehlich, 1985). Further, in an experimental model where methylene blue was administered prior to nitrite administration, in order to specifically prevent methemoglobin formation, sodium nitrite was still an effective antidote (Holmes & Way, 1982; Way, 1983, 1984). Moreover, it has been observed that the effect of sodium nitrite as an antidote in cyanide poisoning is rapid, occurring before the onset of methemoglobinemia (Way, 1984).

Support for methemoglobin formation as a mechanism is provided by the finding that other hemoglobin oxidizers, which form methemoglobin more rapidly than nitrite, such as 4-dimethylaminophenol and hydroxylamine, also have effectiveness as cyanide antidotes (Marrs, 1987; Vick & Froehlich, 1991). Further, stroma-free methemoglobin solution was demonstrated to be an effective antidote for acute cyanide poisoning (Ten Eyck *et al*., 1983–4, 1985), protecting a cohort of rats administered up to eight times the LD_{90} dose of sodium cyanide. Ten Eyck *et al*.'s experiments demonstrated that methemoglobin is an effective antidote in the absence of nitrite, and strongly suggested that, indeed, the production of methemoglobinemia is at least one of nitrite's therapeutic mechanisms.

The second postulated mechanism of therapeutic action for the nitrite compounds is via a conversion to nitric oxide (NO). It has long been observed that nitrite administration causes vasodilation and hypotension, which can be a drawback to sodium nitrite administration. Multiple investigators of cyanide poisoning have hypothesized that the therapeutic benefit of nitrite may be related to its vasodilatory effect (Way, 1984; Hall & Rumack, 1986). Investigating a link between nitric oxide and vasodilation, Modin *et al*. (2001) found that, in a rat model, nitric oxide caused aortic relaxation which was pH dependent, occurring more prominently in acidemic tissue. Cosby *et al*. (2003) established that vasodilation was associated with reduction of nitrite to nitric oxide by deoxyhemoglobin. Select vasodilation would thus occur in hypoxemic tissue, where deoxyhemoglobin is found.

Building on this work, and the work of others, Leavesley *et al*. (2010) found that sodium nitrite antagonizes the cyanide inhibition of cytochrome oxidase through the generation of nitric oxide, which then directly causes a displacement of cyanide from the critical enzyme. Leavesley concluded that the antagonism of cyanide by nitrite appears to be due to both generation of methemoglobin as well as direct rescue of cytochrome oxidase from cyanide by nitric oxide.

Ironically, nitric oxide is one of four gases (carbon monoxide, hydrogen sulfide, and hydrogen cyanide being the other three) which are capable of inhibiting oxygen consumption by poisoning mitochondrial cytochrome oxidase (Cooper & Brown, 2008).

In cases of cyanide poisoning caused by smoke inhalation, carbon monoxide poisoning is usually also present. Because both methemoglobinemia and CO shift the oxygen-hemoglobin dissociation curve to the left, thereby impairing the release of oxygen to peripheral tissues, the use of nitrites in the context of smoke inhalation is ill-advised. Persons with G6PD deficiency should also not receive nitrites, as significant hemolysis may result (Way, 1984). Sodium thiosulfate

may be administered safely as a single agent in both of these circumstances, however.

Sodium nitrite should be dosed very carefully in children (Berlin, 1970) in order to avoid dosing errors, as well as in patients with methemoglobin reductase deficiency.

24.2.2 Sodium thiosulfate

Thiosulfate works as an antidote in cyanide poisoning by acting as a donor of sulfane sulfur, which endogenous sulfurtransferase enzymes, most importantly mitochondrial rhodanese (thiosulfate-cyanide sulfur transferase) and B-mercaptopyruvate sulfur transferase, combine with cyanide (CN^-) to form thiocyanate (SCN^-, CAS RN: 302-04-5). Thiocyanate is a species far less toxic than cyanide (Beasley & Glass, 1998; Marrs, 1988), and was used therapeutically as an anti-hypertensive drug throughout the first half of the 20th century (Barnett *et al.*, 1951). Thiocyanate is renally excreted, and can accumulate in quantities sufficient to cause toxicity in renal failure patients. Should thiocyanate retention occur because of renal insufficiency, hemodialysis is very effective in removing SCN^- (Pahl & Vaziri, 1982).

Sulfane sulfur refers to divalent sulfur attached only to another sulfur atom, and is the only sulfur species used by sulfurtransferase to bind cyanide. Sulfurtransferases are also capable of producing sulfane sulfur from other sulfur species (Westley *et al.*, 1983).

Thiosulfate's distribution would predict that it might not be an effective antidote, as it poorly diffuses into the brain, an organ highly sensitive to the cellular asphyxiant effects of cyanide. It also poorly migrates into mitochondria, the site of both the cyanide inactivation of the electron transport chain enzyme, cytochrome oxidase a_3, which it seeks to reverse, as well as the rhodanese enzyme it needs to form thiocyanate. Rhodanese is located on the cristae of the mitochondria, into which anionic species such as thiosulfate penetrate slowly (Baskin *et al.*, 1992).

Thiosulfate's rate of onset of action has been variably described in the medical literature. While its distribution kinetics would anticipate a slow onset, Sylvester *et al.* (1981, 1983) found in a canine study that within 5 minutes of administration there was a striking difference between the cyanide levels in thiosulfate-treated dogs versus the control animals, with cyanide being much lower in the treated animals. Moreover, cyanide decline occurred with a concomitant rapid rise in thiocyanate levels. It was concluded that cyanide is converted to thiocyanate primarily in the blood, and/or in tissues having close proximity to the blood.

Some authors believe that sodium thiosulfate is more useful to prevent cyanide accumulation, as in the context of sodium nitroprusside therapy, than as a post hoc therapy for acute cyanide poisoning. But again, Chen (Chen *et al.* 1933a, 1933b) found that thiosulfate alone could detoxify three MLDs of sodium cyanide following poisoning, and kinetic studies have shown a threefold increase in cyanide clearance from thiosulfate-induced thiocyanate formation (Paulet, 1984).

24.3 Pharmacokinetics

Amyl Nitrite, a flammable and volatile liquid with an unpleasant odor, is very rapidly absorbed following inhalation, appearing in the bloodstream in under a minute. Its vasodilatory effects begin in about 30 seconds, and last 3–5 minutes. Amyl nitrite undergoes rapid hydrolysis to isoamyl alcohol and nitrite ion (Baselt, 2011).

Sodium nitrite has a plasma half-life estimated to be between 30 and 50 minutes (Baselt, 2011; Pluta *et al.*, 2011). Nitrite undergoes conversion to nitrate, which is then eliminated by the kidney. Caution should be undertaken in re-dosing the patient based on the expected elimination half-life of nitrite, as the endogenous reduction of methemoglobin to hemoglobin occurs far more slowly than does the elimination of nitrite. Thus, the oxidative effect of nitrite on hemoglobin will long outlive the presence of the drug in the blood. Volume of distribution constant is estimated to be between 0.2 and 0.5 l/kg (Baselt, 2011).

Sodium thiosulfate. An estimate of plasma half-life is 16.5 minutes (Schulz *et al.*, 1982). Estimates of volume of distribution constant are below 0.5 l/kg (Sylvester *et al.*, 1983; Ivankovich *et al.*, 1983).

24.4 How supplied

The last United States manufacturer of the traditional three agent cyanide antidote kit developed by Lilly ceased production in 2012. Available now in the U.S. is Nithiodote®, which is marketed by Hope Pharmaceuticals, and which contains one vial of sodium nitrite,

300 mg/10 ml (30 mg/ml) and one vial of sodium thiosulfate, 12.5 grams/50 ml (250 mg/ml). The U.S. Food and Drug Administration had never approved amyl nitrite as an antidote for cyanide poisoning. When Hope sought approval for a 3-antidote kit, the FDA declined to approve the amyl nitrite component, but approved Nithiodote® as a 2-antidote kit.

The American pharmaceutical supply chain is an ever-changing landscape, but as of the end of 2012 amyl nitrite inhalant was available as a stand-alone drug available through Moore Medical and Henry Schein Medical. It comes in a 0.3 ml thin-glassed ampule surrounded by protective cloth, and is designed to be cracked open and inhaled, as the liquid is volatilized from the cloth.

The pearl, or perle (thin glass vial surrounded by protective cloth), containing the amyl nitrite is broken open and the contents are inhaled for periods of 15 seconds interspersed with 15 second periods of breathing without the antidote, for a period of 5 minutes (Lavon & Bentur, 2010). The liquid may be placed into a gauze and interpolated into the airstream/oxygen supply of mask and bag ventilation. The best use of amyl nitrite may be in industrial settings where the generation of cyanide is expected, exposure is by the inhalational route, and where the diagnosis is likely to be made swiftly after exposure (Jandorf & Bodansky, 1946). Amyl nitrite, because of its weak and unpredictable hemoglobin oxidizing effects, should be viewed as a temporizing measure pending administration of IV nitrite and thiosulfate, and not as a stand-alone resuscitative agent (Hall *et al*., 2009).

24.5 Indication and dosing of intravenous antidotes

According to the manufacturer, sequential administration of sodium nitrite and sodium thiosulfate is indicated for the "treatment of cyanide poisoning that is judged to be life threatening" (Hope Pharmaceuticals, n.d.).

Sodium nitrite, because of the potential danger of initiating methemoglobinemia and because of the severe hypotension it may cause, should not be administered empirically or in the absence of a strong suspicion of life-threatening cyanide poisoning. Administration should be accompanied by oxygen administration, by careful blood pressure monitoring and with the anticipation that aggressive fluid resuscitation may be needed.

Though either antidote without the other is far less effective than the combination, sodium thiosulfate alone may be safely administered when cyanide poisoning is in the differential diagnosis but the diagnosis is by no means clear, as it is a largely non-toxic antidote.

The doses of sodium nitrite and sodium thiosulfate are weight-based in children, but not in adults, for whom fixed doses are administered.

24.5.1 Cyanide poisoning

Adults. Sodium nitrite is administered intravenously, 10 ml of a 30 mg per ml solution (300 mg), at a rate not to exceed 2.5 to 5 ml per minute. Immediately following sodium nitrite, *sodium thiosulfate* 50 ml of the 250 mg/ml solution (12.5 grams) is infused at 2.5 to 5 ml per minute. Nitrite and thiosulfate should be administered in rapid sequence, and cannot be mixed together as one infusion. As with any infusion, adverse effects may be fewer and less severe with slower infusion rates, if the clinical situation allows.

Children. Great care must be taken to avoid a dosing error with *sodium nitrite*. A dose of 0.2 ml/kg of the 30 mg per ml solution (6 mg/kg or 6 – 8 ml/m^2 BSA) sodium nitrite is administered not to exceed 2.5 to 5 ml per minute, and not to exceed a dose of 10 ml. The dose of sodium thiosulfate for children is 1 ml of the 250 mg/ml solution per kilogram body weight (250 mg/kg), or 30 – 40 ml/m^2 BSA, not to exceed 50 ml total dose, immediately following sodium nitrite, at a rate of 2.5 to 5 ml per minute.

Pregnant women. Both sodium nitrite and sodium thiosulfate are Pregnancy Category C drugs, and there are no adequate and well controlled studies in pregnant women. However, in moderate to severe cyanide poisoning, the potential benefit to mother and fetus would seem to justify potential risk to the fetus. In a model employing near-term gravid ewes, Curry *et al*. (1997) demonstrated that cyanide crosses the placenta, and that sodium thiosulfate prevented both maternal and fetal cyanide toxicity from nitroprusside administered to the ewe. In a further study using the near-term gravid ewe model, Graeme *et al*. (1999) found that thiosulfate did not cross the placenta, and that the fetus was dependent on maternal detoxification of cyanide following infusion of thiosulfate into the maternal circulation.

Nursing Mothers. It is not known whether sodium nitrite or sodium thiosulfate is excreted in breast milk, but breastfeeding is not a contraindication to use.

The endpoints of therapy are a positive clinical response or toxicity from the antidote. As toxicity from methemoglobinemia climbs rapidly at levels above 30%, there is little point in dosing additional sodium nitrite to achieve levels above that. There appears to be little downside to re-dosing thiosulfate. If more than one dose of either antidote is deemed clinically appropriate, the manufacturer's package insert (Nithiodote "Highlights of Prescribing Information," Hope Pharmaceuticals, Scottsdale, AZ) states that "if signs of cyanide poisoning reappear, repeat treatment using one-half the original dose of both sodium nitrite and sodium thiosulfate".

Prophylaxis against cyanide poisoning. Sodium nitroprusside (C5-Fe-N6-Na2-O, CAS RN: 13755-38-9) is 44% cyanide by weight, as each molecule of contains five cyanide moieties. Infusion of sodium nitroprusside at rates exceeding 2 mcg/kg/min can lead to rising blood cyanide levels, as the rate of CN^- generation exceeds its endogenous conversion to thiocyanate (Schulz *et al*., 1982). Fatal cyanide poisoning resulting from therapeutic use of sodium nitroprusside was reported as early as 1974 (Jack, 1974; MacRae & Owen, 1974; Merrifield & Blundell, 1974.)

If the rate of nitroprusside administration is expected to exceed 2 mcg/kg/min, the clinician must either monitor the patient for signs and symptoms of cyanide accumulation, or prevent cyanide toxicity by prophylactic use of sodium thiosulfate. A 1 : 10 (by weight) intravenous admixture of sodium nitroprusside plus sodium thiosulfate has been shown to be chemically compatible, stable for at least 48 hours when shielded from light (Schulz *et al*., 2010) and effective in preventing the accumulation of cyanide (Schulz *et al*., 1982). The potential for accumulation of toxic amounts of thiocyanate must be kept in mind whenever nitroprusside is administered to patients with renal failure.

24.6 Adverse effects

Amyl nitrite produces hypotension, dizziness, fatigue, and headache (Klimmek *et al*., 1988), but is not expected to substantively contribute to toxic methemoglobinemia.

Sodium nitrite can be anticipated to cause profound hypotension, secondary to decreased peripheral resistance, and methemoglobinemia, both of which the manufacturer warns may be "life threatening". Other ADRs reported by the manufacturer include: (CV) syncope, tachycardia, palpitations, dysrhythmia (CNS), headache, dizziness, blurred vision, confusion, coma, seizures (GI), nausea, vomiting, abdominal pain (respiratory), tachypnea, dyspnea (general), anxiety, diaphoresis, lightheadedness, injection site tingling, cyanosis, acidosis, fatigue, weakness, urticaria, and generalized numbness and tingling (Nithiodote "Highlights of Prescribing Information," Hope Pharmaceuticals, Scottsdale, AZ).

Sodium thiosulfate is usually very well tolerated, though nausea and vomiting can be seen with rapid administration. Other ADRs reported by the manufacturer include: (CV) hypotension (CNS), headache, disorientation (hematologic), prolonged bleeding time (general), salty taste in mouth and warm sensation all over body (Nithiodote "Highlights of Prescribing Information," Hope Pharmaceuticals, Scottsdale, AZ).

24.7 Conclusions

When administered intravenously one after another, sodium nitrite and sodium thiosulfate constitute an effective treatment regimen for acute cyanide poisoning, but are far less effective if only one antidote is given. Sodium nitrite has the potential for serious toxicity via methemoglobinemia and hypotension, and must be dosed carefully. Sodium thiosulfate is far less toxic than nitrite, and may be given empirically for "possible" cases of cyanide poisoning, and also co-administered with nitroprusside in order to prevent cyanide accumulation. Amyl nitrite may still have a role in the treatment of cyanide poisoning, as it is the only antidote which can be administered before initiation of an intravenous access. As with any antidotal treatment of cyanide poisoning, time is of the utmost urgency.

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CHAPTER 25

Cyanide antidotes in current clinical use: hydroxocobalamin

Alan H. Hall and Stephen W. Borron

At a Glance

- Hydrocobalamin is a cobalt-based direct cyanide chelating antidote.
- It also has nitric oxide (NO) scavenging activity.
- Of all the cyanide antidotes in current clinical use, Hydroxocobalamin is at least as efficacious as the others and has the most favorable safety profile.
- Hydroxocobalamin is suitable for pre-hospital or emergency department empirical administration for known or suspected cyanide poisoning, especially for victims of enclosed-space smoke inhalation.
- Nearly all patients administered Hydroxocobalamin develop transient reddish-brown discoloration of the skin, sclera, mucous membranes, and urine.
- Because of Hydroxocobalamin's absorption spectra, there are drug-colorimetric laboratory testing interferences which may result in either falsely elevated or decreased reported values.

25.1 Background and historical perspective

In 1952, Mushett and colleagues first demonstrated the efficacy of hydroxocobalamin as a cyanide antidote in a mouse model (Mushett *et al.*, 1952). One mechanism of action is a direct binding of the cyanide anion (CN^-) to the cobalt moiety of hydroxocobalamin, displacing an OH^- moiety, and forming essentially nontoxic cyanocobalamin (vitamin B12) which is readily excreted in the urine (Bowden & Krenzelok, 1997). A second mechanism of action, nitric oxide (NO) scavenging, results in increasing the blood pressure which, although sometimes cited as an adverse effect (Meridian Medical Technologies, 2010), could actually be a *therapeutic* effect in hypotensive cyanide-poisoned patients (Uhl *et al.*, 2006; Hall *et al.*, 2007).

Hydroxocobalamin is more rapidly acting than sodium thiosulfate, does not produce methemoglobinemia as do some cyanide antidotes (amyl and sodium nitrites, 4-DMAP [4-dimethylaminophenol]), and is not associated with potential life-threatening adverse effects especially when administered in the absence of cyanide poisoning, as does dicobalt-EDTA (Kelocyanor®). It has an acceptable safety profile, such that it can be used empirically for suspected but not confirmed cyanide poisoning, including for victims of smoke inhalation in either the pre-hospital or hospital settings (Hall *et al.*, 2007; Baud *et al.*, 2002; Santiago, 2003).

There may be an antidotal synergy with sodium thiosulfate (Baud *et al.*, 2002; Santiago, 2003; Hall & Rumack, 1987). In fact, the initial hydroxocobalamin cyanide antidote preparation was packaged as a combination of 4 grams of hydroxocobalamin lyophilized base, 8 grams of sodium thiosulfate, 0.32 grams of neutral sodium sulfite, and 80 ml of *QSP* water for injection.

In an editorial accompanying a supplemental edition of *Clinical Toxicology*, Dart (2006) reviewed the various specific cyanide antidotes in clinical use worldwide and

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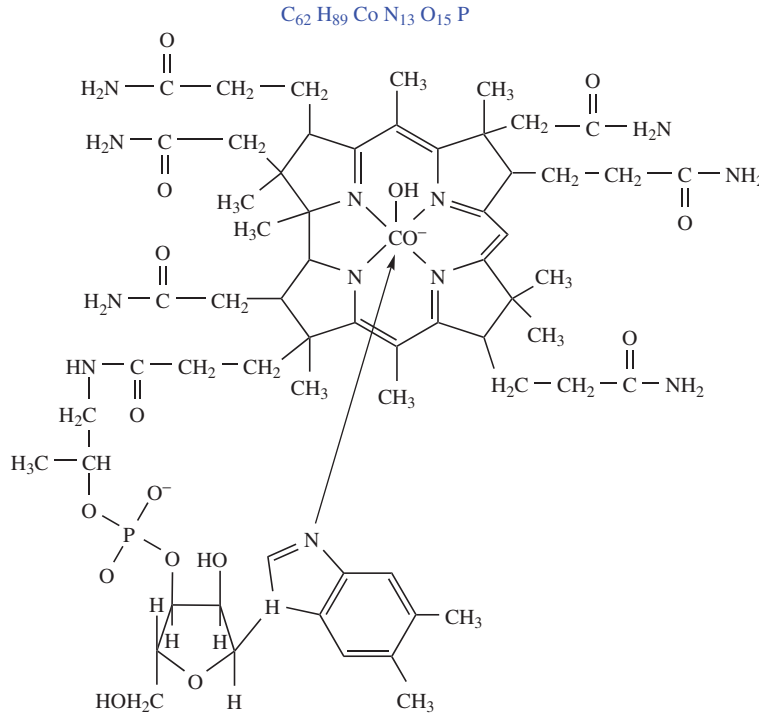

Figure 25.1 Structure of Hydroxocobalamin. Source: National Library of Medicine, Bethesda, MD. ChemID Plus. Hydroxocobalamin [USAN:USP:INN:BAN:JAN]RN: 13422-51-0.

concluded that "These data suggest that hydroxocobalamin offers the potential for improving outcomes for victims of cyanide poisoning" (Dart, 2006).

25.2 Pharmacology

25.2.1 Structure

The chemical structure of hydroxocobalamin is shown in Figure 25.1.

25.2.2 Mechanisms of action

CN^- binding: Hydroxocobalamin, because of its chemical structure, can bind a cyanide ion in 1 : 1 stoichiometry. There was some speculation that there might be a 2 : 1 binding (Hall & Rumack, 1987), but this now does not seem likely.

NO scavenging: The findings of Gerth *et al*. (2006) from a study in rabbits suggest that hydroxocobalamin is a nitric oxide (NO) scavenger (Gerth *et al*., 2006).

25.2.3 Pharmacokinetics

In a small normal volunteer study when sodium thiosulfate was co-administered, hydroxocobalamin pharmacokinetics were determined to be: t ½-alpha, 0.52 hours; t ½-beta, 2.83 hours; Vd-beta, 0.24 l/kg, and peak serum concentration of 753 μg/ml at 0–50 minutes after infusion completion of a 5 gram dose (Forsyth *et al*., 1993).

Houeto *et al*. (1996) and Astier and Baud (1995) found significantly longer elimination half-lives in smoke inhalation victims for both hydroxocobalamin and cyanocobalamin (26.2 ± 2.7 hours and 19.0 ± 5.2 hours, respectively) (Houeto *et al*., 1996; Astier & Baud, 1995). A more recent study in healthy volunteers likewise found longer elimination half-lives for free cobalamins-(III) after administration of hydroxocobalamin, with means ranging from 25.9 ± 2.7 hours to 30.3 ± 2.4 hours after single intravenous doses ranging from 2.5 to 10 grams (Uhl *et al*., 2008).

25.3 Experimental animal studies

Studies of hydroxocobalamin in a number of animal species have shown its efficacy (Bebarta *et al*., 2012; Borron *et al*., 2006; Forsyth *et al*., 1993; Hall & Rumack, 1987).

25.4 Human experience

Smoke inhalation: Please see Chapter 8 on smoke inhalation.

Cyanogenic nitriles: Please see Chapters 10 and 11 on cyanogenic aliphatic nitriles and the special case of acrylonitrile.

25.5 Dosage and route of administration

25.5.1 Adult

Hydroxocobalamin is administered intravenously at a dose of 5 grams over 15–20 minutes (Meridian Medical Technologies, 2010), although in emergency situations it can be given more rapidly. It is supplied as a lypholized preparation and must be reconstituted in a suitable IV fluid such as normal saline or Ringer's lactate. Based on clinical response, second doses of 2.5 grams up to a maximum of 15 grams may be given. Sodium thiosulfate may also be considered, but should not be co-administered in the same IV line unless it has been flushed with a suitable IV fluid. If needed, two separate IV lines should be established. Sodium thiosulfate partially inactivates hydroxocobalamin.

25.5.2 Children

Pediatric doses have not been well-established. In general, a dose of 70 mg/kg is recommended, but this is based on animal studies and extrapolated from adult doses (Geller *et al*., 2006). In a few cases, such a dose has been clinically efficacious and safe (Breton *et al*., 1993).

25.5.3 Elderly

Clinical experience is limited, but a few case reports suggest that the usual adult dose may be efficacious and safe for elderly patients.

25.5.4 Pregnancy

Data are limited to those from a single case report of a smoke inhalation victim (Roderique *et al*., 2012). It is, however, a general principle that treating the mother also treats the fetus.

25.6 Adverse effects

Two studies have been done in normal human volunteers. The first, done in the United States of America, was of a limited number of adult male subjects. Because of an FDA discussion, this study had to involve heavily smoking adults to see if the small amounts of cyanide in the blood of such subjects could be reduced or abolished. Assessing safety issues were also investigated (Forsyth *et al*., 1993). In a few cases, both hydroxocobalamin and sodium thiosulfate were administered, but the protocol was changed because the sodium thiosulfate caused nausea, wretching, vomiting, and localized injection site burning sensations and muscle twitching or cramping (Forsyth *et al*., 1993). A volunteer study of sodium thiosulfate alone reported similar adverse effects (Ivankovich *et al*., 1983).

When hydroxocobalamin was administered alone, it was associated with a transient reddish-brown discoloration of the skin, mucous membranes, and urine (Forsyth *et al*., 1993). Other noted effects were transient and self-limited mean increases of both systolic (13.6%) and diastolic blood pressure (25.9%) with a concomitant 16.3% heart rate decrease. No other clinically significant adverse effects were found (Forsyth *et al*., 1993).

Uhl *et al*. (2006) studied the effects of single intravenous doses of hydroxocobalamin ranging from 2.5 to 10 grams, compared with placebo, in healthy volunteers. Adverse effects were similar to those found by Forsyth *et al*. (1993), including transient reddening of the skin, mucous membranes, urine, and plasma, acneiform rash, and blood pressure elevations peaking at the end of administration and lasting approximately 4 hours. Maximum mean systolic blood pressure changes ranged from 22.6 to 27.0 mmHg, while diastolic blood pressures increased by a mean of 14.3 to 25.4 mmHg. In addition to blood pressure increases, two minor allergic reactions required treatment. Other

minor symptoms (headache, nausea, dysphagia, and others) were reported as well (Uhl *et al.*, 2006)

25.7 Laboratory interferences

Hydroxocobalamin has an intense reddish-brown color and a peak light absorption at 352 and 526 nanometers. It can therefore interfere with certain automated colorimetric analyses for liver function tests, creatinine, and serum iron (Curry *et al.*, 1994). Subsequent studies have identified that at least *in vitro* there are no interferences with standard clinical chemistry tests such as serum calcium, sodium, potassium, chloride, BUN, or GGT (Beckerman *et al.*, 2009). Most standard hematology tests and urinalysis are not clinically significantly affected, although the effects on a PPT and PT (Quick or INR) are unpredictable (Beckerman *et al.*, 2009).

Usual laboratory tests that may have artificially increased results include the following (Beckerman *et al.*, 2009):

- Creatinine
- Bilirubin
- Magnesium
- Triglycerides
- Cholesterol
- Total protein
- Glucose
- Albumin
- Alkaline phosphatase
- Hemoglobin
- MCH
- MCHC
- Basophils

Those that may be artificially decreased are ALT and amylase, while those in which the effects are unpredictable are phosphate, uric acid, AST, CK, CKMB, and LDH (Beckerman *et al.*, 2009). In most cases, these results seem to be within $\pm 10-15\%$ of the actual values and need not prompt further studies, other than repeating the tests once the obvious reddish-brown discoloration of the urine has cleared (usually within 5–7 days).

25.8 Comparison with other antidotes

Hydroxocobalamin does not induce methemoglobinemia as do the nitrite cyanide antidotes and 4-DMAP. Methemoglobin inducers are relatively contraindicated for smoke inhalation victims who quite often have significant carbon monoxide poisoning and may or may not have a significant cyanide poisoning component. Carbon monoxide poisoning decreases the oxygen carrying capacity of the blood to the tissues and also compromises the ability of cells to utilize the oxygen that is delivered. Inducing methemoglobin which also cannot transport oxygen can further decrease tissue oxygen delivery.

25.9 Conclusion

Over many years, experience in various countries has shown that hydroxocobalamin is a safe and efficacious antidote for confirmed or *suspected* cyanide poisoning in either smoke inhalation or other circumstances.

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CHAPTER 26

Cyanide antidotes in development and new methods to monitor cyanide toxicity

Matthew Brenner, Sari Mahon-Brenner, Steven E. Patterson, Gary A. Rockwood and Gerry R. Boss

At a Glance

- Cyanide antidotes in current clinical use in the US (hydroxocobalamin, sodium nitrite/sodium thiosulfate) have limited application in mass casualty situations (large industrial releases, terrorist attacks) because they must be administered IV in large volumes and over a period of minutes.
- In such mass casualty situations, an ideal cyanide antidote should be able to be administered rapidly in small volumes by IM autoinjector, intraosseous injection, or by inhalation.
- Three developmental antidotes that meet the above criteria are: cobinimide (an hydroxocobalamin precursor that can directly bind 2 CN^- moities instead of only 1 for hydroxocobalamin), 3-mercaptopyruvate sulfur transferase (3-MPST), and sulfanegen (a 3-MPST prodrug).
- Cobinimide has been an efficacious cyanide antidote in cultured cells, *Drosophila melanogaster*, mice, and rabbits and is safe at least to 300 mg/kg in the latter 2 species (anticipated human dose is 10–15 mg/kg).
- Sulfanegen has been an efficacious cyanide antidote in a sublethal mouse model and in piglet and rabbit lethal models.
- Cobinimide has also been adapted as a rapid qualitative and quantitative method for assessing cyanide poisoning in the pre-hospital and emergency department settings, based on its tight cyanide binding and resultant marked spectral changes.

26.1 Introduction

Currently approved treatments for cyanide poisoning in the United States are hydroxocobalamin and the combination of sodium nitrite and sodium thiosulfate. All three agents must be given in relatively large volumes by intravenous injection. Even under the best of circumstances, starting an intravenous line takes several minutes, and even more time will likely be required in cyanide-poisoned victims, since they may be hypotensive with collapsed peripheral veins. Compounding this problem is that the drugs must be given slowly over 5–10 minutes. Given that treatment for cyanide exposure must be initiated quickly, the currently available agents do not lend themselves well to treating a large number of cyanide-poisoned people, as could occur in a major industrial accident or a terrorist attack. Thus, a major impetus exists for developing cyanide antidotes that can be administered rapidly and easily, allowing first responders to move quickly from patient to patient. Of possible ways to administer drugs, three

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stand out as potentially useful to treat a large number of cyanide-poisoned people: intramuscular injection via an autoinjector, intraosseous injection, and inhalational delivery. Each has advantages and disadvantages. Intramuscular injection using an autoinjector is quick and can be done through clothing, but, under resting conditions, muscle blood flow is relatively low, and even rapidly absorbed drugs may not reach their peak blood concentrations for 5–10 minutes. Absorption may be further compromised in a hypotensive person, because of peripheral vasoconstriction. Intraosseous injection provides rapid access to the venous circulation and does not require finding a suitable vein. However, it is technically more difficult and requires more time than intramuscular injection via an autoinjector. Inhalational delivery can provide for rapid drug absorption, because of the enormous surface area of the lungs. Moreover, absorption is directly into the pulmonary venous circulation and is not compromised by peripheral vasoconstriction. However, much drug is lost via deposition in the upper airways and current inhalers provide only microgram to low milligram quantities of drug, whereas much larger amounts of an antidote are likely needed to treat cyanide poisoning. Although inhalational delivery requires a patient to be breathing, this is not likely to be a major disadvantage, because realistically it may not be possible to rescue a person who is apneic from cyanide poisoning, particularly in a mass casualty setting. In this chapter, we present data on three drugs in development that are sufficiently potent and soluble to be administered in small volumes. All three have been shown to be effective by intramuscular injection, and preliminary data suggest that all three would be effective by intraosseous injection and inhalational delivery. The three drugs are cobinamide – a cyanide scavenger, dimethyltrisulfide (DMTS) – a sulfur donor and a possible substrate for the two cyanide detoxifying enzymes rhodanese and 3-mercaptopyruvate sulfurtransferase (3-MPST), and sulfanegen, a 3-MPST substrate. In addition, we briefly discuss other cyanide antidotes in development, and present new methods to measure blood cyanide concentrations and real-time methods to monitor cyanide poisoning *in vivo*.

26.2 Cobinamide and sulfanegen

26.2.1 Cobinamide

Cobinamide chemistry

Cobinamide is the penultimate compound in cobalamin (vitamin B_{12}) biosynthesis by micro-organisms. It differs from cobalamin by lacking the dimethylbenzimidazole ribonucleotide group tethered to the corrin ring and coordinated to the lower axial position of the cobalt atom (Figure 26.1). This leads to three major chemical differences between cobinamide and cobalamin. First, both the upper and lower ligand binding sites of cobinamide are free, whereas cobalamin has only an upper ligand binding site. Thus, cobinamide can bind two cyanide ions, whereas cobalamin can bind only

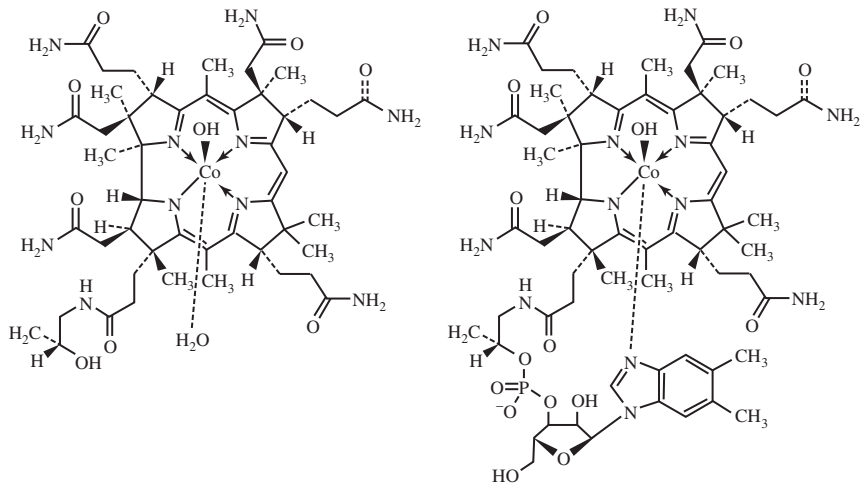

Figure 26.1 Structures of cobinamide (on left) and cobalamin (on right). Cobinamide lacks the dimethylbenzimidazole ribonucleotide group of cobalamin. In aqueous solutions at neutral pH, water and hydroxyl molecules are bound to cobinamide, and a hydroxyl molecule is bound to cobalamin.

one. Second, the dimethylbenzimidazole group has a negative *trans*-effect on cobalamin's upper binding site, pulling the cobalt down out of the plane of the corrin ring, thereby reducing cobalamin's affinity for ligands (Hayward *et al.*, 1965). Thus, cobalamin binds cyanide with an affinity (K_A) of $10^{12}M^{-1}$ (Hayward *et al.*, 1965), which is a comparatively high association constant and the reason cobalamin is an effective cyanide antidote. However, cobinamide binds the first cyanide ion with a K_A of $> 10^{14}\ M^{-1}$ and the second with a K_A of $10^8\ M^{-1}$, leading to a K_A overall of $> 10^{22}\ M^{-2}$ (Hayward *et al.*, 1965). The last value is an enormously high association constant, similar to chelating agents. And third, cobinamide is at least five times more water soluble than cobalamin, allowing for more concentrated solutions of the former compared to the latter.

When dissolved in water, a hydroxyl and a water molecule bind to the cobalt of cobinamide, yielding hydroxoaquocobinamide. Other ligands that bind to cobinamide include nitrite (nitrocobinamide) and sulfite (sulfitocobinamide).

Cobinamide reversal of cyanide toxicity in cultured cells

Mitochondrial cytochrome c oxidase accounts for the majority of cellular respiratory activity and is strongly inhibited by cyanide. In permeabilized Chinese hamster lung fibroblasts, cobinamide restored full respiratory activity to cyanide-treated cells, and was considerably more potent and effective than hydroxocobalamin (Broderick *et al.*, 2006). Cobinamide also completely reversed cyanide-induced inhibition of cell growth (Broderick *et al.*, 2006).

Cobinamide reversal of cyanide toxicity in *Drosophila melanogaster*

D. melanogaster is a recognized model for human disease and is being used increasingly in drug discovery. Because of the flies' high metabolic rate, they are a good model for cyanide poisoning. When administered either orally, by injection, or by inhalation, cobinamide reversed cyanide-induced death in the flies, and, again, was much more potent and effective than hydroxocobalamin (Broderick *et al.*, 2006).

Cobinamide reversal of cyanide toxicity in mice and rabbits

Mice. Mice have been used extensively in the study of cyanide poisoning and development of cyanide antidotes. Hydroxoaquocobinamide is an extremely effective cyanide antidote in mice when given by intravenous or intraperitoneal injection, much more effective than hydroxocobalamin or the combination of sodium thiosulfate and sodium nitrite (Chan *et al.*, 2010). However, it is not well absorbed when given by intramuscular injection. The reason for poor intramuscular absorption seems to be that cobinamide binds to anions like heparan sulfate in the interstitial space, because placing a ligand such as nitrite or sulfite on cobinamide provides for good intramuscular absorption; presumably, the ligand interferes with cobinamide binding to interstitial anions.

Using a custom-made cyanide gas exposure chamber, we have developed a model whereby mice are exposed to cyanide gas, injected with an antidote, and then re-exposed to cyanide gas (Chan *et al.*, 2011). We can make the pre- and post-cyanide exposure periods any length of time, and generally use a 20/20 model: mice are exposed to cyanide gas for 20 minutes, injected intramuscularly with antidote, and then exposed to cyanide gas for another 20 minutes. We chose this model, because we assume about 20 minutes will be required for first responders to reach a disaster scene, and another 20 minutes will be required to evacuate victims; a lethal cyanide gas exposure would most likely occur in an enclosed space. Both nitrocobinamide and sulfitocobinamide administered by intramuscular injection can completely rescue mice from a lethal 20/20 model (Figure 26.2 shows a dose-response curve for sulfitocobinamide).

Rabbits. Similar to the mice, we have found that cobinamide is far superior to hydroxocobalamin as a cyanide antidote in the rabbit cyanide exposure model described later in this chapter (Brenner *et al.*, 2010). Moreover, we have found that an intramuscular injection of nitrocobinamide or sulfitocobinamide rescues rabbits from a lethal infusion of sodium cyanide (Brenner *et al.*, 2009). In this same model, we have shown that cobinamide given by inhalation or intraosseous injection leads to rapid delivery of cobinamide to the systemic circulation, almost as fast as when given by intravenous injection.

Cobinamide safety

From the mouse and rabbits studies, we estimate the cobinamide dose required to rescue a human from a lethal cyanide exposure will be 10–15 mg/kg. We have shown that cobinamide is safe to at least 300 mg/kg in mice and rats. Thus, cobinamide should be safe at the projected human dose.

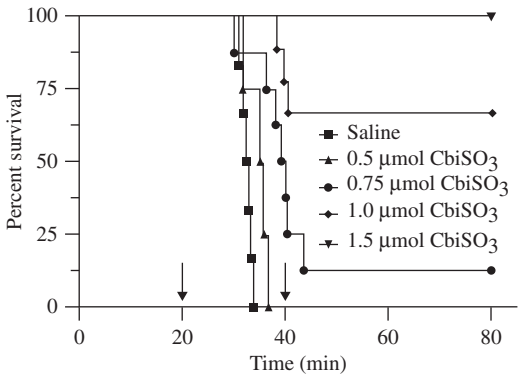

Figure 26.2 Mouse cyanide inhalation model: treatment with sulfitocobinamide post cyanide exposure, with re-exposure to cyanide after treatment. C57BL/6 mice were placed in a sealed chamber with cyanide gas generated at a concentration of 587 ppm. After 20 min of exposure, the mice were removed and injected intramuscularly with saline or the indicated amounts of sulfitocobinamide (noted by arrow at 20 min). The mice were placed back in the chamber and re-exposed to 587 ppm cyanide gas for another 20 min, at which time they were removed from the chamber (noted by arrow at 40 min). They were then observed for another 40 min. Data are shown as Kaplan-Meier survival curves. Mice injected with saline (squares) or 0.5 µmol sulfitocobinamide (triangles) died during the re-exposure period from 30 to 36 min after the initial exposure. One of eight mice injected with 0.75 µmol sulfitocobinamide (circles) and six of nine mice injected with 1.0 µmol sulfitocobinamide (diamonds) survived, while all six mice injected with 1.5 µmol sulfitocobinamide (inverted triangles) survived. Animals that lived for 80 min recovered fully without evidence of abnormalities.

26.2.2 Sulfanegen

Sulfanegens are mercaptopyruvate prodrug derivatives based on a 1,4-dithiane (compound 1 in Figure 26.3) that spontaneously dissociates to the cysteine catabolite, 3-mercaptopyruvate (compound 2 in Figure 26.3). The latter is a substrate of 3-MPST, which catalyzes the release of sulfane (singlet sulfur) from 3-mercaptopyruvate. Nucleophilic attack of cyanide on sulfane results in conversion of cyanide to the less toxic metabolite thiocyanate. Thus, 3-MPST is similar to rhodanese, but has advantages over rhodanese as a drug target: rhodanese is concentrated in the kidneys and liver, chiefly in the mitochondrial matrix, a site poorly accessible to its substrate thiosulfate, while 3-MPST is more widely distributed in tissues, and is found in the cytosol as well as the mitochondria. Targeting 3-MPST may therefore be more effective than rhodanese for cyanide antagonism (Nagahara *et al.*, 1998).

However, 3-mercaptopyruvate is chemically unstable and an attempt to use it as a cyanide antagonist failed (Westley, 1981). Thus, 3-mercaptopyruvate analogs that deliver 3-mercaptopyruvate either by spontaneous dissociation or enzymatic release would be desirable, and sulfanegen sodium (compound 1, sodium salt) was prepared as one of these prototypes (Nagasawa *et al.*, 2007).

Sulfanegen was initially studied using a sublethal murine model: mice receive a toxic but sublethal dose of sodium cyanide (intraperitoneal, 4.8 mg/kg) that causes loss of neuromuscular coordination for approximately 68 minutes in placebo-treated mice. The mice are then inverted on a wire mesh and upon recovery the mice crawl to an upright position on the mesh, that is, "right themselves" (the righting reflex). Antidotal efficacy is evaluated by reduction in time required for the mice to right themselves (Crankshaw *et al.*, 2007). Comparison of sulfanegen sodium with existing antidotes hydroxocobalamin, and the combination of sodium nitrite with sodium thiosulfate demonstrated that sulfanegen sodium is superior (Figure 26.4).

Sulfanegen sodium was then tested in a lethal piglet model that closely follows the Borron beagle dog model (Borron *et al.*, 2006). While sulfanegen sodium was effective when administered intravenously in this model (Belani *et al.*, 2012), it was ineffective when injected intramuscularly due to insufficient aqueous solubility. Therefore, a series of sulfanegen salts were prepared by exchange of sodium for a biocompatible ammonium cation. These salts are equipotent to sulfanegen sodium in the above sublethal murine model, and some, e.g. triethanolammonium, diethanolammonium, glucosammonium, N,N-dimethylethanolammonium (deanol), N-methylethanolammonium, and choline salts are more soluble than sulfanegen sodium.

The triethanolamine and glucosamine salts are effective when administered by intramuscular injection in a lethal rabbit model of cyanide toxicity that closely follows the Borron model. The glucosamine salt successfully rescued 9/10 rabbits, whereas all rabbits that received placebo died. The treatment groups showed a rapid return of lactate and cyanide concentrations to baseline compared to placebo, and an increase in urinary thiocyanate. Comparative studies, including toxicity, solubility/dissolution, and efficacy are being performed to select the optimal candidate for clinical development.

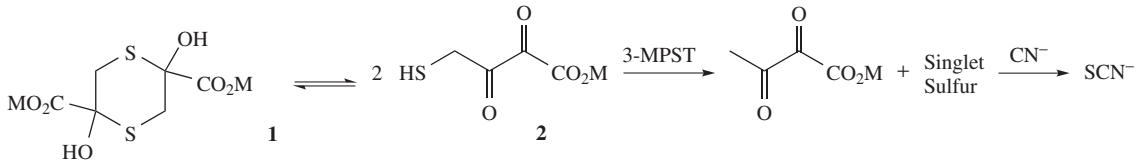

Figure 26.3 Cyanide antagonism by sulfanegen. Sulfangen (compound 1) conversion to two 3-mercaptopyruvate molecules (compound 2) is shown, followed by a 3-MPST-catalyzed reaction with cyanide yielding thiocyanate.

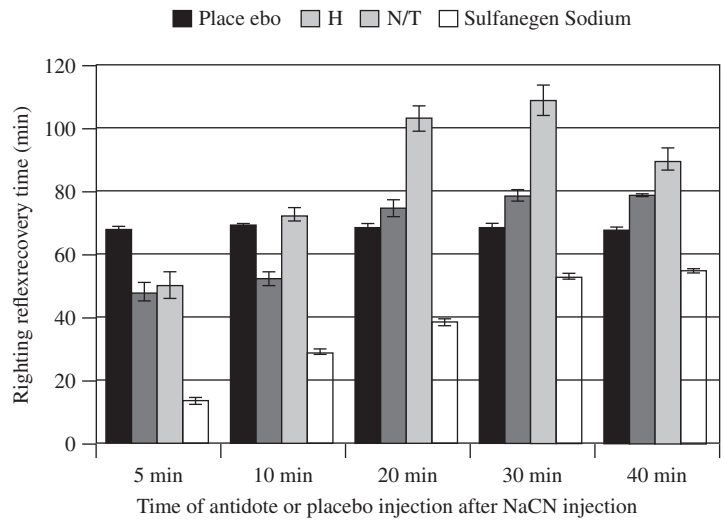

Figure 26.4 Mouse non-lethal righting reflex model of cyanide poisoning. Swiss-Webster ND-4 mice received an intraperitoneal injection of 4.8 mg/kg sodium cyanide at time zero. They then received an intraperitoneal injection of sulfanegen sodium (206 mg/kg), hydroxocobalamin (H, 226 mg/kg), or the combination of sodium nitrite/sodium thiosulfate (N/T, 0.10 and 1.0 g/kg, respectively) at various times after the cyanide injection as indicated on the abscisa. Recovery time was measured as the time required for the mice to right themselves (righting reflex recovery). Controls (CN) performed at each time point received phosphate-buffered saline.

26.3 Other cyanide antidotes in development

Isolated reports of various compounds as potential cyanide antidotes have appeared in the biomedical literature, but those that stand out are aldehydes and ketones, which react with cyanide ion to form the corresponding cyanohydrin. Of the compounds tested in animal models of cyanide poisoning, the best candidates are alpha-ketoglutarate, dihydroxyacetone, oxaloacetic acid, and pyruvate, but even alpha-ketoglutarate, the most potent, must be used at extremely high doses (1–2 grams per kilogram body weight) (Niknahad & O'Brien, 1996; Bhattacharya & Tulsawani, 2008). It seems likely these molecules are metabolized rapidly, and whether any of them will be clinically useful remains to be seen.

26.4 New research methods to diagnose and monitor cyanide poisoning and therapy

26.4.1 Laboratory diagnosis of cyanide poisoning

Laboratory methods to detect cyanide poisoning in acute clinical care settings have been limited by the need for complex and time consuming assays. Results are generally not obtained rapidly enough to assist with immediate therapeutic decisions regarding initiation of antidote therapy (Baskin *et al.*, 2008). Treatment must currently be based on clinical suspicion, and the ability to accurately titrate antidote dose is not possible at this time. However, development of relatively safe antidotes, including hydroxocobalamin, have enabled

greater leeway in decision-making and dosing for suspected exposures.

When assessing for cyanide exposure, most investigators measure the concentration of cyanide in red blood cells (where the majority of it exists), and the concentration of thiocyanate – the major cyanide metabolite – in plasma (Baskin *et al.*, 2008). A variety of methods have been used, including spectroscopic absorption, electrochemical analyses, or gas or liquid chromatography. Measurement of cyanide in red blood cell methods generally involves acidification of the cells to release hydrogen cyanide gas, with subsequent analysis of gas phase or liquid phase collections. Stability, storage, evaporation losses, and artifactual cyanide formation can lead to inaccuracies and variability in red blood cell cyanide measurements (Baskin *et al.*, 2008; Logue *et al.*, 2005). In addition, the methods require some time, and thus there is a recognized need for rapid, field-based assessment of cyanide poisoning.

Two recent approaches to assess cyanide poisoning in the field are under development. The first method exploits the extremely high binding affinity of cobinamide for cyanide and marked spectral changes that occur on cyanide binding to cobinamide; both qualitative and quantitative methods for measuring red blood cell cyanide concentrations have been reported (Ma *et al.*, 2010, 2011). The method requires acidifying samples to release hydrogen cyanide gas, but work is underway to measure cyanide in whole blood without acidification and gas phase steps. The second approach is to measure exhaled breath cyanide concentrations using infrared cavity ring-down spectroscopy with low detection limits and high sensitivity (Stamyr *et al.*, 2009). The potential clinical role for measuring exhaled breath hydrogen cyanide remains to be determined, and further investigations are ongoing regarding simplified detection methods.

26.4.2 *In vivo* methods to assess cyanide poisoning

Even at clinically toxic concentrations, cyanide is present in such low quantities that it cannot be detected directly *in vivo* by current noninvasive monitoring techniques. However, the pathophysiologic effects of cyanide poisoning, including alterations in oxy- and deoxy-hemoglobin concentrations, the cytochrome c oxidase redox state, and gas exchange parameters of anaerobic metabolism can be measured using noninvasive real-time methods (Lee *et al.*, 2007).

One of cyanide's major effects is binding to cytochrome c oxidase, which renders tissues unable to extract oxygen from the blood with a resultant increase in oxy- and decrease in deoxy-hemoglobin concentrations. This effect is evident primarily in capillaries, venules, and veins, and can be measured optically using near-infrared absorption spectroscopy techniques, particularly diffuse optical spectroscopy (DOS). In addition, with progressive cyanide poisoning, cellular anaerobic metabolism results in increased carbon dioxide elimination in exhaled breath and reduced oxygen uptake. These can be measured simultaneously with optical hemoglobin and cytochrome oxygenation state variables to assess the degree of cyanide poisoning. As cyanide poisoning reverses, tissues can again extract oxygen from the blood, with reversal of the above-described changes (Figure 26.5).

The principles underlying DOS are the ability to simultaneously and accurately measure both optical scattering and absorption across the near-infrared spectrum. Once scattering is measured, absorption spectra of tissues can be obtained. This enables accurate, quantitative determination of absolute concentrations of multiple individual constituents comprising the absorption spectra of tissues (Lee *et al.*, 2007). Cytochrome c oxidase, the terminal enzyme in aerobic respiration, consumes > 95% of cellular oxygen and is essential for efficient cellular generation of ATP. Four redox active metal centers are present in cytochrome c oxidase: two hemes (Cyt a and Cyt a3), and two coppers (CuA and CuB). As these four metal centers undergo redox state changes, they change absorption spectra during cyanide poisoning. Cyanide binds to Cyt a3, and prevents oxygen reduction by electrons leaving CuA and Cyt a (Beinert *et al.*, 1980). As a result, both of these near-infrared optically predominant metal centers (which account for > 85% of the optical absorption signals of cytochrome c oxidase) become reduced (Piantadosi *et al.*, 1983), and one can follow them quantitatively and non-invasively using DOS. Furthermore, inhibition of cytochrome c oxidase and oxidative cellular metabolism leads to anaerobic metabolism. This results in increased carbon dioxide production and reduced oxygen utilization. These changes can be detected by examination of exhaled carbon dioxide and oxygen uptake with a concomitant increase in respiratory exchange ratio (ratio of carbon dioxide elimination over oxygen uptake).

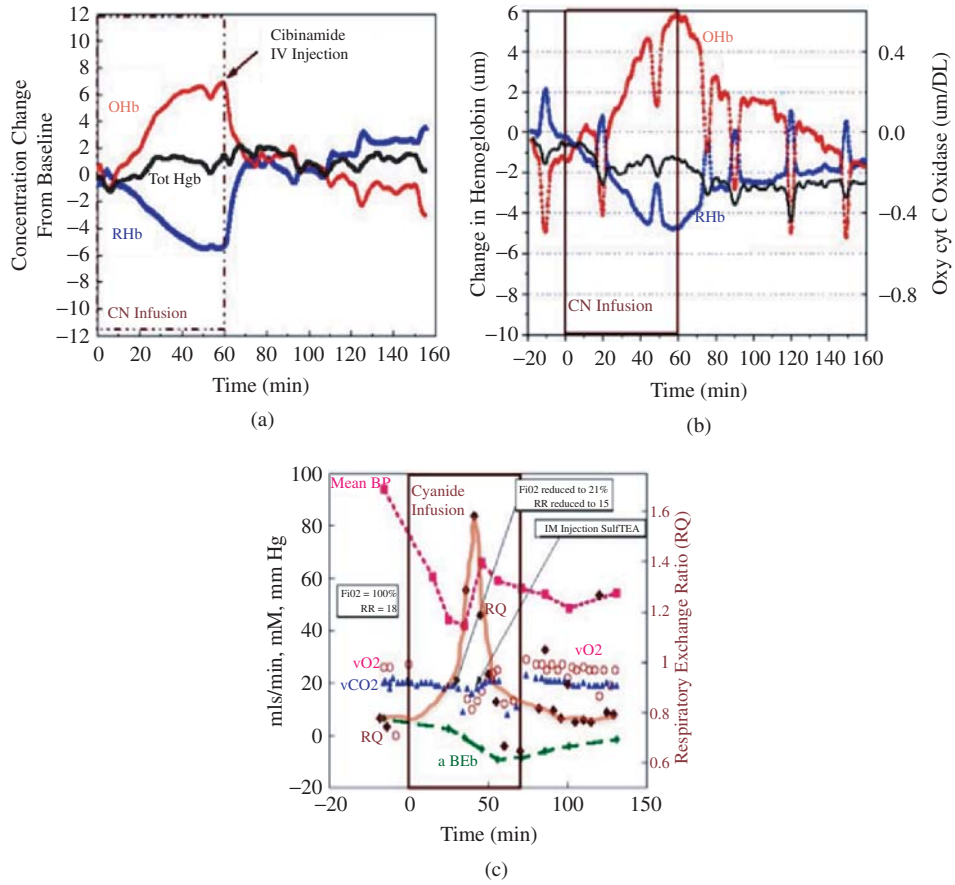

Figure 26.5 Intravenous rabbit model of cyanide poisoning: assessment of tissue hemoglobin saturation, cytochrome c oxidase redox state, and response to oxygen supplementation. Panel A: Oxy- and deoxy-hemoglobin concentrations and effects of cobinamide. Red curve shows that oxy-hemoglobin (OHb) concentrations rise during cyanide infusion from 0 to 60 min as cyanide poisoning progresses. At 60 min, the animal received an intravenous (IV) injection of cobinamide, and tissue oxy-hemoglobin concentrations returned rapidly toward the baseline level. The converse is seen in tissue deoxy-hemoglobin (RHb) concentrations, where deoxygenated hemoglobin decreases during cyanide poisoning due to inability of tissues to extract oxygen from circulating blood. After intravenous cobinamide injection, the oxy- and deoxy-hemoglobin concentrations rapidly return to normal. Panel B: Oxy- and deoxy-hemoglobin concentrations, and change in cytochrome c oxidase redox state. As in Panel A, tissue oxyhemoglobin concentration rises during cyanide infusion, but falls during room air challenges when inhaled oxygen is decreased from 100% to 21%. Ventilated animals are equilibrated on 100% oxygen at baseline. They are then placed for 5 min on room air while ventilation continues. Continuous optical monitoring reveals decreases in tissue oxyhemoglobin concentrations during these challenges, since less oxygen is delivered. The animals then receive a cyanide infusion while on 100% oxygen. As animals become progressively more poisoned with cyanide, a decrease in the cytochrome c oxidase redox state occurs (brown line; the opposite direction of changes in the oxy-hemoglobin signal). In contrast, when oxygen supplementation is briefly dropped to 21% (spikes in the curves), the oxy-hemoglobin concentration decreases, and the cytochrome c oxidase becomes progressively reduced (same direction). These data demonstrate uncoupling of the hemoglobin oxygen signal from the cytochrome c oxidase redox state. Panel C: Gas exchange measures. Standard physiological measurements in a lethal cyanide rabbit model treated with sulfanegen as inspired oxygen (FiO2) was decreased from 1.0 (100% oxygen) to room air (FiO2 .21, 21%) at 35 min. Mean blood pressure falls during cyanide poisoning and reverses quickly with intramuscular injection of sulfanegen-triethanolamine (Sulf-TEA). Oxygen uptake falls during cyanide poisoning, but rises after sulfanegen-triethanolamine. Carbon dioxide elimination remains relatively constant due to increased anaerobic carbon dioxide production countered by decreased carbon dioxide delivery. This results in a rise in the respiratory exchange ratio, RQ, (brown diamonds in figure) during cyanide poisoning. With reversal of cyanide poisoning, the increase in oxygen uptake is greater than the change in carbon dioxide elimination, resulting in return to baseline values.

Using these techniques, noninvasive quantitative assessment of cyanide poisoning in animal models has been shown, as has antidote reversal using approved (nitrites and hydroxocobalamin), and investigational agents (cobinamide, DMTS, and sulfanegen). Their potential clinical role in field-based and hospital-based diagnostics and monitoring remain to be determined.

26.5 Conclusions

Work is under way in several laboratories to develop new cyanide antidotes that could be administered quickly and easily in the field, for example, by intramuscular injection using an autoinjector or by inhalation. For the latter mode to be practical, new devices capable of administering several hundred milligrams of drug will need to be developed. But it is heartening that new agents are on the horizon that will allow for greater flexibility in treating cyanide-poisoned people, and that new monitoring techniques are being developed.

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CHAPTER 27

Recent perspectives on alpha-ketoglutarate

A potential cyanide antidote

R. Bhattacharya

At a Glance

- Alpha-Ketoglutarate (A-KG) has shown exemplary protection against experimental cyanide poisoning *in vitro* and *in vivo*.
- Cyanide is a highly reactive nucleophile that interacts with A-KG to form a cyanohydrin derivative.
- In experimental animals, administration of A-KG provided a 5-fold protection against cyanide and its combination with sodium nitrite and sodium thiosulfate increased this protection to 15- and 19-fold, respectively.
- A-KG is efficacious against experimental cyanide poisoning when it is administered by the oral route. It has been proposed as a prophylactic cyanide antidote as pre-exposure treatment for first responders to fires and unintentional industrial releases.
- Safety and pharmacokinetic studies have been performed in normal human volunteers with doses of 150 mg/kg body weight in divided doses accompanied by adequate hydration.
- A-KG has not been studied for the treatment of human cyanide poisoning to date.

27.1 Introduction

Cyanide is one of the oldest lethal poisons known to mankind but its toxic characteristics still continue to petrify the public at large (Musshoff *et al*., 2002). Cyanide is a strong and fast-acting poison abundantly present in the environment. It is widely used in many industrial processes and its global consumption approximates to 1.5 million tonnes annually (Cummings, 2004). Mass casualty cyanide poisoning can occur in several scenarios such as fire, industrial accident, or terrorist attack (Lavon & Bentur, 2010). Hydrogen cyanide (HCN) and carbon monoxide (CO) are well-known toxic components of fire smoke, which together have been responsible for several lethalities (Ferrari *et al*., 2001; Baud, 2007). Lethal cyanide poisoning is a well-recognized occupational hazard, particularly in the electroplating and metallic luster industries (Seidl *et al*., 2003). Cyanide accounts for numerous suicidal, homicidal, and accidental deaths (Musshoff *et al*., 2002; Labat *et al*., 2004; Hung *et al*., 2009; Garlich *et al*., 2012). Cyanide is naturally found in many plants (cassava) and fruit stones (apricot, peaches, etc.) in the form of cyanogenic glycosides, or in the form of HCN in vehicle exhausts, tobacco smoke or in gas generated upon combustion of nitrogen-containing wool, silk, nylon, synthetic rubber, polyurethanes, and asphalt (Barillo *et al*., 1994; True & Dreisbach, 2002). Iatrogenic release of cyanide from certain drugs like amygdalin, succinonitrile, and sodium nitroprusside has been responsible for several human poisoning cases (Vesey & Cole, 1985; Hall *et al*., 1986; Bromley *et al*., 2005). Use of cyanide as chemical warfare agent has been passably documented elsewhere (Brennan *et al*., 1999; Baskin & Rockwood, 2002). Further, possible use of cyanides and cyanogenic compounds during terrorist actions cannot be ignored (Rotenberg, 2003). U.S. governmental agencies and the

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Department of Homeland Security, consider cyanide to be among the most likely agents of chemical terrorism (Khan *et al*., 2000; Eckstein, 2008). Because of such unscrupulous use of cyanide, it continues to attract the attention of both defense and civil authorities.

There are several antidotes available for the management of cyanide poisoning, which include methemoglobin formers like amyl nitrite, sodium nitrite (SN), and 4-dimethylaminophenol (4-DMAP); sulfur containing compounds like sodium thiosulfate (STS); and cobalt compounds like dicobalt edetate and hydroxocobalamin, but there is no unanimity of opinion on which is the most effective treatment (Van Heijst *et al*., 1987; Van Heijst & Meredith, 1990). In the recent past, sufficient data have been generated on the efficacy of a keto acid, namely oxoglutarate or alpha-ketoglutarate (A-KG), as an effective antidote for cyanide poisoning (Bhattacharya, 2004). This chapter deals with some recent perspectives on A-KG as a potential antidote for cyanide, including its protective efficacy, pharmacokinetics, stability, and toxicity.

27.2 Cyanide toxicity and its treatment

Cyanide exerts its toxicity by inhibiting the iron containing terminal respiratory chain enzyme, cytochrome oxidase a_3 (CCO), within the mitochondria. This leads to diminished oxygen uptake and ATP generation culminating in cellular hypoxia. Consequently, cellular metabolism shifts from aerobic to anaerobic metabolism concurrent with accumulation of lactic acid (Hung *et al*., 2009). Cyanide toxicity may not be attributed solely to a single biochemical lesion but a complex phenomenon. There are several other crucial molecular mechanisms underlying cyanide toxicity, which have been reviewed elsewhere (Borowitz *et al*., 1992; Baskin *et al*., 2004, 2008; Bhattacharya & Flora, 2009). Pathologically, no particular lesions can delineate cyanide toxicity but lesions in the central nervous system (CNS), that is, necrosis in the white matter has been reported after chronic cyanide poisoning (Kamalu, 1995; Banea *et al*., 1997).

The onset of cyanide toxicity is very fast and the prognosis of the victim entails institution of immediate and aggressive specific treatment. Cyanide has several antidotes, with differing mechanisms of action and diverse toxicological, clinical, and risk-benefit profiles (Mégarbane *et al*., 2003; Gracia & Shepherd, 2004; Hall *et al*., 2009). Usually, the specific therapy for cyanide poisoning includes methemoglobin-inducers like amyl nitrite, SN, and 4-DMAP; sulfur donor like STS; and complexing agents like hydroxocobalamin and dicobalt edetate (Mégarbane *et al*., 2003). Therapeutic management of cyanide poisoning has largely relied on inhalation of amyl nitrite as a first aid measure, followed by intravenous administration of SN and STS together with intensive supportive therapy, preferably in a medical facility (Lavon & Bentur, 2010). However, there is lack of international harmony on the efficacy and safety of existing cyanide antidotes (Cummings, 2004; Baud, 2007; Eckstein, 2008). This led to some serious research efforts to develop newer and more effective mechanistic based antidotes (Marrs, 1988; Isom & Borowitz, 1995; Cummings, 2004; Bhattacharya & Flora, 2009). Among several compounds tested, A-KG was one molecule which exhibited exemplary protection against experimental cyanide poisoning in vivo and in vitro (Hume *et al*., 1995; Bhattacharya, 2004).

27.3 A-KG as a cyanide antidote

Cyanide is a highly reactive nucleophile that interacts with various carbonyl moieties like ketones and aldehydes to produce cyanohydrin derivatives. Many carbonyl compounds and their metabolites or nutrients, such as sodium pyruvate, A-KG, dihydroxyacetone, and so on, exhibited significant protection against experimental cyanide intoxication (Schwartz *et al*., 1979; Delhumeau *et al*., 1994; Niknahad *et al*., 1994; Bhattacharya, 2000). Out of these molecules, A-KG was one which alone or with STS was proposed as the most promising treatment for cyanide poisoning, more because these two substances were considered nontoxic for human use (Moore *et al*., 1986; Borowitz *et al*., 1992, 2001). The structure-activity relationship of A-KG with cyanide was demonstrated about twenty-five years back (Norris & Hume, 1986). Subsequently, a mechanism of cyanide antagonism by A-KG was elucidated *in vitro* and *in vivo*, which was ascribed to A-KG binding with cyanide (a highly reactive nucleophile) to form a cyanohydrin complex (Norris *et al*., 1990). The mechanism is illustrated in Figure 27.1.

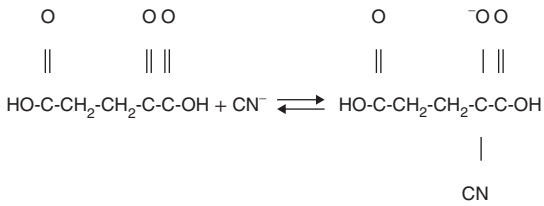

Figure 27.1 A-KG binding with cyanide to form a cyanohydrin complex.

Additionally, pre-treatment of A-KG was shown to prevent the cyanide-induced inhibition of brain CCO activity. 3-Mercaptopyruvate sulfurtransferase (MST) facilitates the enzymatic detoxification of cyanide in vivo, and three alpha-keto acids (alpha-ketobutyrate, A-KG, and pyruvate) were shown to inhibit MST in a concentration-dependent manner. This observation further supported the hypothesis that the mechanism of cyanide detoxification by A-KG was purely mediated through non-enzymatic binding of cyanide and not by stimulation of enzymes involved in transsulfuration of cyanide to thiocyanate (Porter & Baskin, 1996). First detailed experimental evidence on antidotal potency of A-KG against cyanide was provided by Moore *et al*. in 1986. Pre-treatment of A-KG alone afforded five-fold protection against cyanide, which was equivalent to that conferred by SN and STS together. Adjunction of SN and STS further increased the LD_{50} of cyanide by 15-fold and 19-fold, respectively. The study revealed that antidotal potency of A-KG and STS together was better than the conventional treatment of SN and STS. This study prompted others to test the efficacy of A-KG as a novel antidote against cyanide intoxication in dogs (Dalvi *et al*., 1990). Intravenous administration of A-KG was found to resolve the toxicity of cyanide given orally, and was more effective than SN and STS therapy. Further, pre-treatment of A-KG and STS was found to be equally effective against cyanide administered intraperitoneally or HCN given through the inhalation route in mice (Hume *et al*., 1995). During the initial protection studies, both A-KG and cyanide were administered intraperitoneally in mice (Moore *et al*., 1986). Although both the agents were given antipodal, *in situ* binding of both could not be ruled out. This issue was resolved in a separate study, where cyanide given intraperitoneally, subcutaneously, and through the inhalation route was challenged by A-KG given intraperitoneally (Bhattacharya & Vijayaraghavan, 1991). For the first time it was shown that A-KG given parenterally could also mitigate the toxicity of inhaled HCN. The study revealed that A-KG unequivocally protected against cyanide intoxication, regardless of the route of cyanide exposure. In a separate study, various known and potential cyanide antagonists and a glutathione (GSH) depletor were evaluated against acute cyanide poisoning in mice, and A-KG was found to be equipotent to STS (Hatch *et al*., 1990). Further, distribution of cyanide to the brain stem and heart was shown to be significantly reduced by pre-treatment of A-KG, which was also found to be more effective than the therapeutic doses of cobalt edetate and sodium pyruvate (Hume *et al*., 1996). Others also confirmed that A-KG was better than cobalt edetate (Kravzov *et al*., 1994). Other studies revealed that cyanide-induced hyperammonemia, elevated brain neutral and aromatic amino acid levels, convulsions, associated encephalopathy, and loss of consciousness in mice were significantly attenuated by A-KG, more significantly in combination with STS (Yamamoto, 1989, 1990). However, STS alone did not prevent the convulsions, which were accompanied by depleted brain γ-aminobutyric acid (GABA) levels. Since, A-KG is known to play a crucial role in the synthesis of inhibitory neurotransmitter, GABA, its neurochemical implications in cyanide detoxification cannot be overlooked (Yamamoto, 1990; Cynober, 1999). Also, cyanide-induced significant increase in calcium levels in mice brain crude mitochondrial fractions was completely abolished by combination of A-KG and STS (Yamamoto, 1990). In an elaborate study, cytoprotective effects of various glycolytic substrates and keto acid metabolites including A-KG were evaluated against cyanide-induced cytotoxicity in isolated rat hepatocytes (Niknahad *et al*., 1994). Cyanide was found to inhibit CCO *in vitro*, accompanied by impaired cellular respiration and severe ATP depletion. High oxygen concentration together with pyruvate or A-KG effectively prevented the CCO inhibition (Delhumeau *et al*., 1994). The results suggested that oxygen displaced cyanide from the enzyme and the poison was then trapped by the keto acids to form the respective cyanohydrins. The antidotal effectiveness of A-KG could be attributed to its ability to bind cyanide nucleophiles in the vascular system, thus decreasing cyanide distribution to the vital tissues. Such decreased distribution would result in a diminution of consequential histotoxic hypoxia caused during cyanide intoxication.

Cyanide is primarily a neurotoxin but its toxicity in hepatic and renal tissues have also been evidenced (Okolie & Osagie, 1999). Primary culture of rat hepatocytes was employed to elucidate the cytoprotective effects of A-KG and its interaction with cyanide at different molar concentrations. The hepatocytes were found to be relatively resistant to cyanide and the cytotoxicity was independent of oxidative stress and DNA damage (Bhattacharya *et al*., 2012). In a separate study, oxidative stress-mediated cytotoxicity of cyanide was assessed using renal cell line. The cell death was preceded by severe oxidative damage accompanied by oligonucleosomal DNA fragmentation, nuclear fragmentation, together with elevated caspase-3 activity. All these cellular aberrations were prevented by A-KG and *N*-acetyl cysteine (NAC) (Hariharakrishnan, Satpute, *et al*., 2009). Earlier, isolated rat thymocytes were used to delineate cytotoxicity of cyanide and its pharmacological interventions by various molecules (Bhattacharya & Lakshmana Rao, 1997). Further study revealed that pre-treatment and simultaneous treatment of A-KG prevented cytotoxicity of cyanide in thymocytes but failed to attenuate the mitochondrial dysfunction, DNA damage, and depletion of intracellular GSH (Bhattacharya *et al*., 2002). The concentration of cyanide that caused DNA damage was much less as compared to that required to produce cytotoxicity. It is possible that traces of cyanide that could not be sequestered by A-KG was sufficient to cause DNA damage but not cytotoxicity (Bhattacharya & Lakshmana Rao, 1997). In a separate study, protective efficacy of several carbonyl compounds and their metabolites or nutrients like A-KG, citric acid, succinic acid, maleic acid, malic acid, fumaric and oxaloacetic acid, glucose, sucrose, fructose, mannitol, sorbitol, dihydroxyacetone, and glyoxal was evaluated against cytotoxicity of cyanide in thymocytes, and pre-treatment of A-KG was found to afford maximum cytoprotection (Bhattacharya & Tulsawani, 2008). Rat pheochromocytoma (PC12) cells were earlier utilized as a neurotoxicological screen for characterizing anticyanide compound and deciphering the molecular mechanism underlying cyanide toxicity (Borowitz *et al*., 1993; Mills *et al*., 1996). However, most of the *in vitro* protection studies with A-KG were limited to non-neuronal cell types only (Niknahad *et al*., 1994; Bhattacharya & Tulsawani, 2008). The PC12 cells biochemically and phenotypically resemble sympathetic neurons (Isom & Borowitz, 1993). This prompted us to study the effect of cyanide on energy metabolism and other cascading events which could mediate cell death in PC12 cells, and their response to combined treatment of A-KG and NAC. Cyanide-induced cytotoxicity was accompanied by dissipation of mitochondrial membrane potential, decreased cellular ATP content, elevated levels of intracellular calcium ions ([Ca2+]i) and lactic acid, together with generation of peroxides, and all these events favorably responded to treatment with A-KG and NAC (Satpute *et al*., 2008). Subsequent studies indicated that cyanide caused severe oxidative stress in PC12 cells, which was followed by elevated caspase-3 activity and induction of apoptotic type of cell death. Treatment of A-KG and NAC was found to abrogate the cellular insult (Satpute *et al*., 2010). It is well known that in addition to removal of free cyanide, A-KG possesses antioxidant property as well (Andrae *et al*., 1985). On the other hand, NAC is a cysteine donor and a powerful synthetic antioxidant known to increase GSH biosynthesis and reduce lipid peroxidation (Henderson & Hayes, 1994; Sastre *et al*., 1994). Unlike GSH, NAC can easily cross the cell membrane and metabolize to L-cysteine and GSH (Moldeus, 1994). In addition to modulating cellular GSH levels, NAC spares −SH group to directly interact with cyanide (Ballantyne, 1987). This possibly explains the additional protective efficacy of A-KG and NAC together, which was also evidenced earlier during cyanide intoxication in mice (Dulaney *et al*., 1991).

By now sufficient information was available on the efficacy of A-KG against cyanide *in vivo* and *in vitro*. It was also shown that entry of A-KG into fibroblasts occurred through an unmediated diffusion process, and into the liver and kidney through Na^+-dependent transport (Meier *et al*., 1990; Aussel *et al*., 1996). What remained to be observed was the effect of dose, time, and route of A-KG administration against cyanide intoxication. Protection studies were conducted after challenging different doses of cyanide (oral, intraperitoneal, subcutaneous, or intravenous) with pre-treatments of various doses of A-KG (oral, intraperitoneal, or intravenous). Cyanide antagonism by A-KG was best exhibited (7.6-fold) when both A-KG (2.0 g/kg; −10 min) and cyanide were given orally (Tulsawani *et al*., 2007). This implies that cyanide toxicity could be effectively challenged by A-KG given by oral route as well.

27.4 The need for an oral antidote

Mass casualty cyanide poisoning could occur during chemical war, terrorist attack, industrial disasters, or fire accidents. In such cases, the simplicity of administration of amyl nitrite enables first responders to give it by inhalation to patients at the site itself (Marrs, 1988). However, the role of amyl nitrite in the management of pre-hospital mass casualty cyanide poisoning remains ambiguous (Lavon & Bentur, 2010). Further, all other antidotes have to be administered intravenously, which is discouraged during mass casualty management. To address these limitations, novel orally effective cyanide antidotes were suggested as pre-treatment or first aid for mass casualty set-up (Nagasawa *et al*., 2007; Szilasi *et al*., 2011). Similarly, oral prophylaxis by aminophenones was another alternative (Marrs *et al*., 1991). In the U.K., "solutions A and B" (ferrous sulfate dissolved in aqueous citric acid, and aqueous sodium carbonate) are recommended as oral treatment to reduce the absorption of swallowed cyanide (Nicholson *et al*., 1994). The majority of cyanide exposures occur through inhalation or skin contact but intoxication through oral route cannot be overlooked in view of the recent U.S. intelligence report (Fromson, 2012). Oral treatment of A-KG for cyanide poisoning was recognized for some time (Dulaney *et al*., 1991). However, its utility was explicitly substantiated only in the recent past (Bhattacharya, 2004).

27.5 A-KG as an oral antidote

Exogenously administered A-KG is known to be well absorbed in the intestine (Cynober, 1995). However, the point of dispute was that how A-KG given orally would dissipate so fast to challenge cyanide entering through other routes. This fear was dispelled in a study where protective efficacy of A-KG by different routes was shown against cyanide given by various routes (Tulsawani *et al*., 2007). Now, our interest was to narrow down to efficacy of A-KG given orally against cyanide poisoning through the same route. When cyanides are given orally, the gastric acid environment favors formation of the unionized form of HCN, and facilitates its absorption while it diminishes with more alkaline stomach (Ballantyne, 1987). Therefore, alkalinity of A-KG (disodium salt) given orally was anticipated to minimize the luminal absorption of cyanide administered by the same route. Hereafter, most of our studies were focused on neutralizing cyanide by oral route.

27.5.1 Protection Studies

Studies conducted in mice showed that pre-treatment of A-KG (0.125–2.0 g/kg) exhibited a dose and time-dependent efficacy against cyanide. A 10-minute pre-treatment of A-KG increased the LD_{50} of cyanide by 7.6-fold, which further increased to 25.6-fold by addition of SN and STS. Most notable observation was that a 60-minute pre-treatment of A-KG also afforded reasonable protection (Bhattacharya *et al*., 2002). This observation was contradictory to previous reports where 10- to 15-minute prophylaxis of A-KG was only considered for cyanide detoxification. This could be attributed to different routes of A-KG and cyanide administration (Moore *et al*., 1986; Dulaney *et al*., 1991). In a separate study conducted in rats, almost identical protection was observed with A-KG (Bhattacharya & Vijayaraghavan, 2002). A 10-minute pre-treatment of A-KG increased the LD_{50} of cyanide by 7-fold, which further increased to 28-fold by conjunction of SN and STS. Also, cyanide-induced CCO inhibition was significantly prevented by pre-treatment or simultaneous treatment of A-KG and STS, which also minimized animal mortality by 50% if given therapeutically. During these studies, one interesting phenomenon observed was that the unprotected animals receiving very high doses of cyanide would usually die within a few minutes while those protected with A-KG alone or with STS would either survive or perish overnight after protracted struggle. Now, it was of interest to see if the animals receiving such high doses of cyanide could be saved by repeated administration of A-KG. This study revealed that repeated treatments of A-KG in the presence or absence of STS could confer >35-fold protection against cyanide, if given over a period of 4 hours (Bhattacharya & Tulsawani, 2009). This implies that repeated administration of A-KG could challenge super toxic doses of cyanide. In critical cases of cyanide poisoning, repeated infusion of the antidotes is a standard procedure (Van Heijst *et al*., 1987). Using a polygraph, we also showed that pre-treatment of A-KG could significantly resolve the alterations in mean arterial pressure, heart rate, respiratory rate, and neuromuscular transmission caused by cyanide (Tulsawani *et al*., 2007).

The magnitude of protection conferred by A-KG could not be ascribed to cyanohydrin formation alone,

and its other protective mechanisms were also considered (Yamamoto, 1990; Niknahad *et al*., 1994). Antioxidants are known to play a crucial role against cyanide-induced oxidative stress (Ardelt *et al*., 1989; Yamamoto & Tang, 1996). A-KG is not known to have antioxidant properties but various alpha-keto and aldehytic metabolites of carbohydrates and amino acids are reported to protect cells from cyanide toxicity by similar mechanisms (Niknahad *et al*., 1994). In studies conducted in rats, sublethal and lethal doses of cyanide caused significant inhibition of temporal or hepatic CCO activity, accompanied by severe oxidative stress. These changes were resolved by A-KG alone, and the protection was further potentiated by STS (Bhattacharya *et al*., 2004; Tulsawani & Bhattacharya, 2006). However, in a separate study, inhibition of hepatic CCO was not accompanied by extensive liver damage as characterized by several hepatic markers. These findings were in departure from our observations in cultured hepatocytes (Tulsawani *et al*., 2006). Subsequent to acute studies, 14 days of repeated exposure of cyanide was found to cause severe oxidative stress the brain, liver, and kidney, without concomitant gene expression of antioxidant enzymes. However, cyanide increased the expression of HSP-70 activity in brain. All the changes were found to regress in the presence of A-KG (Hariharakrishnan, Anand, *et al*., 2009). In another subacute study, cyanide was found to cause hyperglycemia, inhibition of brain CCO activity, reduction in GSH levels together with enhanced lipid peroxidation, and diminished hepatic rhodanese activity. All these changes were accompanied by various histological changes in the brain, heart, liver, and kidney, which were ameliorated by A-KG and STS (Tulsawani *et al*., 2005). Chronic consumption of cyanogenic food like cassava is known to cause severe neuropathy including motor incoordination and neurochemical alteration in different brain regions (Mathangi *et al*., 1999). Ninety days cyanide exposure was found to produce motor incoordination, oxidative stress and neurochemical changes in different brain regions in rats, which were precluded by A-KG and STS (Mathangi *et al*., 2011). In a similar acute study in rats, cyanide produced changes in both dopaminergic and serotonergic pathways in rats, and the effects were more pronounced in corpus striatum and hippocampus, which were resolved by A-KG and STS (Hariharakrishnan *et al*., 2010).

27.5.2 Pharmacokinetics

Pharmacokinetics and safety studies were earlier performed with L-arginine A-KG in human, but there were no data available on pharmacokinetics of A-KG as such (Campbell *et al*., 2006). Transporters for di- and tricarboxylic acids, including the sodium-dependent NaDC transporters have been located in the small intestine (Pajor, 1999), and possibly due to presence of such transporters, exogenously administered A-KG is well absorbed in the intestine (Cynober, 1995). The distribution half-life, elimination half-life, and C max of A-KG were found to be 0.35 h, 0.53 h, and 36.9 μg/ml, respectively, following oral administration of 2.0 g/kg A-KG in rats. Sixty minutes after treatment, the blood A-KG concentration declined to 16.01 μg/ml and by 8 h to basal levels (Patra, 2005). The bio-availability of A-KG did not commensurate with the dose administered but it coincided with the protection profile in animals (Bhattacharya & Vijayaraghavan, 2002; Bhattacharya *et al*., 2002). The utility of A-KG as cyanide antidote remained occluded because its protective dose in animals (2.0 g/kg) was enormously high (Baskin *et al*., 2004). In order to improve its bio-availability, a pharmacoscintigraphic study with A-KG was carried out in animals and human volunteers (Mittal *et al*., 2010). Time-dependent organ distribution of radiolabeled A-KG (^{99m}Tc-A-KG) was characterized in laboratory animals by gamma scintigraphy. The study suggested gastric retention and poor systemic absorption of A-KG. Thereafter, scintigraphy and radiometry studies were performed in human volunteers receiving various doses of A-KG, and it was observed that bio-availability of A-KG significantly improved if given in split doses followed by hydration. In a separate study, nano-A-KG nebulization formulation was developed, and its pharmacokinetics and safety evaluation were carried out in human volunteers. Serum C max of A-KG was found to be 39 ± 3 μg/ml, while the area under curve after inhalation was 376 ± 23 μg × h/ml, indicating that the drug was rapidly and completely absorbed when targeted directly into the lungs (Sultana *et al*. 2011).

27.5.3 Stability analysis

It is known that carbonyl compounds in solution can exist in a number of tautomeric or hydrated forms (Bell, 1966). Although, carbonyl compounds like pyruvates have shown immense therapeutic value in

various pathological conditions, their aqueous stability remained disputed (Woo *et al*., 2004; Zhou, 2005). On the other hand, A-KG has been shown to have greater stability without any known significant effect on the glycolysis (Varma & Hegde, 2004). We conducted a temperature-dependent stability analysis of A-KG for a period of two years, and observed no significant effect of temperature and storage time on the pH of A-KG. However, protective efficacy of A-KG kept at room temperature ($25 \pm 5°C$) declined as compared to the one refrigerated ($4 \pm 1°C$), more distinctly after six months (Bhattacharya *et al*., 2007). After two years, A-KG stored at room temperature and under refrigeration afforded 2- and 4-fold protection against cyanide, respectively as compared to 7-fold conferred by A-KG prepared fresh. The tautomerism and polymerization of A-KG to its trimers, and traces of succinic acid, particularly at room temperature, were characterized by FTIR, NMR, and GC-MS. The study indicated that aqueous solution of A-KG could be refrigerated for one year without any deterioration. However, is not sufficient from the stability point of view and needs to be addressed by preparing more palatable and stable formulations.

27.5.4 Toxicity studies

There is no antidote for cyanide which is free from side effects (Van Heijst *et al*., 1987; Van Heijst & Meredith, 1990), but A-KG being a biomolecule is not expected to be toxic to animals and human. As a nutritional supplement, A-KG is recommended at a dose up to 3.0 g/day (Pangborn *et al*., 1990). However, due to limited bio-availability of orally administered A-KG, its effective dose for antagonizing cyanide poisoning in animals was as high as 0.5–2.0 g/kg body weight (Bhattacharya & Vijayaraghavan, 2002). Therefore, acute effects of 2.0 and 4.0 g/kg A-KG (oral) on various hematological, biochemical, physiological, and histological parameters were studied in rats (Bhattacharya *et al*., 2001). The LD_{50} of A-KG in rats was found to be > 5.0 g/kg and considering the effective doses of A-KG, a safety margin of 3 to 10-fold was observed. The lower dose of A-KG did not cause any abnormalities but the higher dose decreased mean arterial pressure and neuromuscular transmission. In a few animals, lethargy and occasional purging were observed. In brief, the dose of A-KG (2.0 g/kg) offering maximum antidotal efficacy was found to be non-toxic. There was no prior evidence on toxicity of A-KG, but one review reported gastrointestinal distress after A-KG administration and recommended that it should be avoided during pregnancy and lactation (Hendricks, 2012). During clinical trial with A-KG on human volunteers, a few subjects reported nausea, vomiting and mild tachycardia after oral administration of 20 g A-KG in single or two equally divided doses (Mittal *et al*., 2010). During 14 days' repeated oral administration of A-KG, no serious abnormalities were observed in rabbits and rats. However, high doses of A-KG produced diarrhea, decrease in mean body weight, anemia, and increase in absolute and relative organ weight, and some minor histological changes which regressed after withdrawal of the treatments. The no observed adverse effect level (NOAEL) of A-KG in rabbits and rats were determined as 0.5 and 1.0 g/kg body weight, respectively (Rajesh, 2003; Bhattacharya *et al*., 2011). Also, during our subacute and subchronic protection studies with A-KG, no clinical abnormalities were observed at 1.0 g/kg A-KG in rats (Tulsawani *et al*., 2005; Mathangi *et al*., 2011). Our unpublished work also revealed that A-KG was non-mutagenic and non-clastogenic as tested by *in vitro Ames Salmonella*/microsome mutagenicity test and *in vivo* mouse bone marrow micronucleus test, respectively. These were validated test methods routinely used in our laboratory (Meshram & Rao, 1992).

27.6 Some key functions of A-KG

Several important functions of A-KG in the biological system have been elucidated by many workers (Andrae *et al*., 1985; Pangborn *et al*., 1990; Cynober, 1999; Eghbal *et al*., 2004; Tatara *et al*., 2004; Puntel *et al*., 2005). They include (i) bolstering the citric acid cycle, a biological process that yields energy molecules like ATP; (ii) serving as a natural detoxifying agent as it prevents nitrogen overloading in the body by transporting and eliminating them through urea cycle; (iii) synthesis of glutamine, a cellular energy source and a precursor to GSH, a natural antioxidant present the biological system; (iv) production of inhibitor neurotransmitter GABA; (v) carnitine-mediated metabolism of fat; (vi) non-enzymatic oxidative decarboxylation during hydrogen peroxide decomposition; (vii) production of antibiotics; (viii) bone matrix formation; and (ix) prevention of lipid peroxidation and antioxidant activities.

27.7 Efficacy of A-KG against other toxins

In addition to its ability to neutralize the toxicity of cyanide, A-KG it has been explored as an antidote against several other toxins, more because of its diverse functions. Cyanogens are complex nitrile-containing materials like wool, silk, polyurethane, polyacrylonitriles, melamine resins, and synthetic rubber, and so on, which on combustion can generate free cyanide of toxicological significance (Ballantyne, 1987). For the first time, A-KG was shown to alleviate the acute toxicity of several synthetic cyanogens in rats, particularly malononitrile and propionitrile (Bhattacharya *et al*., 2009). The protective efficacy of A-KG was attributed to its ability to detoxify cyanide liberated by the cyanogens, and its antioxidant properties. Ammonia is yet another widely used industrial chemical and environmental pollutant, which is also a product of catabolism of protein and other nitrogenous substances in the body. Hyperammonemia impairs mitochondrial function, reduces ATP synthesis, leading to generation of free radicals and lipid peroxidation. All these events result in severe neurological disorders of the CNS and hepatic encephalopathy (Conn & Bircher, 1994; Kosenko *et al*., 1997; Felipo & Butterworth, 2002). Excessive ammonia also interferes with phosphorylation and oxidation in the citric acid cycle, leading to depletion of certain energy metabolites and citric acid cycle intermediates including A-KG, which may lead to impaired metabolism of fat (Kosenko *et al*., 1993). Therefore, supplementation of A-KG was expected to improve the efficiency of citric acid cycle and lipid metabolism (Pangborn *et al*., 1990). Protective efficacy of A-KG against ammonia-induced encephalopathy was demonstrated long back (Yamamoto, 1989). Thereafter, A-KG was shown to decrease the elevated levels of circulatory urea and non-protein nitrogen following repeated ammonium acetate intoxication in rats (Velvizhi *et al*., 2002a). In a recent study, oral treatment of A-KG and NAC was found to ameliorate the oxidative stress and temporal, hepatic, and renal pathology caused by repeated oral exposure of ammonium acetate in rats (Satpute *et al*., 2012). In addition to detoxification of ammonia by A-KG, the protective efficacy of both A-KG and NAC was attributed to their antioxidant properties as well (Eghbal *et al*., 2004; Satpute *et al*., 2008, 2010). Ethanol is another toxic chemical which produces acetaldehyde and acetate *in vivo*, and its chronic consumption leads to oxidative stress-mediated hepatocellular damages in mammals (Lieber, 1997). Because of natural detoxifying properties of A-KG, it was found to abrogate the oxidative stress, lipid peroxidation, and decrease in weight caused by chronic ethanol intoxication in rats (Velvizhi *et al*., 2002b; Velvizhi, Nagalashmi, *et al*., 2002). Improved nutritional status during protein depletion, normalization of fat metabolism, increased oxidation of fats, and protection against lipid peroxidation and oxidative stress have often been associated with the protective efficacy of A-KG (Bellei *et al*., 1989; Raul *et al*., 1995). Protective efficacy of A-KG has also been demonstrated against oxidative stress-mediated toxicity of hydrogen sulfide, yet another mitochondrial poison like cyanide (Eghbal *et al*., 2004). Hydrazine is another toxin which is mainly used in polymer chemistry and pharmaceuticals, which has wide actions and considered as potential mutagen and carcinogen. Attenuation of hydrazine toxicity by A-KG, pyruvate, and oxaloacetate alone or in combination has also been reported (Roberts *et al*., 1965). Effect of A-KG and oxaloacetate on brain mitochondrial DNA damage and seizures induced by kainic acid, an excitatory neurotoxin, has also been reported in mice (Yamamoto & Mohanan, 2003). The protection was attributed to alpha-keto acid driven inhibition of reactive oxygen species-dependent oxidative insult to the macromolecules in cell. Similarly, protective effects of A-KG against oxidative stress and lipid peroxidation caused by sodium valproate, an anticonvulsant and antipsychotic drug, has also been documented (Vidya & Subramanian, 2006).

27.8 Role of A-KG as a nutritional supplement

Because of several key biological functions of A-KG, its utility as a nutritional supplement and sport medicine has been cherished for long. Consumption of high-protein diet by athletes and bodybuilders usually leads to accumulation of excessive ammonia in the body, which is eliminated by A-KG supplementation. At the same time, A-KG is also used as a source of energy to increase stamina (Pangborn *et al*., 1990). Glutamine and arginine are two important amino acids involved in protein synthesis and energy supply, and supplementation of artificial nutrition with such

amino acids is considered as pharmacologic nutrition (Cynober, 1999; Pierzynowski *et al*. 2002). Glutamine is an important fuel for all rapidly dividing cells, and a precursor of GSH, pyrimidine, and purines. Glutamine depletion has largely been associated during burn injury, surgical trauma, and other stress conditions. Being a precursor to glutamine, the role of A-KG in nutritional reparation of tissue injury is of immense importance (Le Boucher *et al*., 1997; Cynober, 1999). In burn patients, muscle proteins break down and plasma ammonia and amino acids increase in the urine, leading to weight loss and muscle wasting. Nutrition rich in A-KG provided to the traumatized burn patients exhibited improved nitrogen metabolism and reduced toxicity of ammonium ions in the body (Wernerman *et al*., 1990; Wirén *et al*., 2002). In the last few years there have been several investigations on therapeutic response of A-KG against cataract, ischemic injury, trauma/sepsis, atherosclerosis, rheumatoid arthritis, osteoporosis, carcinogenesis, Alzheimer disease, Parkinsonism, and so on (Varma *et al*., 1984; Riedel *et al*., 1996; Kjellman *et al*., 1997; Varma & Hegde, 2004). Also, there are some indication on possible use of A-KG in treating kidney, intestinal and stomach disorders, bacterial and yeast infections, and improving protein usage in hemodialysis patients (Hendricks, 2012). In addition to A-KG, some of its noteworthy salts including ornithine-A-KG, arginine-A-KG, creatine-A-KG, pyridoxine-A-KG, and citrulline-A-KG have been widely used in clinical nutrition and metabolic care (Cynober, 1991, 1995, 2007; Le Boucher & Cynober, 1998; Campbell *et al*., 2006; Barbul, 2008). Ornithine-A-KG (two molecules of ornithine and one molecule of A-KG), in particular, has been shown to prevent oxidative stress and lipid peroxidation in ammonium acetate treated rats (Dakshayani *et al*., 2002). Also, it has been successfully used in treating burn, traumatized, and surgical patients, and chronically malnourished subjects (Cynober, 1991, 1995; Le Boucher & Cynober, 1998).

27.9 Conclusion

There is sufficient evidence to prove that oral treatment of A-KG could significantly antagonize acute, subacute, and subchronic experimental cyanide poisoning. In particular, it could be a good alternative for amyl nitrite in case of out-of-hospital mass casualty scenarios and also as prophylaxis in case of fire accidents and evacuation operations suspecting cyanide exposure. In severe cases, where both SN and STS are indicated, adjunction of A-KG is likely to potentiate their efficacy. Further, repeated administration of A-KG afforded dramatic protection against unusually high doses of cyanide. As per the pre-clinical studies, the most effective dose of A-KG was found to be 2.0 g/kg but in human studies, this dose could be reduced to divided doses of ~150 mg/kg body weight supported with ample hydration. Also, A-KG at the doses exhibiting antidotal efficacy was nontoxic in animals. The bio-availability and stability of A-KG warrant improvement, and this perhaps could be addressed by optimizing a new A-KG formulation amenable for human use and regulatory approval thereof.

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CHAPTER 28
Azide poisonings

Thomas L. Kurt and Wendy Klein-Schwartz

At a Glance

- Azide poisoning seems to have the same mechanism as cyanide poisoning and hydrogen sulfide poisoning.
- However, the antidotes currently available, worldwide, have been shown to be inefficacious in azide poisoning.
- Most clinical cases seem to be of oral ingestion as accidents or suicide attempts, most in laboratories.
- Occupational exposures are rare and most cases survive.
- Hydrazoic acid inhalation exposure cases usually survive.
- Signs and symptoms are usually those of cyanide poisoning, but symptomatic and supportive treatment are all that are available.

28.1 Introduction

Sodium azide (NaN_3) is a white to clear inorganic ionic solid, which is commonly known as the gas-forming component in automobile airbag systems (Fattah, 1996). In Germany, the United States, and Canada, sodium azide is commonly formed in the two step "Wislicenus process" from ammonia:

$$2\,Na + 2\,NH_3 = 2\,NaNH_2 + H_2 \qquad (28.1)$$

with the second step combining sodium amide with nitrous oxide (Holleman & Wiberg, 2001; Wells, 1984):

$$2\,NaNH_2 + N_2O = NaN_3 + NaOH + NH_3 \qquad (28.2)$$

Azide production in Japan and India from newer plants reacts hydrazine with sodium nitrite (Schirmann & Bourdauducq, 2002; Rippen *et al.*, 1996):

$$N_2H_4 + NaNO_2 = NaN_3 + 2H_2O \qquad (28.3)$$

In automobile airbags and airplane escape chutes, sodium azide mixed with igniters and accelerants is detonated by an electronic controller (Wikipedia, n.d.):

$$2\,NaN_3 = 2\,Na + 3\,N_2 \;\text{ then}: \quad 4\,Na + O_2 = 2\,Na_2O \qquad (28.4)$$

Sodium oxalate (Na_2O) forming as free sodium combines with oxygen in the explosive blast. The end result in the airbag is the dry white powder of the sodium oxalate and the nitrogen gas, which expands the bag. If the sodium oxalate contacts moisture, sodium hydroxide is formed which can cause alkaline surface burns.

Hydrazoic acid ($HN_{3(aq)}$, hydrogen azide, azoimide) is a colorless and volatile explosive liquid with a pungent smell (Chang & Lamm, 2003; Holleman & Wiberg, 2001; Wells, 1984). Hydrazoic acid is formed as a conjugate base ($HN_{3(aq)}$) when sodium azide contacts water. And, if the pH drops with increasing acidity, the mole fraction and vapor pressure of hydrazoic acid increases. In addition, sodium azide is a precursor, or chemical intermediate to other inorganic azides, such as lead azide and silver azide. While azide shares many properties in common with cyanides, remember that azides contain no carbon (Holleman & Wiberg, 2001; Wells, 1984).

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28.2 Lack of cyanide antidote efficacy

It is important to state at the outset that no proven human benefit has been seen from the traditional cyanide antidote in case reports of sodium azide fatalities (Chang & Lamm, 2003). Phenobarbital has reduced seizures in murine studies, but not protected against death (Smith *et al*., 1991). In addition, the FDA-approved intravenous cyanide antidote, hydroxocobalamin, while beneficial in cyanide poisonings, has no proven antidotal benefit in human azide poisonings (Hall, 2012, Colorado School of Public Health, personal communication; Chang & Lamm, 2003).

28.3 Uses of sodium azide

Sodium azide is used as a preservative in aqueous laboratory reagents and biologic fluids (Chang & Lamm, 2003; LaLuna *et al*., 1979). For instance, commercial products are registered with the FDA using a 0.1 percent concentration in Coulter counter preparations for red cell counts, and 4.0 percent is used in reagents for hepatitis antigens (Chang & Lamm, 2003; LaLuna *et al*., 1979; Richardson *et al*., 1975). If such azide-containing reagents are poured down the drain, there may be accumulation in traps where stagnant contact with old copper pipes and lead joint seals can form explosive copper and lead azides, which may be detonated by plumbers (LaLuna *et al*., 1979; Roberts *et al*., 1974).

Sodium azide as the explosive agent in airbags was implemented in December 1991 when federal legislation was passed in the U.S. requiring 95% of new automobiles sold after September 1, 1996, to have airbags; and later, airbags were required in all automobiles (Voelker, 1992). Prior to this, extensive engineering design and testing was performed (Szmant, 1980). Airbags were mandated in all new trucks, vans, and buses starting September 1, 1998 (Voelker, 1992). This has mushroomed annual demand for sodium azide to 10–12 million dollars a year in the U.S., although less toxic substitutes are being evaluated (Fattah, 1996).

Sodium azide is also used in manufacturing applications of rubber, latex, wine, and Japanese beer (Chang & Lamm, 2003). In addition, sodium azide has been tested as a hypotensive, where the release of nitric oxide results in smooth muscular relaxation, similar to that produced by sodium nitroprusside (Black, 1954; Nitropress, n.d.) and sodium azide has been investigated as a herbicide, insecticide, nematocide, fungicide, and bacteriocide (Chang & Lamm, 2003; Ahrens, 1983).

28.4 Review of reported sodium azide human poisoning cases

There are, based upon this review, 80 acute sodium azide poisonings reported in the peer-reviewed literature in different settings (Table 28.1). This chapter adds 11 cases to the 69 of the 2003 Chang and Lamm review (Gaulier *et al*., 2012; LeBlanc-Louvry *et al*., 2012; Schwarz *et al*., 2012; Lopacinski *et al*., 2007; Watanabe *et al*., 2007). This total of 80 cases includes 49 oral ingestions with 21 fatalities. Oral ingestions are commonly suicide attempts or suicides in laboratory personnel and health-care settings. Twelve occupational poisoning incidents have occurred, of which all survived. Nine survived an IV dialysis fluid contamination of the ultra-filters used for preparation of hemodialysis fluid with a preservative containing 0.25 percent sodium azide where they were not rinsed prior to use. There have been five dermal exposures with one death involving a simultaneous major burn. And lastly, sodium azide contamination of a publically located iced tea urn at a restaurant has been reported, and in which all five survived.

The iced tea incident was discovered by a CDC investigation of five ill customers at a restaurant in Dallas, Texas, who required emergency care. The cause was specifically determined to be sodium azide by the FBI crime laboratory, and the source was the iced tea from a self-service urn that customers consumed with paper

Table 28.1 Acute azide poisonings, 80 cases.

	Survivors	Fatalities	Total
Oral ingestions	28	21	49
Occupational exposures	12	0	12
IV dialysis contamination	9	0	9
Dermal exposures	4	1	5
Iced tea contamination	5	0	5

Condensed in part from Chang and Lamm (2003) with permission, supplemented by Gaulier *et al*. (2012), LeBlanc-Louvry *et al*. (2012), Schwarz *et al*. (2012), Lopacinski *et al*. (2007), and Watanabe *et al*. (2007).

cups. The CDC field investigation case-control study showed the iced tea had a high adverse health effects odds ratio (OR) of 65 [CI 2.4-3292]. How the contamination occurred was not determined, because the self-service urn was kept in an open location visible to customers, but out of direct line of vision of employees (Schwarz *et al.*, 2012; Todd, 2012, Southwest Institute of Forensic Science, Dallas, personal communication).

More than two decades earlier, in 1989, a report of three fatal cases and a literature review that described 14 additional acute azide poisoning cases at that time (Klein-Schwartz *et al.*, 1989). This initial case series was followed by others updating additional case literature in 1994 and 1999, further establishing the hypotensive effect and reporting no clear benefit to the Cyanide Antidote Kit (Smith & Wilson, 1994; Chiba *et al.*, 1999). Prior to the Chang and Lamm review (2003), additional case reports further substantiated these updates (Singh *et al.*, 1994; Howard *et al.*, 1990; Wollenek, 1989; Abrams *et al.*, 1987; Albertson *et al.*, 1986; Edmonds & Bourne, 1982; Emmett & Ricking, 1975; Richardson *et al.*, 1975; Gobbi, 1967). This chapter adds the 11 reported cases for the total of 80 acute azide poisonings (Schwarz *et al.*, 2012; LeBlanc-Louvry *et al.*, 2012; Gaulier *et al.*, 2012; Lopacinski *et al.*, 2007; Watanabe *et al.*, 2007).

28.5 Human experimental exposures to sodium azide and hydrazoic acid

In addition, there have been 116 human experimental exposures to sodium azide and hydrazoic acid involving human therapeutic and occupational studies where exposures were by oral, inhalation, and intravenous routes (Table 28.2) (Lamm *et al.*, 1999; Trout *et al.*, 1996; Rippen *et al.*, 1996; Haas & Marsh 1970; Black, 1954; Graham, 1948).

Table 28.2 Human studies: 116 persons, no deaths.

Oral dosing:	Dosage range 0.56 to 3.9 mg, 73 persons
Inhalation:	Dosage range 0.5 to 65 ppm, 42 persons
Intravenous:	Dose 1.3 mg, 1 person

Condensed in part from Chang and Lamm (2003) with permission; Lamm *et al.* (1999), Trout *et al.* (1996), Rippen *et al.* (1996), Haas and March (1970), Black (1954), and Graham (1948).

28.6 Signs and symptoms

The most common signs and symptoms in acute azide poisonings can be categorized by organ systems (Table 28.3). These are (i) general – headache, dizziness, sweating and hypothermia; (ii) neurological – collapse, decreased muscular strength, coma, and seizures; (iii) cardiovascular – hypotension, palpitation, tachycardia, and arrhythmia; (iv) pulmonary – tachypnea, dyspnea, nausea, respiratory failure and pulmonary edema; (v) gastrointestinal – nausea, vomiting and diarrhea; and (vi) metabolic – metabolic (lactic) acidosis. The most important marker among these is hypotension, where early onset hypotension in a few minutes to less than an hour is associated with a benign course, while late onset hypotension of one hour or later is usually fatal (Chang & Lamm, 2003).

28.7 Fatal cases

In fatal cases, typical progression of signs and symptoms are headache, collapse, coma, hypotension, tachypnea, and metabolic (lactic) acidosis. Then arrhythmias appear proceeding to cardiorespiratory arrest. The signs and symptoms from lower exposures (< 700 mg) are

Table 28.3 Signs and symptoms of 80 acute azide poisonings by organ systems, listed in order of frequency.

GENERAL
Headache, dizziness, sweating, hypothermia
NEUROLOGICAL
Collapse, decreased MS, comatose, seizure
CARDIOVASCULAR
Hypotension, palpitation, tachycardia, arrhythmia
PULMONARY
Tachypnea, dyspnea, respiratory failure, pulmonary edema
GASTROINTESTINAL
Nausea, vomiting, diarrhea
OTHER
Metabolic (lactic) acidosis

Top 4 of each listed in order of frequency, organ system not listed if not in more than 2 cases, lower signs and symptoms if not otherwise listed. Condensed in part from Chang and Lamm, 2003 with permission, supplemented by Gaulier *et al.* (2012), LeBlanc-Louvry *et al.* (2012), Schwarz *et al.* (2012), Lopacinski *et al.* (2007), and Watanabe *et al.* (2007).

physiological responses at the vascular level and those at or above are toxicological responses at the metabolic level. Nonlethal doses range from 0.3 to 150 mg (0.004 to 2 mg/kg). While fatal doses occur with exposures equal to or exceeding 700 mg (10 mg/kg). An unusual survival has been reported in a woman who in error drank 1000 mg of sodium azide placed as a preservative in a urine collection container (Watanabe *et al.*, 2007). Her treatment involved gastric lavage, charcoal, intubation, hemodialysis followed by continuous hemodiafiltration, an intra-aortic balloon pump, the pressor dopamine, and the cardiotonic agent, olprinone. With estimated body weight less than 50 kg, she survived a dose in excess of 20 mg/kg.

28.8 Historical perspective

Gosselin *et al.*'s *Clinical Toxicology of Commercial Products* listed sodium azide, hydrazoic acid in the "Ingredients Index, No. 111" (Gosselin *et al.*, 1984). A toxicity rating of 6 was given (Super toxic: Probable lethal human dose for a 70 kg person less than 5 mg/kg). The American Association of Poison Control Centers lists sodium azide as a Poisindex® protocol for poison center calls (accessed through North Texas Poison Center in 2012), but does not have an established category in the annual Toxic Epidemiologic Surveillance System (TESS) report.

28.9 Mechanism(s) of action

Azide in the blood has been shown to be converted to nitric oxide (NO) by erythrocytes; and cyanide, known to be produced during *in vitro* incubation of azide with whole blood, is also a suspected metabolite (Kruszyna *et al.*, 1987). What causes the toxicity of azide has been a topic of extensive discussion (Smith & Wilson, 1994). Originally ascribed to be the inhibition of cytochrome oxidase, then later to nitric oxide (NO), Smith and Wilson (1994) explained that "it is by no means clear whether or not NO generated *in vivo* from sodium azide contributes in a major way to its toxicity." While azide is almost as acutely toxic as cyanide, azide has certain cardiovascular and pulmonary effects in common with nitrite (i.e., hypotension, tachycardia, arrhythmias, respiratory failure, pulmonary edema) (Chang & Lamm, 2003; Smith & Wilson, 1994). In laboratory animals, azide produces non-asphyxial seizures, while human deaths appear more related to organ failure in cardiopulmonary collapse (Smith and Wilson, 1994).

28.10 Autopsy findings

Findings on autopsy typically include: (i) pulmonary edema with frothy pink fluid; (ii) gastric mucosa edema with erythema; (iii) liver congestion; (iv) generalized edema in other organs such as the brain and kidneys; and (v) petechiae on the pleura and pericardium (Chang & Lamm, 2003; Marquet *et al.*, 1996; Herbold *et al.*, 1995; Lambert *et al.*, 1995; Howard *et al.*, 1990; Klein-Schwartz *et al.*, 1989; Klug & Schneider, 1987; Albertson *et al.*, 1986; Emmett & Ricking, 1975; Koźlicka-Gajdzińska & Brzyski, 1966; Kayser, 1928).

28.11 Other outcomes

Other outcomes to sodium azide exposure have been reported. For instance, in chronic or more delayed exposures, myocardial infarction and cardiomyopathy have been reported (Judge & Ward, 1989). Human corneal ophthalmic injury has been reported from alkaline byproducts of sodium azide explosive deployed automobile airbags and rupture (White *et al.*, 1995). Injuries of the cornea from sodium azide have also been described in animal studies that demonstrated changes in retinal electrical potential and lesions in optic nerves and tracts (Grant & Schuman, 1993).

28.12 Occupational health issues

In occupational health, adverse health effects from sodium azide have been described in exposed workers in a sodium azide production plant where headache, nausea, dizziness, fatigue, eye irritation, palpitations, and tremors were more common than in unexposed workers (Miljours & Braun, 2003). Occupational exposures of workers to sodium azide have been assessed by an expanded neuropsychological assessment survey, demonstrating adverse health effects in a sodium azide production plant (Trout *et al.*, 1996). Another report described a group of five workers in a laboratory accidentally ingesting 20 to 80 mg of sodium azide who developed symptoms varying from feeling faint to

severe chest pain (Edmonds & Bourne, 1982). Aggravation of asthma and potentiation of reactive airways dysfunction have also been reported (Weiss, 1996).

28.13 Occupational/environmental exposure limits/recommendations

Sodium azide is listed in the ACGIH and NIOSH guides with a TLV measured as hydrazoic acid vapor of 0.11 mg/m^3, or 0.29 mg/m^3 as sodium azide (ACGIH, 2011; NIOSH, 2005). The NIOSH REL is 0.1 for hydrazoic acid vapor and 0.3 mg/m^3 as sodium azide, but there is no official OSHA PEL for sodium azide (NIOSH, 2005). Federal OSHA's Salt Lake City Technical Center can perform the analysis for sodium azide in samples collected by Federal OSHA, State OSHA family and other governmental agencies. Chemical sampling information for sodium azide can be found at OSHA's public website "Sodium Azide and Hydrazoic Acid in Workplace Atmospheres" accessed from the following sampling link: www.osha.gov/dts/chem/calsampling/data/CH 267505.html. Also use the following link for methodology: www.osha.gov/dts/sltc/methods/inorganic/id211/id211.html.

The online description "Sodium Azide" at the CDC's website quotes from OSHA's comments on January 19, 1989, Final Rule on Air Contaminants Project extracted from 54FR2332, and what follows. This rule was remanded by the U.S. Circuit Court of Appeals and the limits are not currently in force.

> CAS: 26628-22-8; Chemical Formula: NaN_3. There was no former OSHA PEL for sodium azide. The proposed PELs were a ceiling of 0.1 ppm as hydrazoic acid vapor (HN_3) and a ceiling of 0.3 mg/m^3 as sodium azide (NaN_3); NIOSH (Ex. 8-47, table N1) concurred with the Agency's selection. The final rule establishes this limit. In addition, a skin notation is being added to the limit in the final rule. The ACGIH (1986/Ex.1-3) has ceiling limits for sodium azide of 0.1 ppm (as hydrazoic acid vapor) and 0.3 mg/m^3 (as NaN_3). Sodium azide is a colorless, crystalline solid.

28.14 Laboratory evaluation

Laboratory testing of biological specimens (blood, gastric contents) for sodium azide has been most easily accomplished using a microdiffusion process similar to that for isolation of cyanide, followed by ultraviolet spectrophotometry as 214 nm, or colorimetry using ferric chloride (Baselt, 2012). Alternative testing can occur through liquid chromatography, ion chromatography, and gas chromatography–mass spectrometry (Kruszyna *et al.*, 1998). However, while the FBI crime laboratory will test referred specimens through government channels, clinicians need to be aware that commercial reference laboratories such as NMS (National Medical Services) do not offer testing for sodium azide on a regular basis. In addition, decay of azide levels in stored specimens, similar to that reported for cyanide, has been reported with a half-life in whole blood of 4.5 days at room temperature and 12 days at 0°C, thereby making collection in a proper tube with preservative and frozen storage mandatory if analysis is not performed immediately. Be reminded of the importance of proper chain of custody, because civil and criminal legal concerns often evolve with sodium azide cases.

28.15 Conclusion

Sodium azide is a unique, highly toxic inorganic chemical where awareness, caution and prevention are the key, because specific effective antidotal treatment does not exist as for cyanide.

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Conflict of interest

Dr. Kurt has no conflict of interest to disclose.

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