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TOXICOLOGICAL REVIEW

OF

HYDROGEN CYANIDE

AND

CYANIDE SALTS

(CAS No. various)

**In Support of Summary Information on the
Integrated Risk Information System (IRIS)**

September 2010

U.S. Environmental Protection Agency
Washington, DC

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LIST OF ACRONYMS AND ABBREVIATIONS

ACH	acetone cyanohydrin
ADP	adenosine diphosphate
AIC	Akaike's Information Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMD	benchmark dose
BMDL	95% lower confidence limit on the BMD
BMDS	BMD software
BMR	benchmark response
CAP	compound action potential
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
CN$^-$	cyanide ion
(CN)$_2$	cyanogen
CNS	central nervous system
DPO	diphenyl oxide
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EGL	external granular layer
FEV	forced expiratory volume
FVC	forced vital capacity
GD	gestation day
GFAP	glial fibrillary acid protein
GGT	γ-glutamyl transferase
HCN	hydrogen cyanide
i.p.	intraperitoneal or intraperitoneally
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
i.v.	intravenous or intravenously
KCN	potassium cyanide
KSCN	potassium thiocyanate
LD$_{50}$	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
ML	molecular layer
MPST	mercaptopyruvate sulfurtransferase
NaCN	sodium cyanide
NHANES	National Health and Nutrition Examination Survey
NIS	sodium-iodide symporter
NOAEL	no-observed-adverse-effect level

NRC	National Research Council
NTP	National Toxicology Program
OCN⁻	cyanate
OSCN⁻	hypothiocyanate
PBPK	physiologically based pharmacokinetic
PND	postnatal day
POD	point of departure
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RR	relative risk
SCN⁻	thiocyanate
SD	standard deviation
SE	standard error
SEM	standard error of the mean
$\mathbf{T_3}$	triiodothyronine
$\mathbf{T_4}$	thyroxine
TSH	thyroid-stimulating hormone
TWA	time-weighted average
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
$\mathbf{V_{max}}$	maximum velocity
WBC	white blood cell

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to hydrogen cyanide and cyanide salts. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of hydrogen cyanide and cyanide salts.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of hydrogen cyanide (HCN) and cyanide salts. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute ($\leq$ 24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for HCN and cyanide salts has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk*

Assessment (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)*, Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Numbers (CASRNs) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through January 2010.

2. CHEMICAL AND PHYSICAL INFORMATION

The term cyanide refers to any compound that contains the cyanide ion (CN^-), consisting of a carbon atom triple bonded to a nitrogen atom. Hydrogen cyanide (HCN) is a colorless or pale blue liquid or gas with a faint bitter almond-like odor, while sodium cyanide (NaCN) and potassium cyanide (KCN) are white crystalline powders. HCN is a weak acid with a pK_a of 9.2; therefore, HCN and CN^- can interconvert based on pH and temperature. In solution under physiological conditions, the majority of HCN is present in the undissociated form. The simple cyanide salts, KCN and NaCN, are very soluble in water and mildly soluble in ethanol. These compounds readily dissociate in water, and therefore, exposure to any of these compounds in aqueous media results in exposure to CN^-. For the sake of comparability, doses in this review are given as CN^- unless stated otherwise. Physical properties for HCN and other simple cyanide salts are summarized in Table 2-1.

The dissociation constants of metallocyanides vary significantly depending on oxidation states, pH, temperature, and photodegradation (Beck, 1987). As noted above, some of these compounds, such as NaCN and KCN, dissociate completely when dissolved in water, whereas others do not.

Other inorganic and organic compounds containing the CN^- group include the nitriles, in which the CN^- group is covalently bound to the rest of the molecule (e.g., acetone cyanohydrin [ACH]), the cyanogens (i.e., compounds of the form NC-CN or X-CN, where X is a halogen), such as cyanogen chloride, and "cyanogenic" substances in some plant-based foods. These cyanogenic compounds contain cyanogen glycosides that can undergo hydrolysis following ingestion to produce HCN and other cyanide-containing compounds.

Cyanide originates primarily from anthropogenic sources in the environment, but cyanide is also released from biomass burning, volcanoes, and natural biogenic processes from higher plants, bacteria, and fungi (Agency for Toxic Substances and Disease Registry [ATSDR], 2006). Cyanide is also a component of tobacco smoke and can be present at high concentrations in structural fires (Steinmaus et al., 2007; Brauer et al., 2006; Tsuge et al., 2000; Brandt-Rauf et al., 1988). Cyanide compounds are used in a number of industrial processes, including mining, metallurgy, manufacturing, and photography, due to their ability to form stable complexes with a range of metals. Cyanide has been employed extensively in electroplating, in which a solid metal object is immersed in a plating bath containing a solution of another metal with which it is to be coated, in order to improve the durability, electrical resistance, and/or conductivity of the solid. HCN has also been used in gas chamber executions and in chemical warfare. NaCN and KCN are also used as rodenticides. Conversion factors for HCN air concentrations are 1 mg/m^3 = 0.90 ppm and 1 ppm = 1.11 mg/m^3.

Table 2-1. Physical and chemical properties of cyanide compounds

	Hydrogen cyanide	Sodium cyanide	Potassium cyanide	Calcium cyanide	Potassium silver cyanide	Cyanogen
CASRN #	74-90-8	143-33-9	151-50-8	592-01-8	506-61-6	460-19-5
Synonyms	Prussic acid, hydrocyanic acid, Cyclone B	Cyanogran, Cymag, Cyanobrik, white cyanide	Hydrocyanic acid, potassium salt	Calcyanide, calcyan, cyanogas, black cyanide	Potassium dicyanoargentate	Dicyanogen, ethanedinitrile, oxalonitrile
Molecular weight	27	49	65	92	199	52
Form	Colorless gas or liquid	White crystalline powder	White lumps or crystals	White powder	White crystals	Colorless gas
Chemical formula	HCN	NaCN	KCN	$Ca(CN)_2$	$AgK(CN)_2$	$(CN)_2$
Boiling point (°C)	25.7	1,496	1,625	Not applicable	Not found	-21.17
Melting point (°C)	-13.4	563.7	634.5	640	Not found	-27.9
Density (g/mL)/specific gravity (unitless)	0.6884	1.6	1.52	1.85	2.36	0.9537
Solubility	Ethanol, ether	Water, ethanol	Water, ethanol	Water, ethanol, weak acid	Water, ethanol	Water, ethanol

Cyanide or cyanogenic compounds are found in many foods. Cyanide compounds occur naturally as part of sugars or other naturally occurring compounds in certain plant-derived foods, including almonds, millet sprouts, lima beans, soy, spinach, bamboo shoots, sorghum, and cassava roots. The parts of these plants that are eaten in the United States, however, contain relatively low amounts of cyanide (ATSDR, 2006).

Around the world, cassava is a vital staple for about 500 million people. Cassava is a major source of carbohydrate in parts of Africa, South America, and Southeast Asia. Its starchy roots produce more food energy per unit of land than any other staple crop, and it can be dried and ground into flour. Its leaves, commonly eaten as a vegetable in tropical regions, provide vitamins and protein. However, the storage root of the cassava plant contains linamarin, a cyanogenic glycoside that is easily hydrolyzed by the enzyme linamarase (a β-glucosidase) to release HCN. Although HCN can be readily removed during processing of cassava, cyanide liberated from residual linamarin is associated with goiter in iodine-deficient populations with chronic intake of cassava-based food products (Taga et al., 2008; Teles, 2002; Abuye et al., 1998).

Information on the concentration of cyanide in drinking water is available from the National Drinking Water Contaminant Occurrence Database (U.S. EPA, 2003). In this database, a cross-sectional study of 16 states was used to develop a statistical estimation indicative of the national occurrence of contaminants in drinking water. Based on these data, the overall mean cyanide concentrations in treated surface water and groundwater systems were 2 and 8 μg/L, respectively. Cyanide was detected infrequently; the average among the public water systems that detected cyanide was 60 μg/L (parts per billion), although some systems had levels in the parts per million range.

Although concentrations of HCN in foods are expected to be low, one study (Fiksel et al., 1981) estimated that HCN intake from inhalation of air and ingestion of drinking water would be less than the intake from food. Estimates of the HCN concentration in the total diet of U.S. populations are not available in the peer-reviewed literature. An average daily SCN⁻ intake of 16.3 μmol/day was estimated for Koreans based on vegetable intake surveys obtained from the Korean National Health and Nutrition Examination Survey (Han and Kwon, 2009). ATSDR (2006) estimated an atmospheric concentration of 170 ppt (188 ng/m^3), corresponding to an inhalation exposure to the general U.S. nonurban, nonsmoking population of 3.8 μg HCN/day, corresponding to 54 ng/kg-day HCN.

Smokers and those exposed to second-hand tobacco smoke make up a subset of the general population that may be exposed to elevated levels of HCN. Smokers could be exposed to 10–400 μg HCN per cigarette, whereas nonsmokers exposed to sidestream smoke could be exposed to 0.06 to 108 μg HCN per cigarette (ATSDR, 2006). Serum and urinary levels of thiocyanate (SCN⁻), the primary metabolite of HCN, are generally about two- to fivefold higher

in smokers versus nonsmokers, indicating significantly elevated cyanide exposure through tobacco smoke (Steinmaus et al., 2007; Brauer et al., 2006; Tsuge et al., 2000).

3. TOXICOKINETICS

3.1. ABSORPTION

The available data show that cyanide is rapidly and extensively absorbed via the oral, inhalation, and dermal routes, although quantitative data on the percent or extent of absorption are limited. Oral absorption has been reported as being lower at lethal doses. Some cyanide salts, including potassium cyanide (KCN) and sodium cyanide (NaCN), rapidly dissociate in water. Because hydrogen cyanide (HCN) is a weak acid (pKa of 9.2), the acidic environment in the stomach favors the nonionized form (HCN) (U.S. EPA, 1992). The nonionized form is also favored under neutral conditions. Thus, HCN and the dissociated sodium and potassium salts are predominantly present as HCN at the acidic pH levels of the stomach and lower gastrointestinal tract. Accordingly, these compounds are presumably absorbed by passive diffusion across the lipid matrix of the intestinal microvilli. The moderate lipid solubility and small size of the HCN molecule also indicate that HCN crosses mucous membranes rapidly. HCN is absorbed rapidly after inhalation, and it penetrates the epidermis. KCN and NaCN are corrosive to the skin, which can increase dermal absorption. In the absence of such corrosion, however, these ionic forms of cyanide are absorbed less completely than HCN via the dermal route.

Limited data are available on oral absorption of cyanide in humans. In a case report (Liebowitz and Schwartz, 1948) of a suicide attempt by an 80-kg male who ingested an estimated 15–25 mg/kg cyanide ion (CN^-) as KCN, the authors estimated that, 2 hours after ingestion, the patient had 2.5 g CN^- in the body, of which 1.2 g was in the blood, based on a concentration of 200 mg HCN/L in the blood at that time. This study does not provide any information on the disposition of the remaining cyanide.

Gettler and Baine (1938) reported data indicating that, at doses above historical lethal doses, absorption decreases with increasing dose levels. Absorption was estimated at 19.5, 18.1, and 15.7% in people estimated to have ingested 297, 557, and 1,450 mg HCN in suicide attempts. The low absorption at the highest dose may have been due to death occurring before absorption was complete. The assumption that absorption is lower at higher lethal doses is supported by a case report where absorption was approximately 82% in an individual, estimated as having ingested 30 mg HCN, who died more than 3 hours after exposure.

Only limited data are available on absorption of inhaled cyanide by humans. Landahl and Herrmann (1950) measured the pulmonary retention of HCN in 10 volunteers exposed to concentrations of 0.0005–0.02 mg/L (0.5–20 mg/m^3) for up to 3 minutes. All subjects breathed through their mouths. The percent retained in the lung (and, presumably, the percent absorbed) was approximately 60% and ranged from 58 to 77% among people who were breathing normally. Rapid, shallow breathing appeared to decrease absorption.

Dermal absorption of HCN gas has also been observed in humans. Drinker (1932) reported that three workers who entered an atmosphere containing 2% HCN (20,000 ppm [22,100 mg/m^3]) became dizzy and weak and were on the verge of unconsciousness, despite wearing gas masks providing respiratory protection. The observed effects were attributed to dermal absorption of the gas. Potter (1950) reported on a worker, wearing respiratory protection and protective clothing, who was accidentally exposed to liquid HCN. Within 5 minutes, the worker became dizzy, had difficulty breathing, and fell unconscious.

Animal data also indicate extensive absorption. In male Sprague-Dawley rats treated by gavage with 1 mg/kg KCN, a peak blood level of 6.2 nmol/mL CN$^-$ (160 µg/L) was observed 2 minutes after treatment, indicating rapid absorption (Leuschner et al., 1991). As described above for oral poisonings in humans, absorption by dogs decreased as the dose increased well above lethal levels (Gettler and Baine, 1938). Oral absorption was essentially comparable (16.6 and 15.7%) in dogs that ingested 100 and 50 mg HCN, respectively. However, absorption increased to 72% in a dog that ingested 20 mg HCN (1.5 mg/kg).

No animal studies were located that quantitatively evaluated the rate or extent of absorption via the inhalation or dermal routes. However, Walton and Witherspoon (1926) reported toxic effects and death in guinea pigs exposed by holding the open end of a test tube containing liquid HCN against their shaved stomachs and concentration-related signs of toxicity (including death) in dogs given whole-body exposure to HCN (excluding the head). These results support the human data, demonstrating that absorption via the dermal route occurs in animal species and can produce toxic effects, including death.

3.2. DISTRIBUTION

Cyanide distributes rapidly and uniformly throughout the body following absorption. HCN enters the systemic circulation when inhaled or dermally absorbed (Yamamoto et al., 1982; Potter, 1950; Drinker, 1932). Limited qualitative and quantitative data are available regarding the tissue distribution of cyanide in humans from inhalation exposure studies to high doses of cyanide. For example, cyanide was found in the lung, heart, blood, kidneys, and brain of humans who died following cyanide inhalation (Gettler and Baine, 1938). In addition, Knowles and Bain (1968) evaluated the relationship between blood concentrations of cyanide and short-term accidental exposures to lethal levels of HCN in human case reports. Air concentrations of >300 ppm (333 mg/m^3 HCN), >200 ppm (222 mg/m^3 HCN), >100 ppm (111 mg/m^3 HCN), and >50 ppm (55 mg/m^3 HCN) corresponded to blood concentrations of >10, >8–10, >3–8, and >2–4 mg/L, respectively. The authors noted that there is considerable variability in this relationship, presumably reflecting both interindividual variability and uncertainty of exposure duration and concentrations estimated or measured retrospectively.

Limited data on distribution of cyanide in humans, following oral exposure, are available. Immediately following oral cyanide exposure, the stomach contents appear to contain the highest

concentration of cyanide. Other tissues containing cyanide included the liver, brain, spleen, blood, kidneys, and lungs (Ansell and Lewis, 1970; Gettler and Baine, 1938).

Several animal studies are available that demonstrate the tissue distribution of cyanide following both inhalation and oral exposures. In dogs exposed to lethal concentrations of cyanide by inhalation, the highest concentrations of cyanide were found in the lungs, blood, and heart (Gettler and Baine, 1938), while in rabbits exposed to 2,714 ppm HCN (3,000 mg/m^3) for 5 minutes by inhalation, the highest tissue concentrations were detected in the heart, lung, and brain, with lower levels in the spleen and kidneys (Ballantyne, 1983, as cited in ATSDR, 2006).

In rats and rabbits exposed by the oral route, the highest tissue concentrations of cyanide were in the liver, lung, blood, spleen, and brain (Ballantyne, 1983, as cited in ATSDR, 2006; Ahmed and Farooqui, 1982; Yamamoto et al., 1982). Yamamoto et al. (1982) compared cyanide distribution in rats following gavage exposure to NaCN (7 or 21 mg/kg CN) or inhalation exposure to HCN (average of 356 or 1,180 ppm, equivalent to 393 or 1,303 mg/m^3 HCN, respectively). These exposure levels resulted in death in ≤10 minutes. Elevated concentrations were found in all tissues evaluated following exposure via either route, but the relative concentrations were route dependent. There was also some dose dependence, which may have been related to the faster time to death at higher exposures for each route (approximately 10 minutes at the lower exposure levels vs. 3–5 minutes at the higher levels). Focusing on the lower oral dose, the highest tissue concentration of cyanide following exposure was in the liver, followed by the blood and lungs, and then the spleen and brain. After inhalation exposure, the highest concentration was in the lungs, followed by the blood and liver, and then spleen and brain. The kidney was not evaluated for either exposure route. The route-specific difference may be related to first-pass metabolism in the liver, following oral dosing, and initial deposition at the portal of entry, following inhalation exposure.

Okoh and Pitt (1982) investigated the tissue distribution of cyanide in rats fed KCN in the diet at 77 µmol/day (approximately 5.5 mg/kg-day CN) for 3 weeks and then injected intraperitoneally (i.p.) with radiolabeled NaCN. Radioactivity was widely distributed, with the highest concentrations in the gastrointestinal tract, blood, kidneys, lungs, spleen, and liver. Radioactivity appeared in the stomach as early as 10 minutes after injection, with 18% of the injected dose found in the stomach contents within 60 minutes of dosing. More than 80% of the radioactivity in the stomach was present as thiocyanate (SCN$^-$), with small portions present as cyanide and radiolabeled carbon dioxide.

In a subchronic study, male Sprague-Dawley rats (26–40/group) received KCN in their drinking water at doses of 0, 40, 80, or 140/160 mg/kg-day for 13 weeks (Leuschner et al., 1991). Blood was collected every 2 weeks for analysis of CN$^-$ and SCN$^-$ levels; both were found to be dose related. Within each dose group, however, the levels of both cyanide and thiocyanate remained fairly constant over the 13-week exposure period. Cyanide levels in the blood were 16–25 nmol/mL CN$^-$ (420–650 µg/L); thiocyanate levels were 341–877 nmol/mL SCN$^-$ (20–

51 mg/L). Small amounts of thiocyanate were also detected in the control animals at concentrations of 11–53 nmol/mL SCN⁻ (0.64–3.1 mg/L) in plasma.

Howard and Hanzal (1955) exposed rats to HCN in the diet at an average daily dose of up to 10.8 mg/kg-day CN⁻ for 2 years and found virtually no cyanide in the plasma or kidneys. Cyanide was detectable in the red blood cells (RBCs) of less than half of the rats at an average concentration of 2 µg/100 g tissue. However, as the food was treated with HCN gas, some volatilization likely occurred.

McMillan and Svoboda (1982) incubated cyanide with washed erythrocytes, resuspended in phosphate-buffered saline containing glucose and bovine serum albumin, and found that cyanide concentrated in the RBCs. The major portion of cyanide in blood is sequestered in the erythrocytes, and a relatively small proportion is transported via the plasma to target organs (International Programme on Chemical Safety [IPCS], 2004). The RBC:plasma ratio for cyanide is approximately 200:1 (IPCS, 1992). Binding to RBCs is primarily due to cyanide reacting with ferric iron (Fe^{3+}) in methemoglobin to form the nontoxic complex cyanomethemoglobin (Chen and Rose, 1952).

In rats treated orally with [^{14}C]KCN (5 mg/kg), radioactivity levels in plasma and whole blood were initially (at 3 hours) much higher than levels in RBCs (Farooqui and Ahmed, 1982). Levels in plasma and whole blood decreased rapidly, and red cell levels increased slightly, so that at 24 hours, plasma levels were only slightly higher than whole blood levels. Most of the radioactivity in the red cells was in the heme fraction of hemoglobin rather than the membranes. The reason for the finding of higher levels in the plasma than blood in this study is not clear, but it may have been due to differences in sampling times. Approximately 60% of the cyanide in plasma is bound to protein (IPCS, 1992).

Limited information indicates that cyanide can cross the placenta. Pettigrew et al. (1977) compared cyanide and urinary thiocyanate levels in a small group of smoking and nonsmoking pregnant women matched for age, height, parity, and social class. Maternal plasma and urinary thiocyanate levels were statistically significantly increased in smokers during gestation at weeks 28, 32, and 36; at delivery, only plasma thiocyanate was measured and was also statistically higher in mothers who smoked. Mean urinary thiocyanate levels of neonates of smoking mothers were elevated compared to those of nonsmokers (40.6 compared to 23.1 µmol/L, respectively), although the difference was not statistically significant, probably due to the small sample size (n = 10).

Lactational transfer of cyanide and thiocyanate has been shown to occur in goats. Soto-Blanco and Gorniak (2003) dosed lactating goats with 0, 1.0, 2.0, or 3.0 mg/kg-day KCN (equivalent to 0, 0.4, 0.8, or 1.2 mg/kg-day CN⁻) from lactation days 0 to 90 and measured whole blood cyanide and thiocyanate concentrations on lactation days 30, 60, and 90. Both whole blood cyanide and plasma thiocyanate concentrations were increased in a dose-dependent manner in treated mothers, with mixed results regarding time dependence. In the offspring, both

blood cyanide and plasma thiocyanate increased with increasing maternal cyanide dose at lactation day 30 and decreased with lactation time. By lactation day 90, the concentration of these compounds in the blood/plasma of the offspring was low or undetectable. The study authors attributed these findings to a decrease in milk consumption, accompanied by a concomitant increase in solid food (grass and feed) during the latter part of lactation.

Small levels of cyanide are normally present in blood plasma at 0–140 µg/L and in other tissues at <0.5 mg/kg CN^- (ATSDR, 2006; Feldstein and Klendshoj, 1954). Chandra et al. (1980) found that nonsmokers with no occupational exposure to cyanide had an average of 3.2 µg/100 mL CN^- (32 µg/L) in blood; smokers had average blood cyanide levels of 4.8 µg/100 mL (48 µg/L). The background level is attributed to exposure to cyanogenic food, vitamin B_{12}, and passive tobacco smoke. Cyanide preferentially binds to hemoglobin in RBCs but does not appear to accumulate in tissues after chronic oral exposure to inorganic cyanides (Leuschner et al., 1991; Chen and Rose, 1952).

3.3. METABOLISM

The major metabolic pathway for cyanide is conversion to the less acutely toxic compound, thiocyanate, primarily by rhodanese, with some conversion occurring via 3-mercaptopyruvate sulfur transferase. Conversion to thiocyanate accounts for 60–80% of a cyanide dose. Minor pathways include incorporation into a 1-carbon metabolic pool or conversion to 2-aminothiazoline-4-carboxylic acid (ATSDR, 2006). Conversion to 2-aminothiazoline-4-carboxylic acid via reaction with cystine accounted for approximately 15% of an injected dose of cyanide in rats (Wood and Cooley, 1956). Small amounts are also converted to carbon dioxide in exhaled air or excreted unchanged as HCN in exhaled air. These pathways are shown in Figure 3-1.

Rhodanese, a mitochondrial enzyme that converts cyanide to thiocyanate, facilitates transfer of a sulfur atom to cyanide from a sulfane-sulfur donor. Because these donors must contain an S-S bond, glutathione, thiosulfate, and cystine are sulfur donors for rhodanese, whereas the thiols, cysteine, and reduced glutathione are not donors. Rhodanese is widely distributed throughout the body. Using immunohistochemical staining techniques, rhodanese in rabbits has been located in the liver, where it is most abundant in the hepatocytes near blood vessels (Sylvester and Sander, 1990). It was also found in the lung, localized in epithelial cells that formed the barrier between inhaled air and blood vessels. In the kidneys, rhodanese was present in tubules closest to the glomeruli. The authors concluded that sites with the greatest abundance of rhodanese are located to maximize conversion of cyanide to thiocyanate following both oral and inhalation exposure. The presence of rhodanese in these tissues also indicates the importance of first-pass metabolism in determining the toxicity of inhaled and ingested cyanide.

CN⁻

Cyanide

Major Path (80%)

Thiocyanate
(SCN⁻)

Urinary Excretion

Minor Path

2-Aminothiazoline-4-carboxylic acid &
2-Iminothiazolidine-4-carboxylic acid

HCN
(Pool)

HCN
Hydrogen Cyanide
(in expired air)

CNO^-
Cyanate

HCOOH
Formic Acid

Metabolism of one-
carbon compounds

Formates

CO_2
Carbon dioxide

Some
excreted
in urine
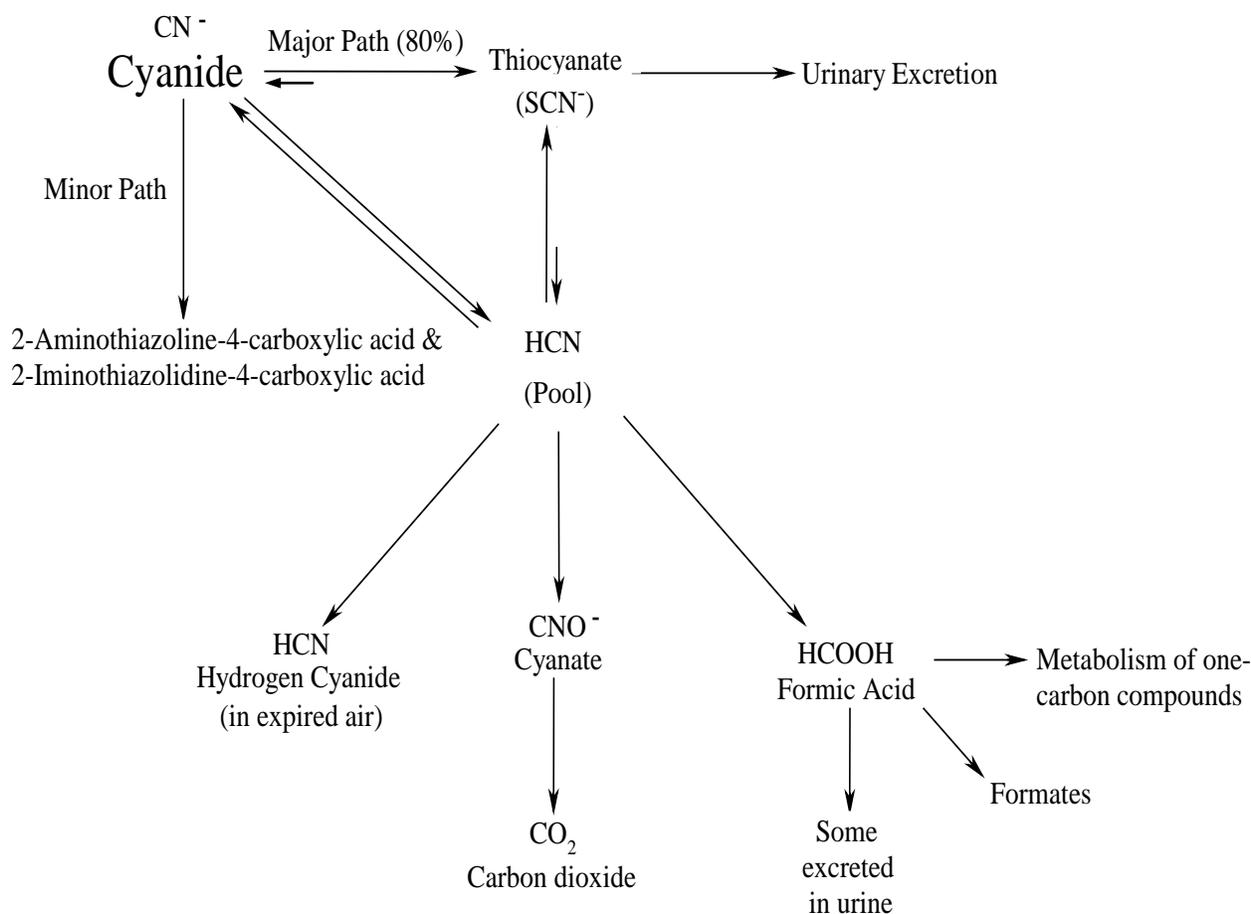
Source: Adapted from Ansell and Lewis (1970).

Figure 3-1. Cyanide primary metabolic pathways.

Metabolism of cyanide by rhodanese exhibits zero-order kinetics relative to cyanide; the concentration of sulfur-containing donor molecules is the rate-limiting factor. The primary endogenous sulfur donor is thiosulfate; others include glutathione and cystine. Schulz et al. (1982) evaluated the metabolism of cyanide in humans continuously infused with the hypotensive drug sodium nitroprusside, $Na_2[Fe(CN)_5NO]\cdot 2H_2O$, which completely releases the CN^- in the blood. The authors estimated that the detoxification rate of cyanide in humans

(in the absence of antidotes) is about 1 µg/kg-minute. McNamara (1976) estimated the detoxification rate in humans as 17 µg/kg-minute based on a study in men injected intravenously (i.v.) with HCN. Lawrence (1947, reported in an extended abstract) found that continuous i.v. infusion of NaCN into dogs at a rate of 0.013 mg/kg-minute (apparently as milligrams of CN^- but not explicitly stated) was "tolerated over 37 hours" and speculated that this rate of infusion could be tolerated indefinitely. Infusion rates of 0.028 mg/kg-minute or higher resulted in lethality. Based on these findings, the whole-body rate of cyanide detoxification in dogs can be estimated to be approximately 13 µg/kg-minute. The actual rate may be lower because the

portion of cyanide that remained unmetabolized at this dose may have been insufficient to cause lethality. These findings suggest that the rate of cyanide detoxification in humans and dogs may be similar.

Devlin et al. (1989a) evaluated rhodanese activity in rat liver and skeletal muscle. Using histochemical staining techniques, the authors determined that only low levels of rhodanese activity were present in the blood vessels. In contrast, high levels of rhodanese activity were detected in the liver and skeletal muscle. Although the activity of rhodanese in muscle was lower than in the liver, the authors concluded that the total skeletal muscle mass makes a significant contribution to whole-body metabolism of cyanide. In a follow-up study in perfused liver and hind-limb muscle, Devlin et al. (1989b) observed that the liver cleared 80% of the available cyanide compared to 18% for the hind limbs. However, when the hind-limb data were extrapolated to total muscle mass, muscle cleared cyanide 2.6-fold faster than did liver in the absence of exogenous thiosulfate. When thiosulfate was included in the perfusion medium, liver clearance was dependent on flow rate, but muscle tissue clearance was unaffected. Westley (1981) found that purified bovine liver rhodanese has a high turnover rate of almost 20,000/minute in vitro (i.e., 1 mol of rhodanese could convert 20,000 mols of cyanide to thiocyanate in 1 minute). This high turnover rate, coupled with the basal amount of rhodanese in the liver and other tissues, means that the rate of cyanide metabolism should not depend critically on the enzyme content of the tissue. Therefore, the limiting factor for cyanide metabolism is the availability of the sulfur donor rather than the rhodanese metabolic capacity. Similarly, differences between muscle and liver in ability to detoxify cyanide appear to be related to the availability of sulfur donors.

Lewis et al. (1991) observed the presence of rhodanese in the epithelium of human nasal tissue. Rhodanese activity in human nasal epithelium was higher in nonsmokers than smokers. Individual enzyme kinetic data (V_{max} and K_m) suggested that decreased activity in smokers may be due to decreased affinity. In kinetic studies with adequate sulfur present, rhodanese in human nasal tissue exhibited a higher affinity (lower K_m) for cyanide and a lower maximum velocity (lower V_{max}), compared to rhodanese in human liver. Human rhodanese exhibited a higher K_m and lower V_{max} than did rat rhodanese. Dahl (1989) also found that rat nasal tissue exhibited high levels of rhodanese activity, particularly in the olfactory region, which had almost sevenfold more activity on a per milligram mitochondrial protein basis than did rhodanese in rat liver. Rhodanese activity was also observed in the respiratory tracts of dogs, particularly in the nasal cavity (Aminlari et al., 1994).

The tissue distribution and activity of rhodanese is highly variable among species. In dogs, Himwich and Saunders (1948) observed that the highest activity of rhodanese was observed in the adrenal glands, followed by the liver. The brain, spinal cord, kidneys, and testes also had large amounts of rhodanese. In general, monkeys, rats, and rabbits had much higher rhodanese activity (mg CN^- converted to SCN^- per g tissue) than dogs, with the liver and

kidneys containing the highest activity. Drawbaugh and Marrs (1987) also studied the tissue distribution of rhodanese in several species, including marmosets, rats, hamsters, rabbits, guinea pigs, dogs, and pigeons. The highest rhodanese activities were found in rats, hamsters, and guinea pigs; the lowest were found in pigeons, marmosets, and dogs. Except for rabbits, rhodanese activity was higher in the liver than in the kidneys of the species studied. However, the authors noted that the biological significance of species differences in rhodanese activity is unclear.

The study by Drawbaugh and Marrs (1987) has been used to suggest that the dog is not an appropriate animal model for cyanide toxicity in humans, due to significantly lower levels of rhodanese in this species as compared with humans. However, as noted above, the amount of sulfur donor, not the amount of rhodanese itself, is the rate-limiting factor for detoxification of cyanide by rhodanese, even at bolus doses resulting in high acute toxicity. Schulz (1984) reported that the rate of cyanide detoxification in humans is slower than the rate in rodents or dogs despite the higher levels of rhodanese in humans. Other data (McNamara, 1976) suggest that the cyanide detoxification rate in humans is slightly higher than in dogs. Furthermore, urinary concentrations of thiocyanate have been shown to be higher in dogs than rats (NTP, 1993; Kamalu, 1993). Lower thiocyanate levels would be expected if metabolism via the rhodanese pathway were limited in this species. Although this analysis did not normalize by urine specific gravity or other factors, it suggests that cyanide was metabolized to a similar degree in dogs and rats.

Chronic exposure to cyanide resulted in increased rhodanese levels in rabbits, suggesting that rhodanese is inducible, at least in this species (Okolie and Osagie, 1999). Alternatively, the increased levels could be due to other factors, such as increased protein stability. Data were not located regarding whether chronic exposure increases rhodanese levels in other species.

Several polymorphisms in rhodanese have been identified in human populations, although only a minimal effect on cyanide detoxification was detected (Billaut-Laden et al., 2006). A second enzyme that converts cyanide to thiocyanate is mercaptopyruvate sulfurtransferase (MPST). This enzyme differs from rhodanese in that it catalyzes the transfer of sulfur from an organic thiol to cyanide (Wing and Baskin, 1992). Therefore, this enzyme breaks a carbon-sulfur bond to facilitate transfer of sulfur to cyanide, whereas rhodanese breaks a sulfur-sulfur bond. MPST is most active at pH 9.5, while rhodanese is most active at pH 8.6, which is closer to physiological pH. MPST also appears to have a different tissue distribution from that of rhodanese; this enzyme has been reported as being located in the RBCs and kidneys. MPST is located in both the mitochondria and the cytosol, making it more accessible for conversion of cyanide than rhodanese, which occurs only in the mitochondria (Wing and Baskin, 1992). Support for the role of MPST in cyanide detoxification was provided in in vitro studies by Huang et al. (1998). These authors demonstrated that addition of L- or D-cysteine to hepatocytes in cell culture prevented cyanide cytotoxicity and enhanced the formation of

thiocyanate. Mercaptopyruvate and thiocystine, metabolites of L- and D-cysteine, are substrates of MPST. Huang et al. (1998) observed that, when formation of these metabolites in isolated hepatocytes was prevented, the formation of thiocyanate was also inhibited. However, it is not clear whether MPST directly transfers sulfur to cyanide or whether it acts indirectly by transferring sulfur to albumin in the liver. The modified albumin could then be excreted to form a sulfane-sulfur pool that is available to react with cyanide via rhodanese (Wing and Baskin, 1992).

Although the reaction of rhodanese with cyanide is irreversible, thiocyanate can be converted back to cyanide and sulfate by the action of thiocyanate oxidase located in the RBCs, lymphocytes, mammary gland, and thyroid (Wood, 1975). Thiocyanate oxidase has been found in the erythrocytes of humans, dogs, rabbits, and rats (Goldstein and Rieders, 1953). This enzyme catalyzes the reaction of hydrogen peroxide and thiocyanate to form cyanide and sulfate. In addition, these enzymes produce an intermediate oxidation product of thiocyanate, the $OSCN^-$ ion known as hypothiocyanate, which reacts with cyanide to form cyanate (CNO^-), which is then hydrolyzed to ammonia and carbon dioxide (Wood, 1975).

The minor pathway shown in Figure 3-1 involves the spontaneous reaction of cyanide with cystine to yield 2-aminothiazoline-4-carboxylic acid, which tautomerizes to 2-iminothiazolidine-4-carboxylic acid. This pathway accounted for approximately 15% of the cyanide dose in a female rat receiving daily i.p. injections of NaCN for 8 days (Wood and Cooley, 1956). In another experiment in the same publication, the percentage metabolism via this pathway was higher when rats were injected i.v. with labeled cystine and subsequently were administered NaCN subcutaneously.

The mean blood thiocyanate level in smokers with untreated tobacco amblyopia (a condition causing visual defects that has been attributed to cyanide exposure) was significantly lower than the concentration in smokers overall, suggesting that people with this condition have a decreased ability to convert cyanide to thiocyanate (Pettigrew and Fell, 1973). Blood cyanide levels were low in both smokers and nonsmokers in this study, with no significant effect of smoking or tobacco amblyopia, perhaps because approximately 1–2 hours had elapsed between the time the last cigarette was smoked and when cyanide levels were measured. The authors suggested that the excess cyanide was bound up as cyanocobalamin (one form of vitamin B_{12}), but they did not investigate this hypothesis. Cyanide also reacts with methemoglobin (hemoglobin that has been oxidized either by normal metabolism or by xenobiotic oxidant stressors) in RBCs to form cyanomethemoglobin. Schulz (1984) noted that, theoretically, 1 g of methemoglobin can bind approximately 60 µmol of HCN and that 1 L of erythrocytes should be able to bind approximately 50–200 µmol (1.4–5.4 mg) HCN at physiological levels of methemoglobin (0.25–1%). This readily reversible reaction is considered to be a naturally occurring detoxification pathway for low levels of cyanide in the blood (Lundquist et al., 1985) and forms the basis of the first phase of treatment for acute cyanide poisoning, which consists of

administering amyl nitrite or sodium nitrite to cyanide-poisoned individuals (Klaassen, 2001). Amyl nitrite and sodium nitrite are oxidants that increase the conversion of hemoglobin to methemoglobin, thus providing a sink for CN^- to reduce interaction with tissue cytochrome c oxidase. Under this treatment approach, cyanide is then detoxified by slow release from cyanomethemoglobin and cytochrome c oxidase and subsequent conversion by the enzyme rhodanese to SCN^-, which has much lower acute toxicity than cyanide. Sodium thiosulfate administered as the second phase of treatment for acute cyanide poisoning accelerates detoxification by supplying a sulfur substrate for the reaction. Other substances used to detoxify cyanide include hydroxycobalamin (vitamin B_{12a}), an antidote used outside the United States that binds cyanide to form cyanocobalamin (vitamin B_{12}), and cobalt edetate, which is used as an antidote in some countries due to the high affinity of cobalt for cyanide (Klaassen, 2001).

3.4. ELIMINATION

Data in humans and animals indicate that cyanide is primarily excreted in the urine as thiocyanate following both inhalation and oral exposure. Smaller amounts are excreted as urinary cyanide or as HCN or carbon dioxide in exhaled air. Following occupational exposure to 0.19–0.75 ppm HCN, urinary thiocyanate levels in nonsmoking exposed workers were approximately 7 times the levels in nonsmoking controls (Chandra et al., 1980). Urinary cyanide levels were also elevated in the exposed workers, but they were approximately 2 orders of magnitude lower than thiocyanate levels.

Following a single subcutaneous injection of rats with [^{14}C] KCN, 89% of the excreted radioactivity was detected in urine within 24 hours; about 4% of the excreted radioactivity was expired in air, primarily as carbon dioxide (Okoh, 1983). The authors found that 71–79% of the urinary activity was in the form of thiocyanate. The excretion pattern was not affected by prior exposure to cyanide in diet for 6 weeks.

In a related study of rats injected i.p. with radiolabeled NaCN after being fed KCN in the diet at 77 μmol/day (approximately 5.5 mg/kg-day CN) for 3 weeks (Okoh and Pitt, 1982), 86% of the radioactivity in the expired air was present as carbon dioxide and 14% was present as HCN. Boxer and Rickards (1952) also observed that exhaled air contained both radiolabeled HCN and carbon dioxide after dogs were injected subcutaneously with radiolabeled NaCN. However, the primary path of excretion was still in the form of urinary thiocyanate, although small amounts of cyanide and cyanocobalamin were also found in the urine. Sylvester et al. (1983) treated dogs with i.v. NaCN and found that <1% of the total cyanide dose was eliminated through exhaled air.

Leuschner et al. (1991) evaluated the elimination of KCN following both acute and subchronic exposure. For the acute study, three male Sprague-Dawley rats were treated by gavage with 1 mg/kg KCN. Blood was collected at regular intervals for up to 1 hour following administration. A peak blood level of 6.2 nmol/mL (160 μg/L) CN^- was observed 2 minutes

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after treatment; by 60 minutes, the blood levels had dropped to the analytic detection limit. The authors calculated an elimination half-life of 14 minutes.

In the subchronic portion of Leuschner et al. (1991), male Sprague-Dawley rats (26–40/group) received KCN in their drinking water at doses of 0, 40, 80, or 160 mg/kg-day for 13 weeks. Blood was collected every 2 weeks for analysis of cyanide and thiocyanate levels. Urine was collected over a 16-hour period during weeks 6 and 13 of the study to determine cyanide and thiocyanate levels. Similar patterns of excretion were observed for both urinary levels of cyanide and thiocyanate. A dose-response relationship was observed for the concentration of both cyanide and thiocyanate in urine, and a small amount of thiocyanate was observed in the urine of the controls. The levels of cyanide in the urine were much lower than the thiocyanate levels; the ratio of cyanide to thiocyanate was about 1 to 1,000. Approximately 11% of the administered cyanide was eliminated per day as urinary thiocyanate during the dosing period, while only about 0.003% was excreted per day unchanged. The study authors did not report how they estimated the percent of total dose eliminated; radiolabeled material was not used. Elimination half-life was not calculated for the subchronic study. Blood levels of cyanide and thiocyanate were fairly consistent with time. Some elimination may have occurred as exhaled HCN or carbon dioxide, but data indicate that this route accounts for <10% of a dose of cyanide following acute dosing (Okoh, 1983). The study authors (Leuschner et al., 1991) also noted that the percent of administered cyanide excreted via the urine was unchanged between weeks 6 and 13, indicating that detoxification pathways were not saturated and the mode of cyanide excretion was not affected over this period.

In cynomolgus monkeys exposed via inhalation for up to 30 minutes to approximately 100–170 mg/m^3 HCN, levels of cyanide in the blood remained nearly constant after approximately the first 10–15 minutes of exposure and for 60 minutes following termination of exposure (Purser et al., 1984). Thus, the half-life under these conditions was longer than in the Leuschner et al. (1991) gavage study in rats. The difference may have been due to saturation of metabolism, first-pass metabolism following oral exposure, or species-related differences.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A pharmacokinetic analysis of the distribution and metabolism of cyanide was conducted in dogs following a single i.v. dose (Sylvester et al., 1983). Dogs (six/group) were administered i.v. saline, NaCN (20.4 µmol/kg), or sodium thiocyanate (12.3 µmol/kg); cyanide concentration was determined in whole blood, and thiocyanate concentration was determined in plasma. Blood levels of CN$^-$ and SCN$^-$ measured after administration were used to develop a pharmacokinetic model in dogs. The conversion of cyanide to thiocyanate was found to follow first-order kinetics. Three hours following i.v. dosing with cyanide, 90% of the total dose had been converted to thiocyanate. The half-life of thiocyanate was determined to be 29 hours. The

authors also found that <8% of the cyanide dose was eliminated through non-thiocyanate routes and only 1% of the total cyanide dose was eliminated through exhalation.

Additionally, some data exist on the comparative toxicokinetics of cyanide and thiocyanate in several species (Sousa et al., 2003). Rats (n = 42), pigs (n = 6), and goats (n = 6) were studied up to 24 hours after a single gavage dose of 3.0 mg/kg KCN. Cyanide was quickly absorbed in all species. The peak plasma concentration of cyanide was highest in goats, followed by rats and pigs. Goats also had the highest volume of distribution, highest area under the curve (AUC), and slowest elimination compared with the other two species. The similarities in absorption data between species indicated that pH differences between the monogastric stomachs of rats and pigs (pH 1–2) and ruminant stomachs (pH 6.8) did not noticeably impact absorption of cyanide. Toxicokinetic parameters for thiocyanate indicated the peak plasma concentrations and AUC to be greatest in rats, followed by goats and pigs. Blood levels of cyanide in each species indicate rapid decreases in cyanide blood concentration by 3 hours following dosing, with the half-life of elimination for thiocyanate for all species about 9–11 times longer.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Acute Oral, Inhalation, and Dermal Studies

The effects of acute, high-level exposure to cyanide (CN^-) are well characterized (reviewed in ATSDR, 2006; IPCS, 2004; U.S. EPA, 1992; and Hall and Rumack, 1990). Although acute oral doses of cyanide cause cardiovascular, respiratory, and neurophysiological changes, the brain appears to be the organ most sensitive to acute cyanide toxicity (IPCS, 2004). Several studies of the acute effects of cyanide in humans, following suicide attempts or accidental poisoning by the oral and inhalation routes, provide additional details, although most such studies include only a limited characterization of exposure. Symptoms of severe cyanide poisoning include vomiting, nausea, weakness, confusion, lethargy, cyanosis, weak and ataxic movements, increased respiratory and heart rates progressing to coma with respiratory depression, seizures, cardiovascular collapse, and death. The principal feature of the acute toxicity profile for cyanide includes lethality by all routes of administration, with a steep rate-dependent dose-response curve. Death from cyanide poisoning is believed to result from central nervous system (CNS) depression, subsequent to inhibition of brain cytochrome oxidase activity (Way, 1984). The toxic effects and lethality associated with acute exposure to CN^- in humans and animals are generally similar and are believed to result from inactivation of cytochrome oxidase and inhibition of cellular respiration during the terminal reaction of the electron transport chain. This inhibition prevents the formation of adenosine triphosphate (ATP) via oxidative phosphorylation. The IPCS (2004) noted that the lowest reported oral lethal dose in humans is 0.54 mg/kg body weight; the average absorbed dose at the time of death was estimated at 1.4 mg/kg body weight (calculated as hydrogen cyanide [HCN]). Rapid recovery from relatively low, short-term inhalation exposures often occurs once the exposed individual is moved to fresh air. Individuals suffering from higher oral and inhalation exposures may benefit from supplemental oxygen and the use of antidotes. Oral exposure results in slower absorption, passage to the liver, and faster detoxification. If the patient responds to treatment and survives, recovery is usually prompt and complete; however, delayed neurological symptoms, including neuropsychiatric manifestations and Parkinson-type disease, can occur (IPCS, 2004). Exposure to lower levels can cause flushing, light-headedness, dizziness, headache, and other symptoms indicative of hypoxia (Wolfsie and Shaffer, 1959).

Liebowitz and Schwartz (1948) reported a case of cyanide poisoning following ingestion of an estimated 38–63 mg/kg of potassium cyanide (KCN) (15–25 mg/kg CN^-). The patient was comatose with muscular rigidity and a thready pulse on admission. By 8 hours after admission, the patient was alert and the symptoms had begun to subside (with the exception of weakness,

nausea, and an enlarged heart). The authors suggested that the reason that the patient recovered from exposure to a dose that was about 30 times the estimated lethal dose may have been because he was a chemist who frequently immersed his hands in thiosulfate. Therefore, exposure to thiosulfate may have had an antidotal effect.

Several authors (Grandas et al., 1989; Rosenberg et al., 1989; Carella et al., 1988; Uitti et al., 1985) reported the development of symptoms of Parkinsonism in patients who recovered from ingestion of a single dose of cyanide. The four cases included an 18-year-old male who ingested 5.6–7.6 mg/kg cyanide in a suicide attempt (Uitti et al., 1985), a 46-year-old woman who ingested an unreported amount of cyanide by accidental poisoning (Carella et al., 1988), a 46-year-old man who ingested 8.6 mg/kg cyanide in a suicide attempt (Rosenberg et al., 1989), and a 39-year-old man who ingested an unknown amount of cyanide in a suicide attempt (Grandas et al., 1989). In all cases, the patients recovered from the acute symptoms of cyanide poisoning with treatment, and neurologic examinations immediately following the poisoning were normal. Follow-up neurologic examination at times of 3 weeks (Rosenberg et al., 1989), 4 months (Uitti et al., 1985), or 1 year (Grandas et al., 1989; Carella et al., 1988), however, revealed that the patients had developed symptoms of parkinsonism, including generalized rigidity, bradykinesia, tremors of tongue and eyelids, slow-shuffling gait, and a weak dysphonic voice. A computerized tomography scan or magnetic resonance imaging showed lesions in the putamen and globus pallidus regions of the brain (Grandas et al., 1989; Rosenberg et al., 1989; Carella et al., 1988; Uitti et al., 1985). Similarly, Lam and Lau (2000) reported mild impairment of recent memory and concentration, which was confirmed by neurological testing, a year after a 19-year-old woman experienced an episode of acute inhalation exposure to cyanide.

Potter (1950) reported a case history of a worker accidentally exposed via inhalation to an undetermined concentration of HCN. Early symptoms were dizziness, dyspnea, and weakness of the legs followed by a period of deep unconsciousness accompanied by absent reflexes, stertorous respiration, rapid pulse, fixed and unreactive pupils, and convulsions. The subject recovered with treatment, and no after-effects were reported. In another case report, a worker was found unconscious, lying in tank sludge after working without protective gear in a plating tank containing silver-cyanide sludge (Singh et al., 1989). The duration of exposure was unknown, but the tank air was later measured to contain 200 ppm HCN (220 mg/m^3 HCN). He had dermal evidence of chemical burns and was not breathing, with a rapid pulse, fixed and dilated pupils, and no recorded blood pressure or response to pain. Blood cyanide was 804 µmol/L within 0.5 hours of hospital arrival and decreased to 15 µmol/L at 18 hours after arrival and following detoxification efforts. The patient died within 3 days despite extensive treatment.

Cyanide is readily absorbed through the skin (Lam and Lau, 2000; Potter, 1950); therefore, systemic toxicity can readily result from dermal exposure to cyanide fumes or direct dermal contact with HCN. A case report of a worker accidentally exposed to a brief stream of

liquid HCN on his hand (amount not specified) reported that the worker became deeply unconscious within 5 minutes of exposure (Potter, 1950). Breathing was hoarse, his face was flushed, and reflexes were absent. The subject recovered with sodium nitrite and sodium thiosulphate treatment.

Dizziness, weakness, and a throbbing pulse were reported when three workers wearing gas masks entered an atmosphere containing 2% HCN gas (Drinker, 1932). These effects were attributed to the dermal absorption of the gas. The men developed symptoms of dizziness, weakness, and throbbing pulse after about 10 minutes of exposure and eventually became unconscious. The symptoms persisted for several hours following exposure.

Relatively mild symptoms of cyanide poisoning (flushing, dizziness, headache, throat discomfort, chest tightness, skin itchiness, and eye irritation) were reported in firefighters who were wearing self-contained breathing apparatus when they responded to a HCN gas release (Lam and Lau, 2000). The effects were attributed to dermal cyanide absorption and direct contact with skin and eyes.

4.1.2. Subchronic and Chronic Oral Studies

No subchronic or chronic dose-response studies of human exposure to cyanide by the oral route were located. However, a number of studies have examined populations exposed to cyanogenic compounds in foods, particularly cassava root, which can be dried and ground into flour and is a primary source of carbohydrate in many parts of Africa and Southeast Asia (Bonmarin et al., 2002; Okafor et al., 2002; Makene and Wilson, 1972). Due to concern about effects of cyanogenic compounds, many of the recent studies of chronic cyanide toxicity have been conducted in the developing world, where exposure to cyanogenic compounds in food is a significant public health and agricultural (for livestock) concern. Symptoms reported in these populations include ataxic tropic neuropathy, spastic paraparesis (paralysis, particularly of the lower extremities), optic atrophy, and decreased nerve conduction velocity. Effects seen in these studies are often confounded by dietary deficiencies (particularly low dietary intake of protein, vitamin B_{12}, and/or iodine) and overall malnutrition. In addition, animal studies comparing the effects of cassava ingestion and ingestion of cyanide indicate that some of the observed effects are due to compounds besides cyanide in cassava (Banea-Mayambu et al., 1997; Kamalu, 1993; Olusi et al., 1979). Because of the confounding factors of dietary deficiencies in the studied populations and the presence of other potentially toxic compounds in cassava, human and animal studies of cassava are of limited use for the hazard assessment of cyanide.

4.1.3. Subchronic and Chronic Inhalation Studies

Several reports of occupationally exposed workers indicate that chronic exposure to low concentrations of cyanide can cause alterations of thyroid function and neurological symptoms (Banerjee et al., 1997; Blanc et al., 1985; El Ghawabi et al., 1975). Occupational exposure to

cyanide occurs primarily via inhalation, although dermal and limited oral exposure also can occur. HCN is noted for its systemic toxicity, which would be expected to occur at concentrations below those at which any direct respiratory tract effects would be anticipated.

El Ghawabi et al. (1975) evaluated the effects of long-term occupational exposure to cyanide in 36 male workers employed in the electroplating sections of three factories in Egypt. Cyanide exposure was from a plating bath that contained 3% copper cyanide, 3% sodium cyanide (NaCN), and 1% sodium carbonate. Individual breathing zone air samples were taken to determine the levels of airborne cyanide to which the men were exposed. Fifteen-minute air samples were collected by using a Midget impinger. Twenty male volunteers of the same age group and socioeconomic status who had no occupational exposure to cyanide were chosen as controls. Information on how or from where the controls were recruited was not provided. None of the exposed or control workers were cigarette smokers at the time of the study. Participants were prohibited from ingesting cyanide-containing foods during the course of the investigation. Cyanide-exposed workers and controls were given medical examinations (with special focus on thyroid abnormalities), interviewed regarding medical history, and questioned regarding symptoms experienced. Thyroid function (as measured by uptake of radiolabeled iodide) was assayed, and urinary levels of SCN^- were recorded over a 2-month period and reported as average daily excretion (in mg). No investigation of thyroid hormone levels was reported. Of the 36 workers, 14 had been exposed for 5 years, 14 for 5–10 years, 7 for 10–15 years, and 1 for >15 years. The mean and median exposure times for the worker population were not reported. The mean cyanide air concentrations in the breathing zones of workers at each of the three plants were 10.4, 6.4, and 8.1 ppm (11.5, 7.1, and 8.9 mg/m^3, respectively, with a range of 4.2–12.4 ppm (4.6–13.7 mg/m^3) HCN. The authors reported that workers were exposed to other chemicals during the electroplating process (e.g., gasoline, alkali, and acid), although concentrations to these other chemicals were not quantified. Urinary SCN^- concentrations from exposed workers were measured during 2 successive months. Graphically presented data of mean individual urinary SCN^- levels plotted against the concentration of HCN in the air indicated a strong positive linear relationship between urinary SCN^- and HCN concentration in the air (Figure 4-1).
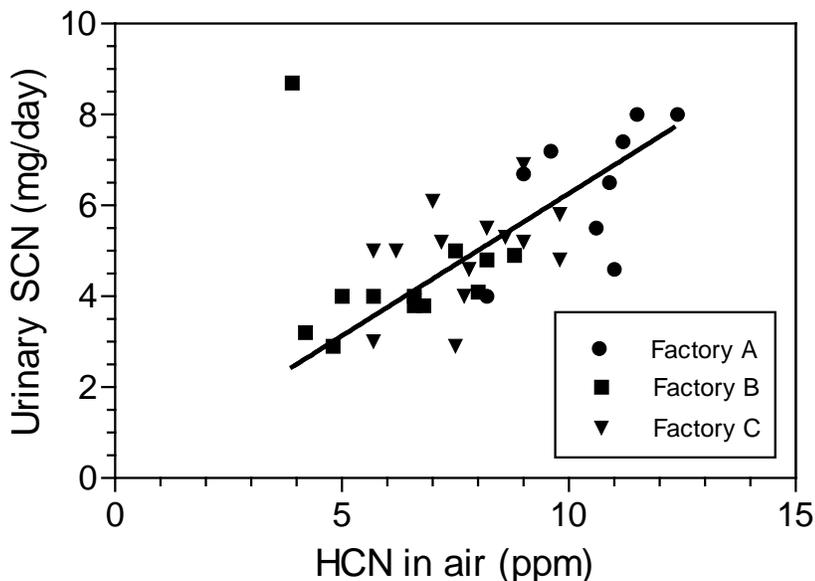
Source: El Ghawabi et al. (1975).[1]

Figure 4-1. Urinary SCN⁻ of exposed workers plotted against individual breathing concentrations of HCN.

Twenty of the 36 exposed workers (56%) had thyroid enlargement rated as being mild to moderate; however, there was no correlation between duration of exposure and either incidence or magnitude of enlargement. The authors reported that none of the workers showed clinical symptoms of either hypo- or hyperthyroidism, although the basis of this assessment was not provided. Radioactive iodine uptake measured following a 2-day break in HCN exposure indicated statistically significantly elevated iodide uptake after 4 hours (38.7 compared to 22.4%) and 24 hours (49.3 compared to 39.9%) as compared with controls (Table 4-1). In a separate assay, blood samples taken 72 hours after administration of the iodide tracer indicated that protein-bound ^{131}I in the blood was similar between controls and workers (0.11 ± 0.041 compared to 0.12 ± 0.039).

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[1]Reproduced by EPA from a graph published in El Ghawabi et al. (1975) by estimation of original data points and regeneration of graph. EPA's reproduced linear regression line had a slope of 0.63 compared to the slope of 0.65 reported in the published study and a statistically significant Pearson correlation coefficient of 0.5 ($p < 0.001$).

Table 4-1. Thyroid uptake of ^{131}I in electroplating workers

	After 4 hrs	After 24 hrs
Percent ^{131}I thyroid uptake (mean ± standard deviation [SD])		
Controls (n = 20)	22.42 ± 7.21	39.95 ± 4.80
Exposed (n = 36)	38.722 ± 6.63[a]	49.33 ± 10.61[a]

[a]Significant difference ($p < 0.001$) by Student's t-test.

Source: El Ghawabi et al. (1975).

The authors noted that the radioactive iodide uptake test was conducted after the workers had been away from work for 2 days, allowing time for thiocyanate (SCN⁻), with a 3-day elimination half-life (as established by Schulz et al. [1979]), to be partially cleared from their systems. The authors suggested that the sudden cessation of cyanide exposure may have caused the thyroid gland to rapidly accumulate iodine. Increased 24-hour uptake of radioactive iodide has been reported to occur in hyperthyroidism, iodine deficiency, and goiter (NLM, 2008a; Ravel, 1995).

Other findings noted in this study included significantly higher hemoglobin (14.8 vs. 13.4 g/dL) and lymphocyte counts (42 vs. 30%) and punctate basophilia of erythrocytes in 78% of workers. The authors indicated that the observed punctuate basophilia was not characteristic of HCN exposure and may be related to other concomitant chemical exposures. Symptoms reported more frequently in the exposed workers than in the controls included (in decreasing order of frequency) headache, weakness, and changes in the senses of taste and smell. Incidences of symptoms at individual plants were not reported, and no evaluation of symptoms by exposure concentration was presented. Based on the observed thyroid effects, the lowest mean concentration recorded in the three factories of 6.4 ppm (7.07 mg/m^3) HCN was designated as a lowest-observed-adverse-effect level (LOAEL) for this review.

An unpublished study by Leeser et al. (1990) compared the health of 63 male cyanide salt (NaCN, KCN, and Cu(CN)$_2$) production workers with a control group of 100 British workers from a diphenyl oxide (DPO) plant in a cross-sectional study. Cyanide workers were exposed for periods ranging from 1 to 32 years with a mean exposure duration of 12.6 years. Air cyanide was monitored with static floor monitors that would set off an alarm at ≥11 mg/m^3 (the floor monitor alarms were never triggered), with Draeger pump tests of area samples, and with personal monitoring. Personal samples were collected on four to five occasions on different people for each of the eight job categories in NaCN production (34 samples total). The geometric means for the eight job categories ranged from 0.03 to 1.05 mg/m^3 cyanide. Draeger pump samples were reported to range from 1.1 to 3.3 mg/m^3 cyanide. Blood samples were collected from workers to measure hematological parameters and serum levels of cyanide, carboxyhemoglobin, vitamin B$_{12}$, and thyroxine (T$_4$). Each cyanide worker had a complete

medical examination and was given a self-administered questionnaire that included questions addressing 15 symptoms (feeling unwell, gaining weight, losing weight, pain in chest, short of breath, headaches, smell problem, sleep problem, hand shake, lacking energy, dizzy spells, nausea, indigestion, nose bleeds, and taste problem). Analysis of continuous variables (e.g., T_4, hematological parameters) was conducted using linear regression adjusting for age, alcohol use, and smoking status; body mass index (kg/m^2) was also included in the models for blood pressure. The authors noted that log-transformation was used for some of the dependent variables (but did not specify which variables). Results of two sets of questionnaires (given at different time periods) showed that cyanide workers had more self-reported symptoms than the control group (66.6 vs. 50.0% reporting one or more symptom). Symptoms with a greater frequency in the cyanide group than in the control group included gaining weight (25.4 vs. 12.0%), shortness of breath (14.3 vs. 7.0%), headaches (6.4 vs. 3.0%), smell problems (9.5 vs. 3.0%), sleep problems (12.7 vs. 8.0%), shaky hands (6.4 vs. 1.0%), lack of energy (14.3 vs. 5.0%), dizzy spells (7.9 vs. 2.0%), nausea (3.2 vs. 0%), and taste problems (3.2 vs. 1.0%). The difference in sleep problems was attributed to differences in the proportion of shift workers in the two groups (89 and 43%, respectively, in cyanide and control worker groups).

Mean (± standard error [SE]) cyanide levels of blood collected prior to the block of shifts were higher in nonsmoking exposed workers than in non-smoking controls (3.32 ± 1.25 vs. 1.14 ± 1.11 µmol/100 mL), and in ex-smokers (2.16 ± 1.13 vs. 1.46 ± 1.10 µmol/100 mL), but not among current smokers (2.94 ± 1.11 vs. 3.14 ±1.11 µmol/100 mL). Thus, the blood cyanide levels in nonsmoking exposed workers were similar to that of smokers. Blood SCN^- was measured, but the authors reported that it could not be analyzed due to unspecified technical reasons. Mean hemoglobin levels in cyanide workers were statistically significantly increased compared to controls (15.57 ± 0.14 vs. 15.08 ± 0.10 g/dL). Ratios associated with hemoglobin, such as mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also statistically significantly elevated, although the difference in these parameters was low (about 3%). Lymphocytes were statistically significantly elevated in cyanide workers compared to controls (mean 2.87 ± 0.11 vs. 2.55 ± 0.08 × 10^9/L). Serum T_4 levels in cyanide exposed workers were decreased in controls, but the difference was not statistically significant according to the study authors (mean 85.13 ± 2.51 vs. 89.04 ± 1.81 nmol/L). The number of workers for which serum T4 was measured was not reported; therefore, the lack of statistical significance could not be confirmed for this review. Serum T_4 was below the clinical reference range (60–160 nmol/L) in 3 cyanide-exposed workers.. The authors claimed that these three workers were otherwise normal and other thyroid functional tests showed that there was no functional problem, although the authors did not state which additional tests were conducted to confirm normal thyroid function. A LOAEL of 1 mg/m^3 cyanide for increased lymphocyte count and increased hemoglobin concentration was established for this

review. A no-observed-adverse-effect level (NOAEL) for thyroid effects was not identified for this study based on the lack of measurement of sensitive thyroid parameters.

A group of 36 male workers who had been exposed to HCN fumes in a silver-reclaiming facility in Illinois were retrospectively studied by Blanc et al. (1985), following the death of one employee from cyanide overexposure and the closure of the factory due to health and safety violations. The authors attempted to recruit all previous employees. In this study, data collection for the former workers included physical examinations (including examination of neurological effects), serum biochemistry, hematology, urinalysis, serum enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), and thyroid hormone analysis (thyroid-stimulating hormone [TSH] and T_4), and a questionnaire designed to determine exposure, symptoms during employment, and current symptoms. Workers were qualitatively categorized into low-, moderate-, or high-exposure groups based on their primary job activities. The median time elapsed since last employment at the facility was 10.5 months; the median duration of employment was only 8.5 months. Environmental monitoring conducted the day after the plant was shut down found that the 24-hour time-weighted average (TWA) exposure was 15 ppm (16.6 mg/m^3) HCN. None of the former workers were found to have palpable thyroid gland abnormalities, mucosal erosion, or focal neurological deficits. Clinical tests revealed decreases in the absorption of vitamin B_{12} (a possible factor protecting against cyanide toxicity) and decreased folate levels that may have been secondary to the decrease in vitamin B_{12}. Serum TSH levels were also reported as being significantly elevated in workers relative to laboratory controls (concurrent controls were not used in this study design). Triiodothyronine (T_3) uptake in the highest exposed workers (n = 9) was statistically significantly elevated compared to that in laboratory controls (Table 4-2). The authors reported that this elevation may reflect a postinhibitory response.

Table 4-2. Thyroid parameters in former silver-reclaiming workers

Population	Percent T_3 uptake[a]	TSH[a] (µU/mL)
Laboratory controls (n = 100)	30.0 ± 2.8	1.7 ± 1.2
All workers (n = 33)	30.9 ± 2.6	2.2 ± 1.6[b]
Low-exposure workers (n = 24)	30.4 ± 2.3	2.2 ± 1.7
High-exposure workers (n = 9)	32.4 ± 2.4[c]	2.4 ± 1.3

[a]Values are mean ± SD.
[b]Statistically significant by Student's t-test at $p < 0.05$ compared to laboratory controls.
[c]Statistically significant by Student's t-test at $p < 0.01$ compared to laboratory controls.

Source: Blanc et al. (1985).

A statistically significant positive trend for self-reported weight loss was demonstrated against the exposure index, supporting an exposure-response relationship. A statistically

significant trend was also found between the incidence of symptoms reported during active employment (headache, dizziness, nausea, and bitter almond taste), as well as those reported at the time of the survey (after adjustment for time elapsed since exposure) and the qualitative index of exposure, providing evidence of another exposure-response relationship. Some of the symptoms were reported as persisting for ≥ 7 months following exposure termination. The reported CNS effects suggest the occurrence of neurotoxicity associated with exposure to cyanide or its metabolite, thiocyanate. Dermal exposure to cyanide was reported by half of the workers, and additional exposure by ingestion was likely due to poor general hygiene in the factory in addition to inadequate personal protective equipment and worker training. Because there were multiple possible routes of cyanide exposure, including dermal exposure and contamination of food, data do not support for the selection of a LOAEL for inhalation. This study does demonstrate, however, the occurrence of nontransient effects of thyroid function (as measured by percentage of T_3 uptake) from occupational exposure to HCN.

In a study of electroplating workers in a factory in India, Banerjee et al. (1997) compared levels of the thyroid hormones T_3, T_4, and TSH in 35 male workers who had been exposed to cyanide via inhalation for >5 consecutive years to a randomly selected control group of 35 unexposed male workers matched for age and dietary habits. None of the subjects used tobacco products or had a prior history of thyroid disease. No environmental monitoring data were provided on HCN levels in the factory. However, serum SCN^- levels, a measure of internal dose, were reported in both workers and controls. The average serum thiocyanate level in the exposed workers was 316 µmol/L compared with 90.8 µmol/L in the controls, a difference that was statistically significant ($p < 0.01$). The exposed workers had significantly lower levels of T_3 and T_4 (48 and 37% lower, respectively) and significantly higher levels of TSH (142%) compared with controls (Table 4-3). In addition, there was a significant negative correlation between serum T_4 and thiocyanate concentrations (r = -0.363, $p < 0.05$) and a significant positive correlation between TSH and thiocyanate concentrations (r = -0.354, $p < 0.05$). There was also an apparent negative correlation between T_3 and thiocyanate (r = -0.245), but this difference was not statistically significant. Levels of T_3 and T_4 in exposed workers were outside the reported normal range for these endpoints, indicating a potentially clinically relevant alteration of thyroid hormone levels.

Table 4-3. Thyroid parameters in HCN-exposed and unexposed electroplating workers

	SCN⁻ (µmol/L)[a]	T$_4$ (µg/dL)[a]	T$_3$ (µg/dL)[a]	TSH (µU/mL)[a]
Controls (n = 35)	90.8 ± 9.02	6.09 ± 0.601	111.0 ± 9.3	1.2 ± 0.301
Exposed (n = 35)	316 ± 15.0[b]	3.81 ± 0.3181[c]	87.2 ± 8.1[c]	2.91 ± 0.201[c]

[a]Values are mean ± SD.
[b]Statistically significant by Student's t-test at $p < 0.01$ compared to unexposed workers.
[c]Statistically significant by Student's t-test at $p < 0.05$ compared to unexposed workers.

Source: Banerjee et al. (1997).

As part of a report on excretion of cyanide and its metabolites, Chandra et al. (1980) reported on a group of 23 electroplating workers chronically exposed to HCN fumes at 0.2–0.8 mg/m^3, with a mean value of 0.45 mg/m^3. The title of this report indicated that workers were exposed chronically, although exposure durations were not provided. The concentration in the breathing zone was reported as 0.1–0.2 mg/m^3, with a mean of 0.15 mg/m^3. The authors noted that the workers complained of symptoms typical of cyanide poisoning but provided no additional information on specific symptoms or further analysis. In the absence of further information, no independent assessment of this study is possible.

Chatgtopadhyay et al. (2000) investigated the effect of exposure to cyanide fumes on pulmonary function in workers at a metal-tempering plant. The authors evaluated 24 workers in an initial assessment and conducted a follow-up study on 17 of these workers 2 years later. The control group for the initial study consisted of 14 unexposed workers matched for socioeconomic status and race. The follow-up study did not include a concurrent control group; data from the control group in the initial assessment were used for comparison. No information was provided on cyanide concentrations in the air. The mean duration of exposure was 21.0 ± 5.03 years at the time of the first assessment. In the initial study, there were statistically significant decreases in pulmonary function as assessed by reduced peak expiratory flow rate, forced vital capacity (FVC), and forced expiratory volume (FEV) in 1 second as a percentage of FVC (FEV$_{1\%}$); decreases in other pulmonary function parameters were not statistically significant. In the follow-up study, statistically significant decreases were observed in all measured pulmonary function parameters. However, concurrent controls were not utilized in the follow-up study. Furthermore, adjustments for smoking and other co-occurring chemical exposures as sources of potential confounding were not conducted.

Population-based studies examining inhalation of cyanide at ambient levels and potential health outcomes are limited. However, studies examining smokers, a subgroup with higher inhalation exposure to cyanide through tobacco smoke, have indicated an association between smoking and thyroid disorders. Specifically, a meta-analysis of eight studies found a statistically significant association between smoking and the development of goiter in women (odds ratio =

1.29, 95% confidence interval [CI] 1.01–1.65) (Vestergaard, 2002). A more recent epidemiological study of a population in an industrialized area of Germany with relatively low intake of iodine has indicated that SCN^- urinary excretion is a cofactor or indicator for goiter in nonsmokers as well as in smokers. These same authors found urinary ratios of iodide to SCN^- to be predictive of increased risk for development of goiter as compared to iodide status alone (Brauer et al., 2006). This study population was believed to have high exposure to HCN due to industrial activities in the area, although exposure levels of HCN were not presented.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Studies

The National Toxicology Program (NTP, 1993) reported the results of a subchronic bioassay of NaCN administered in drinking water to rats and mice. F344 rats were administered NaCN in drinking water at concentrations of 0, 3, 10, 30, 100, or 300 ppm for 13 weeks. These concentrations are equivalent to the following doses, estimated by the study authors and based on measured body weights and water consumption (converted to CN^- equivalents for this assessment): 0, 0.16, 0.48, 1.4, 4.5, or 12.5 mg/kg-day CN^-, respectively, in male rats and 0, 0.16, 0.53, 1.7, 4.9, or 12.5 mg/kg-day CN^-, respectively, in female rats. The parameters evaluated included body weight, clinical signs, water consumption, clinical chemistry, hematology, urinalysis, extensive histopathology, selected organ weights (heart, kidneys, liver, lungs, thymus gland, testes, epididymis, cauda epididymis), testicular sperm measures (spermatid count and spermatid heads), epididymal sperm measures (spermatozoa count and motility), and vaginal cytology. Thyroid weight or levels of thyroid hormones were not evaluated in this study.

In rats, no treatment-related effects on mortality or clinical signs of toxicity were seen in either males or females. Body weight was statistically significantly decreased by 6% in high-dose males, but this was not considered to be biologically significant by the study authors. No body weight changes were observed in females. There was a dose-related decrease in water consumption that was >10% in both sexes exposed to 100 or 300 ppm NaCN, compared with controls. Decreased urine volume and increased urine specific gravity were observed in the high-dose male rats and were attributed to decreased water consumption. Urinalysis data were not reported for females. Urinary thiocyanate concentration was statistically significantly increased at drinking water concentrations of ≥30 ppm NaCN at the end of the study. There were no observed effects on nonreproductive organ weights in males, but there was a statistically significant increase in absolute (16%) and relative (12%) liver weights in high-dose females relative to controls. For all examined organs, there were no histopathologic changes that were attributed to cyanide exposure. In particular, no histologic effects were observed in either the thyroid or the brain. Female rats in the 100 and 300 ppm dose groups (4.9 and 12.5 mg/kg-day

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CN⁻, respectively) spent significantly more time in proestrus and diestrus compared with controls, but there was no clear dose response and the authors did not consider these results to be exposure related.

Male reproductive endpoints in the testis and epididymis were evaluated only in rats exposed to ≥30 ppm NaCN (≥1.4 mg/kg-day CN⁻). All reproductive parameter measurements were conducted with the left reproductive organ (Table 4-4). In addition to evaluation of epididymis weight, the weight of the cauda subsection of the epididymis was also measured. This section of the epididymis functions as a site of sperm maturation and storage. Because the cauda is part of the epididymis, these weights are not independent endpoints. Reproductive organ weights were reported by NTP (1993) as absolute organ weights. For this review, relative weights of reproductive organs were also calculated based on the individual animal data downloaded from NTP's web site (http://ntp-server.niehs.nih.gov/) and were statistically tested by using analysis of variance (ANOVA) followed by Dunnett's test.

Table 4-4. Reproductive effects in male rats administered NaCN in drinking water for 13 weeks

Study parameter	0 ppm	30 ppm	100 ppm	300 ppm
Number of animals[a]	10	10	10	10
Dose (mg/kg-d CN⁻)	0	1.4	4.5	12.5
Weights (g)				
Body weight[a]	338 ± 5	335 ± 5	338 ± 4	319 ± 5^b
Epididymis, absolute (relative weight, 10^{-4})[c]	0.448 ± 0.006 (13.7 ± 0.14)	0.437 ± 0.005 (13.3 ± 0.21)	0.425 ± 0.007 (13.0 ± 0.18)	0.417 ± 0.005^d (13.3 ± 0.41)
Cauda epididymis, absolute (relative weight, 10^{-4})[c]	0.162 ± 0.003 (4.92 ± 0.05)	0.150 ± 0.004^b (4.54 ± 0.07)^b	0.148 ± 0.004^b (4.51 ± 0.12)^d	0.141 ± 0.003^d (4.49 ± 0.09)^d
Testis, absolute (relative weight, 10^{-4})[c]	1.58 ± 0.03 (48.2 ± 0.64)	1.56 ± 0.02 (47.3 ± 0.58)	1.52 ± 0.02 (46.4 ± 0.40)	1.46 ± 0.02^d (46.6 ± 0.80)
Testicular spermatid measurements				
Spermatid heads (10^7/g testis)	11.35 ± 0.38	10.88 ± 0.53	10.92 ± 0.37	10.57 ± 0.33
Spermatid heads (10^7/testis)	17.86 ± 0.61	16.94 ± 0.81	16.58 ± 0.63	15.42 ± 0.44^b
Spermatid count (mean/10^{-4} mL suspension)	89.28 ± 3.05	84.68 ± 4.03	82.90 ± 3.16	77.10 ± 2.20^b
Epididymal spermatozoal measurements				
Motility (%)	94.24 ± 0.58	90.67 ± 1.25^b	92.09 ± 0.85^b	90.66 ± 1.46^b
Concentration (10^6/g cauda epididymal tissue)	615 ± 42	684 ± 40	699 ± 33	709 ± 45
Spermatozoa count (10^6/cauda epididymis)[c]	99.4 ± 6.8	102.9 ± 7.5	102.8 ± 4.9	99.4 ± 5.8

[a]Data reported as mean $\pm$ standard error of the mean [SEM]. Statistical significance determined by NTP, using Dunnett's test (for body weight only) or Shirley's test.
[b]Statistically different from control at $p \leq 0.05$.
[c]Calculated for this assessment based on individual animal data available at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. Statistical significance tested by one-way ANOVA followed by Dunnett's test.
[d]Statistically different from control at $p \leq 0.01$.

Source: NTP (1993).

NTP (1993) reported statistically significant decreases in several reproductive parameters including epididymis weight, cauda epididymis weight, testis weight, number of spermatid heads, testicular spermatid concentration, and epididymal spermatozoa motility. Absolute and relative cauda epididymis weights were statistically significantly decreased at all doses examined ($\geq$1.4 mg/kg-day CN⁻). In contrast, absolute epididymis weight was statistically significantly depressed only at the highest dose (12.5 mg/kg-day). At the highest dose tested (12.5 mg/kg-day), cauda epididymis weight (absolute) was decreased 13% below controls. The whole epididymis weight (absolute) was significantly depressed 7% at this dose, and absolute testis weight was significantly depressed 8%. Relative epididymis and testis weights were not

significantly different at any dose level. Additionally, standard histopathology did not demonstrate any morphologic effects in any reproductive organ.

Testicular spermatid parameters, including spermatid count and spermatid heads per testis, were statistically significantly depressed at the highest dose tested (12.5 mg/kg-day). No effect was seen on epididymal spermatozoa concentration; however, spermatozoa motility was statistically significantly reduced at all tested concentrations ($\geq$1.4 mg/kg-day), although motility did not exhibit a clear dose-related trend. At the lowest and highest dose, the percent of mobile spermatozoa was reduced 4%, a magnitude of change within the range of historical controls and not considered by the study authors to be biologically significant. Because fertility may be a function of total spermatozoa count, rather than the concentration per gram cauda epididymal tissue, and because decreased cauda epididymis weight can mask changes in spermatozoa content, the number of total spermatozoa/cauda epididymis was also calculated for this review (U.S. EPA, 1996). This number did not vary with dose. The unaltered spermatozoa count, coupled with the decreased cauda epididymal weight, explained the slight dose-related (but not statistically significant) increase in cauda spermatozoa concentration (see Table 4-4). For the purpose of this review, a LOAEL of 1.4 mg/kg-day was identified, based on significantly decreased relative and absolute cauda epididymis weights in male rats.

NTP (1993) also conducted a subchronic bioassay of NaCN administered in drinking water in mice. B6C3F$_1$ mice (10/sex/group) were administered NaCN in drinking water at concentrations of 0, 3, 10, 30, 100, or 300 ppm for 13 weeks. These concentrations are equivalent to the following doses, estimated by the study authors based on measured body weights and water consumption (converted to CN$^-$ equivalents for this assessment): 0, 0.26, 0.96, 2.7, 8.6, or 24.4 mg/kg-day, respectively, in male mice and 0, 0.32, 1.1, 3.3, 10.1, or 28.8 mg/kg-day, respectively, in female mice. The parameters evaluated were identical to rats and included body weight, clinical signs, water consumption, clinical chemistry, hematology, urinalysis, extensive histopathology, selected organ weights (heart, kidneys, liver, lungs, thymus gland, testes, epididymis, cauda epididymis), testicular sperm measures (spermatid count, spermatid heads), epididymal sperm measures (spermatozoa count and motility), and vaginal cytology. Thyroid weight and level of thyroid hormones were not evaluated.

In mice, no significant treatment-related effects on mortality, body weight, or clinical endpoints were observed. Water consumption in both males and females was decreased in the mid- and high-dose groups. Absolute and relative liver weights were significantly increased by 18 and 23%, respectively, in the high-dose females, and relative liver weight (but not absolute liver weight) was significantly increased in high-dose males (12%). However, there was no clear dose response. No treatment-related effects were observed in clinical chemistry, hematology, urinalysis, nonreproductive organ weights, or histopathology in any of the assessed organs. Reproductive effects were only evaluated in mice exposed to the highest three doses ($\geq$2.7 mg/kg-day). As in the rat component of this study, relative organ weights were not

originally reported by NTP, but were calculated for this review and statistically analyzed by using ANOVA followed by Dunnett's test.

In male mice, NTP (1993) found that the absolute weights of the epididymis and cauda epididymis were statistically significantly decreased in the high-dose group (24.3 mg/kg-day) relative to controls (Table 4-5). At the high dose, absolute epididymis and cauda epididymis weights were reduced 10 and 18%, respectively. Relative cauda epididymis weight was significantly decreased (18%) at 8.6 mg/kg-day. Neither relative epididymal and testis weights (relative and absolute) nor sperm parameters (spermatozoa per gram cauda epididymis, total spermatozoa per cauda epididymis, and spermatozoa motility) were statistically significantly decreased. No reproductive effects were reported at any of the dose levels tested for female mice. For this review, a LOAEL of 8.6 mg/kg-day was determined based on a statistically significant decrease in relative cauda epididymis weight.

Table 4-5. Reproductive effects in mice administered NaCN in drinking water for 13 weeks

Study parameter	0 ppm	30 ppm	100 ppm	300 ppm
Number of animals[a]	9	10	10	9
Dose (mg/kg-d CN⁻)	0	2.7	8.6	24.3
Weights (g)				
Body weight	37± 1.0	39.2 ± 1.3	38.6 ± 1.1	35.5 ± 1.1
Epididymis (relative weight, 10^{-4})[b]	0.049 ± 0.001 (13.5 ± 0.54)	0.047 ± 0.002 (12.1 ± 0.52)	0.047 ± 0.001 (12.1 ± 0.42)	0.044 ± 0.001[c] (11.8 ± 0.29)
Cauda epididymis (relative weight, 10^{-4})[b]	0.017 ± 0.001 (4.74 ± 0.24)	0.016 ± 0.000 (4.12 ± 0.15)	0.015 ± 0.001 (3.88 ± 0.22)[c]	0.014 ± 0.001[c] (3.68 ± 0.17)[d]
Testis (relative weight, 10^{-4})[b]	0.121 ± 0.002 (33.4 ± 1.8)	0.113 ± 0.008 (29.2 ± 2.2)	0.117 ± 0.002 (30.3 ± 0.90)	0.118 ± 0.003 (31.7 ± 0.92)
Testicular spermatid measurements				
Spermatid heads (10^7/g testis)	18.47 ± 1.13	21.48 ± 2.34	17.42 ± 1.34	18.17 ± 1.62
Spermatid heads (10^7/testis)	2.24 ± 0.14	2.26 ± 0.14	2.03 ± 0.15	2.11 ± 0.16
Spermatid count (mean/10^{-4} mL suspension)	69.94 ± 4.34	70.80 ± 4.25	63.28 ± 4.53	66.06 ± 4.87
Epididymal spermatozoal measurements				
Motility (%)	92.38 ± 0.81	90.63 ± 1.34	91.43 ± 0.55	89.52 ± 0.96
Concentration (10^6/g cauda epididymal tissue)	1,235 ± 82	1,393 ± 70	1,386 ± 70	1,462 ± 101
Spermatozoa count (10^6/cauda epididymis)[b]	21.2 ± 1.2	22.3 ± 1.3	20.5 ± 1.1	19.6 ± 0.85

[a]Data reported as mean ± SE. Statistical significance determined by NTP using Dunnett's test (for body weight only) or Shirley's test.
[b]Calculated for this assessment based on individual animal data available at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. Statistical significance tested by one way ANOVA, followed by Dunnett's test.
[c]Statistically different from control at $p \leq 0.05$.
[d]Statistically different from control at $p \leq 0.01$.

Source: NTP (1993).

As part of a study evaluating the effects of cyanogenic compounds in cassava, Kamalu (1993) evaluated the toxicity of inorganic cyanide administered in a rice diet to male dogs (six/group) for 14 weeks. The diet was supplemented at feeding time with NaCN at a dose calculated to release 10.8 mg HCN per kg cooked food. Based on a reported daily food consumption of 0.1 kg/kg body weight, this corresponds to 1.08 mg/kg-day HCN or 1.04 mg/kg-day CN⁻. Further information about the study animals was not provided in this study, but animal selection and study pretreatment were described in an earlier publication by the same author (Kamalu, 1991), apparently describing the same study. That publication reported that dogs of mixed breeds were purchased from local African markets at 6 weeks of age; treatment was initiated when the dogs were approximately 22 weeks old. The authors noted that the dogs were

repeatedly treated for ecto-and endoparasites. It is unclear what impact the compromised health status and repeated treatment for parasites had on the observed effects in the dogs. The basal diet used rice as the carbohydrate source, supplemented with pork, bone meal, and a vitamin and mineral supplement that included iodine.

Blood was obtained from each dog at study weeks 1, 3, and 14; urine was collected at weeks 1, 3, 5, 7, and 14. Plasma and urinary thiocyanate concentrations were determined for each collection period. Serum enzymes (including γ-glutamyl transferase [GGT], ALT, isocitrate dehydrogenase, total serum protein, serum albumin, serum globulin, and urinary protein), as well as sodium, magnesium, and phosphorus, were measured. Histopathologic evaluation was performed on the liver, kidneys, heart, testes, and adrenal glands of each dog. The thyroid gland was not evaluated. At all time points evaluated, both plasma and urinary thiocyanate concentrations were significantly increased in the treated dogs compared with controls. Relative to controls, treated dogs had significantly increased urinary protein concentration at weeks 5 and 14. No treatment-related effects were observed in serum enzymes, total serum protein, albumin, or globulin or in sodium, magnesium, and phosphorus concentrations. No histopathologic changes were observed in the liver or heart of treated dogs; however, treatment-related effects were observed in the kidneys, testes, and adrenal glands. Kidneys of the treated dogs had casts in the lumens of the renal tubules, accompanied by occasional desquamation. In the testes, specialized reproductive morphologic analysis indicated that the treated dogs had a significantly decreased percentage of tubules in stage VIII of the spermatogenic cycle (characterized by elongated spermatids lining the lumen of the seminiferous tubules) as compared with controls ($p < 0.01$). This percentage was $1.6 \pm 1.07\%$ (mean $\pm$ standard error of the mean [SEM]) in the treated group compared with $14.4 \pm 0.94\%$ in the controls. Treated dogs also had an increased incidence of abnormal cells and sloughing of germ cells in the seminiferous tubules. Hyperplasia and hypertrophy were observed in the adrenal gland. The adrenal medulla was unaffected by inorganic cyanide treatment. Although the width of the adrenal cortex did not differ significantly between the cyanide-treated and control groups, the zona glomerulosa (the most superficial layer of the adrenal cortex) was significantly wider in treated dogs. The results of this study indicate that cyanide may be a reproductive toxicant in male dogs. Based on histopathologic changes in the kidneys, testes, and adrenal glands, the only dose tested (1.04 mg/kg-day) was considered to be a LOAEL.

An evaluation of the thyroid from this study was presented in Kamalu and Agharanya (1991). At week 14, serum T_3 was significantly decreased by 55%, and thyroid weight was significantly increased by 23% in the cyanide-exposed group (compared to control animals at the same time point). A histopathologic evaluation of the thyroid gland found decreased colloid content compared to that of controls.

A 40-week study in New Zealand white rabbits that reported both liver and kidney lesions supports the kidney as a possible target organ for toxicity following exposure to cyanide

(Okolie and Osagie, 1999). In this study, groups of six male rabbits were fed a diet of growers' mash only or mash containing 702 ppm CN^- as KCN for 10 months. Based on daily food consumption and weekly body weight measurements, the study authors estimated that the average CN^- intake was 0.39 mg/day per rabbit in the control group and 36.5 mg/day per rabbit in the exposure group. Initial and final body weights were averaged to estimated daily doses of 0.2 and 20 mg/kg-day CN^-, respectively. Decreased body weight (33%) and decreased food efficiency were observed in the high-dose group (33%). At the end of 10 months of treatment, serum levels of ALT, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and sorbitol dehydrogenase were increased. ALP levels were reduced in the lung but not in the heart. LDH was increased in the liver and kidneys, a finding that the study authors interpreted as indicative of a shift from aerobic to anaerobic metabolism, thereby increasing the production of lactic acid. Biochemical evidence of tissue injury in the liver and kidney was supported by histopathologic findings of focal areas of hepatic necrosis and congestion and renal tubular and glomerular necrosis. No abnormal histopathology was reported for the pancreas or the heart. Neither a full list of tissues examined nor additional information on histopathologic changes in other organs was provided in the study. However, the occurrence of focal pulmonary edema and necrosis in treated rabbits was reported in a second paper on the same study (Okolie and Osagie, 2000). Based on necrosis in the liver and kidney, the only dose tested (20 mg/kg-day CN^-) is considered for this review to be a LOAEL.

Manzano et al. (2007) examined the effects of subchronic (70-day) KCN ingestion in 45-day-old Lanrace-Large white pigs. The number and sex of animals used in this study are unclear since study details in the published report are conflicting, with indications of 6 animals per group in the materials and methods section, but 5–10 animals indicated in the tables. Animals were administered KCN in the diet, twice per day, for total daily doses of 0, 2, 4, or 6 mg/kg-day (0, 0.8, 1.6, or 2.4 mg/kg-day CN^-). Blood samples were collected prior to the experimental period and then every week thereafter and analyzed for ALT, glucose, cholesterol, blood urea nitrogen, creatine, T_3, T_4, and thiocyanate. At the conclusion of the experiment, thyroid glands were weighed, and tissues from the CNS, thyroid, pancreas, liver, and kidneys were examined histologically. Significantly decreased serum ALT was observed at ≥ 0.8 mg/kg-day. Additionally, significantly increased urea and creatinine were observed at doses ≥ 1.6 mg/kg-day. Thyroid weight was significantly increased (24%) in animals in the highest dose group, although significant alterations in thyroid hormones were not observed. Histological alterations of the thyroid gland, characterized by numerous vacuoles in the colloid of the thyroid follicles, were observed in all dosed animals. The authors also reported histologic alterations in the liver, kidney, and CNS. Liver lesions were reported as karyolysis and pyknosis (nuclear DNA changes denoting cell death) and distortion of the normal lobular architecture. Degeneration of the renal tubular epithelial cells was reported in the kidney. In the brain, minimal degeneration of Purkinje cells and loss of cerebellar white matter were reported. All

histologic lesions were reported by the authors to occur in a dose-related manner, although neither incidence nor statistical analysis of these findings was presented. A LOAEL of 2.4 mg/kg-day based on increased thyroid weight was determined for this review.

Jackson (1988) evaluated the effects of oral administration of KCN on behavior and thyroid function in miniature pigs. Doses of KCN equivalent to 0, 0.4, 0.7, or 1.2 mg/kg-day CN^- were administered to three pigs per group via gavage. A total of five females and seven males were used; each dose group contained both male and female animals (one dose group contained two females and one male while the others contained two males and one female). The solutions were administered once daily for 24 weeks, prior to feeding, in order to increase the gastrointestinal absorption of cyanide. Regularly measured serum thiocyanate levels were positively correlated with cyanide dose. Serum levels of T_3, T_4, and glucose were measured every 6 weeks. Behavioral evaluations were conducted daily. Two categories of behavior were evaluated: performance measures, including innate behavior, and learning measures, including the acquisition and retention of new behaviors. No other endpoints were evaluated. Changes in thyroid hormones were portrayed graphically as means without reporting variance (SEM or standard deviation [SD]) or individual animal data. Individual animal data were requested from the study authors by EPA, but were not provided. Both T_3 and T_4 demonstrated a dose-related decrease (23 and 13%, respectively) that was statistically significant by week 18 of the study. Thyroid histopathology was not evaluated in this study. A variety of behaviors were significantly altered in treated animals, including a decrease in dominance behavior (high-dose group), a decrease in fighting (mid- and high-dose group), an increase in flight response (all treated groups), a decrease in exploratory behaviors (all groups), and less aggressive feeding patterns (high-dose group). The authors concluded that the overall pattern of behavioral changes in the group administered 1.2 mg/kg-day was different from that of the control animals, but that the changes at lower doses were inconsistent. This study supports the large body of evidence demonstrating that the thyroid is a target organ for cyanide toxicity. Based on reported behavioral changes and decreased thyroid hormones, the LOAEL and NOAEL values identified for this review are 1.2 and 0.7 mg/kg-day CN^-, respectively.

Philbrick et al. (1979) evaluated the long-term health effects of oral exposure to cyanide in rats. Male rats from Woodlyn Laboratories (10/group, strain not specified) received diets (10% casein supplemented with 0.3% methionine, potassium iodide, and vitamin B_{12}) containing either 0 or 1,500 ppm KCN or an equal molar amount (2,240 ppm) of potassium thiocyanate (KSCN) for 11.5 months. Parallel studies were conducted with rats provided a diet deficient in methionine, iodine, and vitamin B_{12}, containing 0 or 1,500 ppm KCN (44 mg/kg-day CN^-)[2]. At 4 and 11 months, plasma T_4 levels, T_4 secretion rates, and urinary thiocyanate levels were measured in five animals per group. After sacrifice, brain, heart, liver, and thyroid weights were

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[2]Based on the average food intake across rat strains (U.S. EPA, 1988a) and adjusting for the molecular weight ratio of cyanide to potassium cyanide.

recorded. Histopathologic evaluation was conducted on the brain, optic and sciatic nerves, spinal cord, and thyroid gland. This study design, although limited by the use of only one dose level, allowed for the comparison of effects mediated directly through cyanide versus the primary metabolite, thiocyanate. It also allowed for the comparison of cyanide and thiocyanate treatment in control rats compared to rats fed nutritionally restricted diets.

Body weight gains of the KCN-treated animals were significantly lower than those of controls, beginning at week 8; administration of KSCN did not affect body weight. Urinary thiocyanate levels were reported as μg/g food ingested (instead of μg/mL urine) and were significantly higher than in controls in all treated groups. Urinary thiocyanate levels were lower at 11 months than at 4 months of treatment in KCN-treated groups but not in KSCN-treated groups. This appears to indicate reduced metabolism of KCN with chronic exposure. Additionally, although animals were fed an equal molar amount of KCN and KSCN, at 11 months, urinary levels of SCN^- in the KCN-treated animals were only approximately one quarter of the urinary excretion of SCN^- in KSCN-treated animals. This appears to indicate only partial metabolism of cyanide into thiocyanate. Administration of cyanide altered serum T_4 levels at 4 months but not at 11 months; thiocyanate altered serum T_4 levels at both 4 and 11 months. After 4 months of treatment, rats in the cyanide-exposed groups had significantly decreased plasma T_4 levels (53%) and decreased T_4 secretion rates (68%) compared to controls; however, after 11 months of cyanide treatment, T_4 levels no longer differed from those of controls, although T_4 secretion rates were depressed 27%. KSCN-treated animals also showed a significant reduction (62%) in T_4 secretion rates (at 4 months but not at 11 months) and decreased T_4 levels (55% at 4 months and 26% at 11 months). At the termination of the study, relative thyroid weights were significantly increased in both KCN- and KSCN-treated animals by 43 and 33%, respectively. In the nutritionally restricted control animals, levels and T_4 secretion rates were lower compared to controls fed a standard diet. However, alterations of T_4 levels, T_4 secretion rates, and thyroid weights in animals on the restricted diet treated with KCN and KSCN were of similar magnitude compared to treated animals on the standard diet. No histopathologic lesions were observed by light microscopy in the optic or sciatic nerves or thyroid gland of any group. Increased vacuolation was observed in the spinal cord white matter of treated animals (with both KCN and KSCN) receiving sufficient or deficient methionine compared to controls, and spinal cord demyelination induced by methionine deficiency was exacerbated by treatment. No information on incidence or severity of the observed histologic lesions was reported by the authors. No measurable differences were reported in spinal cord pathology between cyanide- and thiocyanate-treated rats. Based on increased thyroid gland weight, decreased thyroid hormone levels, and histopathologic changes in the spinal cord, the single dose tested (44 mg/kg-day CN^-) is considered to be a LOAEL.

Howard and Hanzal (1955) conducted a 2-year dietary study in which 10 Carworth Farms rats per sex per group were administered food fumigated with HCN. The authors indicated that

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only rats surviving to the end of the study were analyzed histologically because the accuracy of necropsies performed on animals that died early were compromised by autolysis. It appears that seven, five, and nine males and six, seven, and six females were examined histologically in the 0, 4.3, and 10.8 mg/kg-day CN^- dose groups, respectively. Although special feeding jars were used to minimize air circulation and evaporation, the study authors noted that it was necessary to measure the loss of HCN due to evaporation from the chow and to prepare fresh rations every other day to keep the HCN concentration near the target values of 100 and 300 ppm (mg HCN per kg diet). The average daily concentrations were 73 and 183 mg CN^- per kg diet. These average concentrations of cyanide in the food were estimated based on Howard and Hanzal's (1955) data for concentrations at the beginning and end of each food preparation period and assuming a first-order rate of loss for the intervening period (U.S. EPA, 1992). From the data reported on food consumption and body weight, estimated doses were 0, 4.3, and 10.8 mg/kg-day. There were no treatment-related effects on growth rate, no gross signs of toxicity, no hematologic effects, and no histopathologic lesions in the tissues evaluated from an undisclosed subset of animals (heart, lungs, liver, kidneys, spleen, stomach, small and large intestines, adrenals, thyroid, testes or uterus and ovaries, cerebrum, and cerebellum). Histopathology of the spinal cord was not examined. Howard and Hanzal (1955) also reported that there appeared to be no effect on relative organ weight of the liver, kidneys, spleen, brain, heart, adrenals, testes, or ovaries. Thyroid weight was not investigated. The highest dose tested, 10.8 mg/kg-day CN^-, is considered to be the NOAEL.

An unpublished study by Leuschner and Neumann (1989) administered KCN to male Sprague-Dawley rats (26-40/group) in drinking water for 13 weeks. Administered doses were 0, 40, 80, and 160 mg KCN/kg-day or 0, 16, 32, and 64 mg/kg-day CN^-. Doses were lowered in the highest dose group at week 12 due to excessive toxicity/mortality. Water consumption was decreased in all dose groups, prompting the authors to add a control group matching the water consumption observed in the highest KCN treatment group. Examinations included hematology, clinical chemistry, and urinalysis. After 3 months of exposure, organ histology was performed on the kidney, heart, liver, testes, thyroid, and brain. Organ weights were measured for the following organs: adrenals, heart, kidneys, lungs, thymus, brain, liver, pituitary, testis, and thyroid (only the left lobe). Epididymis weight was not weighed independently, but was included as part of testicular weight.

Early mortality was observed at the high dose, with 11 animals dying prematurely. Statistically significant body weight decreases were observed in the highest two doses. Body weight was statistically significantly decreased 42% at the high dose and 15% in the mid-level dose. Average body weight decrease (4%) at the low dose was not statistically different than controls. Water consumption was decreased in all dose groups (17, 21, and 32% in the low, mid, and high dose, respectively) compared to controls. No changes in hematology or clinical biochemistry were reported. Urinalysis demonstrated increased protein, which appeared to be

dose-related. No dose-related histopathological changes were reported. Organ weight changes were not observed at the lowest dose level tested (16 mg CN⁻/day). At the mid-dose level (32 mg CN⁻/day), absolute thymus weight was statistically significantly decreased (20%). Statistically significant relative and absolute organ weight changes were observed at the highest dose level (64 mg CN⁻/kg-day), although these changes were inconsistent. At the highest dose, absolute heart, liver, spleen, kidney, and brain weights were statistically significantly decreased compared to the controls. However, when relative weights were calculated, all organs showed increased weight compared to controls (except for the thymus, which was decreased). For this review, a LOAEL of 32 mg/kg-day and a NOAEL of 16 mg/kg-day were identified based on decreased body weight in male rats treated with KCN for 13 weeks.

Two studies by Soto-Blanco et al. (2002a, b) provide evidence of neurological changes associated with cyanide ingestion in rats and goats. In the first study, Soto-Blanco et al. (2002a) evaluated the toxicity of KCN administered daily by gavage (vehicle not stated) to male Wistar rats for 12 weeks. Administered doses of KCN were 0, 0.15, 0.3, or 0.6 mg/kg-day, equivalent to 0, 0.06, 0.12, or 0.24 mg/kg-day CN⁻, respectively. The number of animals included in each group was seven, six, six, and seven for the control, low-, mid-, and high-dose groups, respectively. Endpoints evaluated included clinical signs of toxicity, body weight, food consumption, serum cholesterol, glucose, T_3, and T_4. Histopathologic examination was limited to the CNS, thyroid gland, and pancreas. No treatment-related effects were reported for clinical signs of toxicity, body weight gain, food consumption, serum T_3 and T_4, or serum glucose. Plasma cholesterol was significantly decreased in the high-dose group. No histopathologic changes were observed in the thyroid gland or the pancreas. Reported CNS effects in the high-dose group included neuron loss in the hippocampus, damaged Purkinje cells (further details not reported) and loss of white matter in the cerebellum, and the occurrence of a dose-related increase in spheroid bodies on white matter in the spinal cord. EPA was unsuccessful in obtaining incidence data from the study authors for the observed histologic lesions. Because quantitative information was not reported on these histologic observations, neither a LOAEL nor a NOAEL could be identified from this study.

Soto-Blanco et al. (2002b) also evaluated the neurotoxicity of cyanide, administered daily as KCN, to male Alpine-Saanen goats in milk or water for 5 months at doses of 0, 0.3, 0.6, 1.2, or 3.0 mg/kg-day (equivalent to 0, 0.12, 0.24, 0.48, and 1.2 mg/kg-day CN⁻). The test compound was administered twice daily in milk (one-half of the daily dose per treatment) for the first 3 months and in water for the remainder of the treatment period. The number of animals per dose group ranged from six to eight. The CNS was evaluated histologically for the presence of glial fibrillary acid protein (GFAP), a marker for glial cells. The only clinical signs of toxicity were transient muscular tremors and ataxia in one high-dose animal on days 121–123 of the study. Neuropathology, including congestion, hemorrhage, and gliosis in the cerebellum, spinal cord, and pons, as well as spheroids on the gray matter of the spinal cord, was observed at

0.58 and 1.2 mg/kg-day CN⁻. Additional findings in the high-dose group included damage and loss of Purkinje cells in the cerebellum, spongiosis in the pons, and spheroids, axonal swelling, gliosis, spongiosis, and ghost cells in the medulla oblongata. GFAP immunostaining confirmed the gliosis observed by histopathology. While this study confirmed that the CNS is a target organ of subchronic cyanide administration, no information on the incidence or severity of histologic findings was reported. Therefore, a NOAEL or LOAEL could not be identified from this study.

4.2.2. Inhalation Studies

No chronic or subchronic animal studies of cyanide inhalation exposure were located. However, several subchronic inhalation studies of related compounds, including cyanogen $(CN)_2$ and ACH, are available. A 6-month inhalation study in monkeys (5 males/group) and rats (30 males/group) exists for the gas $(CN)_2$. $(CN)_2$ is thought to break down in aqueous solution to CN^- and cyanate (CNO^-) ions (Cotton and Wilkinson, 1980). Lewis et al. (1984) exposed three groups of male Rhesus monkeys or male albino rats (Sprague-Dawley) to 0, 11, or 25 ppm $(CN)_2$ 6 hours/day, 5 days/week for 6 months. This would be equivalent to 0, 12, or 28 mg/m³ HCN (based on the creation of 1 mol CN^- per mol $(CN)_2$ in water). Pathology evaluated for both monkeys and rats included gross and microscopic examination of heart, liver, kidney, cerebellum, lungs, thyroid, spleen, and bone marrow. Hemoglobin, hematocrit, T_3, and T_4 were also evaluated. Additionally, behavioral tests and electrocardiograms were administered to the monkeys. No significant changes were seen in monkeys other than decreased lung moisture content in both dose groups. The only effect noted in rats was significantly depressed body weight (13%) in the high-dose group.

Inhalation studies of subchronic ACH exposure in male and female rats are available. ACH is a liquid at room temperature, with a boiling point of 95°C and a vapor pressure of 0.75 mm Hg. In neutral to basic aqueous environments, ACH is reported to dissociate readily to acetone and cyanide (U.S. EPA, 1985). Sprague-Dawley rats (15/sex/group) were exposed by inhalation at average concentrations of 10.1, 28.6, or 57.7 mg/m³ ACH 6 hours/day, 5 days/week for 14 weeks (Monsanto Co., 1985a, b). This dose corresponds to the molecular equivalent of HCN concentrations of 3.2, 8.8, and 18.2 mg/m³. Endpoints analyzed included hematology, clinical chemistry (including T_3 and T_4 levels), and gross and microscopic histopathology on a wide range of organs and tissues. No effects on mortality, body weight, or behavior were observed in treated animals. Blood and urine levels of thiocyanate were elevated in a dose-dependent manner, although no alterations in T_3 or T_4 were observed. No significant gross or microscopic histology was observed in treated animals compared with controls. In summary, the study authors found no gross signs of toxicity attributable to subchronic inhalation exposure to ACH in rats. Reproductive and developmental studies by the inhalation route have also been conducted for ACH (Monsanto Co., 1985a, b; IRDC, 1984) and are described in Section 4.3.2.

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4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Oral Studies

Imosemi et al. (2005) fed 20 pregnant female Wistar rats 0 or 500 ppm KCN (equivalent to 20 mg/kg-day CN⁻) in the diet during gestation and up to postnatal day (PND) 50. Offspring (five/group) were sacrificed on PNDs 1, 9, 14, 21, 28, and 50, and the cerebellar tissues were examined grossly. Parameters examined included body weight, brain weight, cerebellar weight, maximum vermis length (length between cerebellar hemispheres), maximum side-to-side dimensions of the cerebellum, and maximum thickness (anteroposterior dimension) of the cerebellum. Aggressive and restless behavior was noted in the exposed dams but not in controls. Additionally, significantly decreased body weight (6%) and brain weight (19%) were observed in the treated pups on PNDs 14 and 9, respectively. No significant changes in body weight or brain weight were found at the additional five time points examined. Cerebellar weight was significantly reduced on PNDs 14, 21, and 28. The maximum vernal length was significantly reduced on day 50 and the maximum side-to-side width of the cerebellum was reduced on day 29. In a separate publication, the authors also reported on microscopic parameters of the cerebellum (Malomo et al., 2004). A significantly thicker external granular layer (EGL) was seen in the experimental group on PNDs 14 and 21. Reduced thickness of the molecular layer (ML) was also observed on PNDs 28 and 50. The density and size of the Purkinje cells were not different between groups. Additionally, staining of the white matter was similar between groups, suggesting normal myelination. The authors concluded that maternal consumption of 20 mg/kg-day CN⁻ did not significantly affect microscopic indicators of cerebellar development, but caused mild changes later in postnatal life. The authors noted that the presence of a thicker EGL layer in the experimental group suggested delayed maturation and migration of cells in the cerebellum. A LOAEL of 20 mg/kg-day CN⁻ was identified for this review based on altered maturation of the cerebellum.

Soto-Blanco and Gorniak (2004) evaluated effects of gestational exposure to cyanide in pregnant mixed-breed goats (six per group). Starting on day 24 of pregnancy, goats were administered, by gavage, 0, 1, 2, or 3 mg/kg-day KCN (equivalent to 0, 0.4, 0.8, or 1.2 mg/kg-day CN⁻) until parturition (day 150). Blood samples were collected every other week and analyzed for plasma glucose, cholesterol, and thiocyanate. T_4 and T_3 concentrations in plasma were measured from the offspring at birth and at 1 week old. One control dam and one dam from the highest dose group were sacrificed at day 120. Three months after birth, the male offspring and one dam from each group were sacrificed and the pancreas, thyroid, and entire CNS (including spinal cord) were collected for histologic examination. Two dams from the highest dose group experienced clinical signs of cyanide intoxication, specifically ataxia and convulsions. Cyanide treatment did not significantly alter the number of live offspring or the length of gestation, although average length of gestation in all treated groups was about 2.5 days

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shorter than controls. T_3 levels in dams and offspring tested at birth were significantly elevated over controls in the highest dose group, while T_4 levels did not appear to be different. The dam sacrificed at day 120 of pregnancy revealed increased reabsorption vacuoles in the thyroid follicular colloid and severe spongiosis of the cerebral, internal capsule, and cerebellar peduncle white tracts, suggestive of myelin edema of the white matter. The histopathologic study of dams and offspring 3 months after birth revealed no lesions. Due to the lack of incidence and severity data for the observed histologic effects, a LOAEL could not be identified from this study.

Soto-Blanco and Gorniak (2003) dosed mixed-breed, lactating goats (six per group) with 0, 1.0, 2.0, or 3.0 mg/kg-day KCN (equivalent to 0, 0.4, 0.8, or 1.2 mg/kg-day CN^-) by gavage (in water) from lactation days 0 to 90 and measured whole-blood cyanide and thiocyanate concentrations in dams and offspring on lactation days 30, 60, and 90. On the 90th day, glucose, cholesterol, plasma urea nitrogen, creatinine, T_4, T_3, AST, ALT, and GGT were also determined. After 90 days, one dam from each dose group and every male goat from all litters was sacrificed. The pancreas, thyroid glands, liver, kidneys, and the whole CNS were collected for histologic examination. No clinical signs of toxicity were seen in any group, although one dam in the highest dose group died on the 55th day of lactation. Both whole-blood cyanide and plasma thiocyanate concentrations were increased in a dose-dependent manner in treated dams. In the offspring, both blood cyanide and plasma thiocyanate increased with increasing maternal cyanide dose, peaking at lactation day 30, and decreased with lactation time. Plasma parameters in all groups of dams and offspring appeared to be unaffected by KCN treatment, except for the level of T_4 in dams, which was significantly elevated (20%) over controls ($p \leq 0.01$ by a t-test conducted for this review). T_3 and T_4 levels also appeared elevated in the high-dose animals, although these differences were not significant. In the thyroid, histopathologic changes, characterized by an increased number of reabsorption vacuoles on the colloid of the thyroidal follicles were observed in dams and offspring. Additionally, histologic changes in the liver and kidney were noted, characterized by hepatocellular vacuolization and degeneration and mild vacuolization of tubular epithelial cells, in dams and offspring. The authors noted that observed histologic lesions were most intense in the highest KCN dose group. No histologic lesions were noted in other examined tissues. In the absence of incidence data or statistical analysis on any histologic changes, a LOAEL was not identified for this study.

Tewe and Maner (1981) fed female rats (20 per group, strain not specified) either a basal diet prepared from low-HCN cassava meal or the basal diet supplemented with 500 ppm of KCN throughout mating, gestation, and lactation. In addition, two female weanling rats per litter were maintained on each diet for 28 days following weaning. Adult rats on the basal diet alone received a dose of 1.2 mg/kg-day (based on a dietary HCN concentration of 12 mg/kg and average food intake among female rats of 0.102 kg/kg body weight). Adult rats on the basal diet plus 500 ppm KCN (high-cyanide diet) received a total CN^- dose of 21.6 mg/kg-day, including the 1.2 mg/kg-day from the basal diet and 20.4 mg/kg-day as KCN. For the weanling rats, the

corresponding doses were approximately 1.9 mg/kg-day CN$^-$ for the basal diet and 34.3 mg/kg-day CN$^-$ for the basal + KCN diet, based on average food intake (0.162 kg/kg body weight) for female weanling rats (U.S. EPA, 1988). As compared with controls, the high-cyanide diet had no effect on body weight of pregnant rats, food consumption, maternal liver or kidney weights, litter size, birth weight of pups, or pup mortality. In the weanling rats, the high-cyanide diet resulted in significant decreases in food consumption and growth rate and an increase in the ratio of food consumption to body weight gain, indicating that the decreased weight gain was not due solely to poor palatability. The high-cyanide diet also resulted in a significant increase in serum thiocyanate in both dams and weanlings compared with animals on the basal diet alone. The activity of rhodanese, the enzyme that metabolizes cyanide to thiocyanate, in the liver and kidneys was comparable in all groups. A LOAEL of 34.3 mg/kg-day was identified from this study, based on decreased daily weight gain in weanlings; a NOAEL of 1.2 mg/kg-day (in adults) and 1.9 mg/kg-day (in weanlings) was identified (for this review) based on cyanide content of the basal cassava diet.

Teratogenicity has been reported in some nontraditional developmental studies that administered cyanide or cyanogenic foods to animals. Doherty et al. (1982) administered NaCN to hamsters subcutaneously via osmotic minipumps at doses around 0.13 mmol/kg-hour (approximately 80 mg/kg-day) on gestation days (GDs) 6–9 and observed fetotoxic effects, including significantly increased fetal resorptions and malformations and decreased crown-rump length. Doses utilized in this study covered a narrow range from about 78 to 81 mg/kg-day. At these doses, clinical signs of toxicity were evident in the dams, including weight loss, ataxia, and dyspnea. At the lowest dose tested (78 mg/kg-day), there were 63% resorptions as compared to 10% in controls. Additionally, at this dose, 62% of fetuses were malformed vs. 5% of controls. The majority of malformations in treated groups were characterized as neural tube defects. Coadministration of cyanide with the cyanide poisoning antidote sodium thiosulfate, which serves as a sulfur donor in the conversion of cyanide to thiocyanate by the enzyme rhodanese, protected against maternal toxicity and teratogenic effects. Another developmental study by Frakes et al. (1986) observed reduced ossification and decreased body weight in offspring of hamsters administered a protein-deficient cassava diet containing low- or high-cyanide levels during days 3–14 of gestation. The developmental effects of dietary cyanide were not evaluated in animals fed a protein-sufficient diet. Low (mean: 0.65 mmol CN$^-$/kg food) and high (mean: 8 mmol CN$^-$/kg food) cassava-containing diets equaled daily doses of approximately 1.3 or 14 mg/kg-day cyanide, respectively, and averaged only 4% protein (the standard laboratory diet contained 25% protein). Body weight in the cassava-fed dams was approximately 30% lower than in control dams fed a standard, protein-sufficient diet, regardless of whether they were in the high- or low-cyanide group. The numbers of implantations, resorptions, live fetuses, and malformed fetuses in cyanide-treated groups were not statistically different from those in controls. Fetal body weight was significantly decreased by 14 and 8% in low- and high-cyanide

treatment groups, respectively (compared with the low-protein controls). Significantly decreased ossification centers (28–37%) were observed in portions of the fetal skeletons, including the sacrocaudal vertebrae, metatarsals, and sternebrae. No dose-related trend was observed for the decreases in ossification or in maternal or body weight between the low- and high-cyanide dose groups.

A Japanese study (Amo, 1973) reported that 0.05 mg/kg-day of cyanide administered in drinking water decreased the fertility and survival rate in the F1 generation and produced 100% mortality in the F2 generation in mice. Although no other studies exist on F2 animals treated with cyanide, the data presented by Amo (1973) on decreased survival of the F1 generation are not consistent with the body of available literature for cyanide, which indicates no decrease in survival of the F1 generation of goats treated gestationally with doses twice as high (Soto-Blanco and Gorniak, 2004) or in rats treated gestationally with doses ≥20 mg/kg-day (Imosemi et al., 2005; Tewe and Maner, 1981). Additionally, studies in rats with ACH, which breaks down into cyanide and acetone following inhalation or oral exposure, have not observed decreases in reproductive parameters or F1 survival at inhalation exposures equivalent to 66 mg/m^3 HCN (Monsanto Co., 1985a, b). Furthermore, gavage dosing of pregnant Sprague-Dawley rats with doses of ACH equivalent to 3 mg/kg-day during GDs 6–15 did not decrease survival in offspring compared with controls, nor were there any difference in number of viable fetuses, postimplantation losses, mean fetal body weight, fetal sex distribution, or fetal malformations between treated animals and controls (IRDC, 1984).

4.3.2. Inhalation Studies

No studies exist on the potential reproductive or developmental toxicity of inhaled cyanide. However, male and female fertility indices were investigated in rats exposed via inhalation to the cyanide precursor ACH, which decomposes to acetone and cyanide (IPCS, 2005). At room temperature, ACH is primarily a liquid (boiling point: 95ºC); however, in these inhalational studies, the ratio of target and analytical air concentrations were close to unity, indicating that ACH was primarily present as a vapor.

In a male fertility study (Monsanto Co., 1985a), Sprague-Dawley rats (15/group) were exposed by inhalation to ACH at 0, 35, 104, or 209 mg/m^3 for 6 hours/day, 5 days/week over a period of 69 days. These doses are equivalent to 0, 11, 32, or 65 mg/m^3 HCN. Following the exposure period, males were mated with three nonexposed females each. Pregnant females were sacrificed at mid-gestation (GDs 13–15), and pre- and postimplantation losses were determined. Males were sacrificed 3 weeks following cessation of exposure. Histologic analysis of reproductive organs, including the testis, epididymis, prostate gland, and seminal vesicle, was conducted; reproductive organ weight and sperm parameters were not evaluated. No treatment-related differences were seen in mean body weight, clinical chemistry, or histology of treated

males. The mating efficiency, number of live implants, and pre- and postimplantation losses were not different between treated and control groups.

Female Sprague-Dawley rats (24/group) were exposed by inhalation 6 hours/day, 7 days/week for 21 days to 0, 38, 108, or 207 mg/m^3 ACH (0, 12, 33, or 64 mg/m^3 HCN) and then mated with untreated males (Monsanto Co., 1985b). Exposure of the females was continued until the day of mating, and the females were sacrificed at mid-gestation (GDs 13–15) to determine pregnancy status, nidations, pre- and postimplantation loss, and histology of the ovaries and uteri. No clinical signs of toxicity were observed in treated animals except for dose-related observations of red nasal discharge or encrustation in some animals. No treatment-related differences were seen in mean body weight, clinical chemistry, or histology. Mating efficiency, pregnancy rates, number of live implants, and pre- and postimplantation losses in treated animals were comparable to control values.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute Oral Studies

In evaluating the oral toxicity of cyanide, both the total amount administered and the rate of absorption are important (U.S. EPA, 1992), because toxicity results from exceeding the body's capacity for detoxification of cyanide, which occurs mainly in the liver. At high doses of cyanide, the availability of the sulfur donor needed for detoxification by the enzyme rhodanese can become rate limiting. If absorption of ingested cyanide proceeds too quickly, then the capacity of the liver to form thiocyanate via first-pass metabolism may be exceeded. In contrast, slow absorption of the same total oral load of cyanide may allow complete metabolism by the liver. Similarly, an acute cyanide dose is more toxic when administered by inhalation compared with the same dose administered by ingestion, because the inhalation route bypasses first-pass metabolism in the liver and directly enters systemic circulation.

The significant impact of absorption on the rate of detoxification of cyanide is responsible for the observation that median lethal dose (LD$_{50}$) values for NaCN (presented below) are *lower* than the acute and chronic LOAEL values. The LD$_{50}$ values are based on bolus doses that result in rapid absorption of a large amount of cyanide that overwhelms the detoxification capacity of the body. In contrast, the acute and chronic LOAEL values are based on cyanide administration at a lower dose rate over the course of a day. This slower dose rate means that the body is able to detoxify higher doses of cyanide (on the basis of administered mg/kg) without being overwhelmed, and thus, it can handle a higher total dose load.

Acute oral LD$_{50}$ values for cyanide in rats range from 3 mg/kg (Ballantyne, 1988) to 8 mg/kg (Smyth et al., 1969) for cyanide administered as NaCN. Single daily doses of 4 mg/kg in rats and 6 mg/kg in mice as KCN resulted in 95% mortality (Ferguson, 1962). Dermal LD$_{50}$ values in rabbits range from 4.1 to 8.9 mg/kg. Clinical signs observed following single dermal

doses ranging from 0.9 to 2.5 mg/kg include rapid breathing, dizziness, weakness, convulsions, and loss of consciousness (ATSDR, 2006).

Palmer and Olson (1979) administered KCN to groups of seven male Sprague-Dawley rats at 0 or 200 ppm in drinking water or 0 or 200 ppm in feed for 21 days. Using an average body weight of 0.12 kg and a water consumption rate of 0.17 L/kg-day as per U.S. EPA (1988), this assessment estimated the daily CN^- intake to be approximately 14 mg/kg-day. The only endpoints evaluated were body weight gain and liver weight. A statistically significant 17% increase in absolute liver weight was observed relative to controls; thus, the LOAEL was the single dose tested, 14 mg/kg-day.

The dietary part of this study was inadequate for evaluation of toxicity due to instability of cyanide concentrations in feed. Although CN^- ingestion was estimated at 9 mg/kg-day by using default assumptions for body weight and food consumption (U.S. EPA, 1988), the study authors noted that subsequent analysis of cyanide in feed resulted in <20% recovery of the predicted value compared to 95% recovery for cyanide added to feed immediately prior to analysis. Therefore, due to uncertainties regarding actual animal dosage, a LOAEL could not be identified from the dietary part of this study.

Sousa et al. (2002) administered KCN to adult male Wistar rats in drinking water at target doses of 0, 0.3, 0.9, 3.0, or 9.0 mg/kg-day for 15 days, equivalent to CN^- doses of 0, 0.12, 0.36, 1.2, and 3.6 mg/kg-day. There were 10 rats/group, except for the high-dose group, which included 6 rats. Weight gain was significantly decreased at the high dose to about a third that of the controls; however, weight gain was normal in the next lower dose group. There were no effects on serum levels of T_3, T_4, or serum levels of ALT, while AST exhibited sporadic statistically significant changes that were determined not to be dose related. Serum levels of urea or creatinine were unaffected by treatment. "Moderate" to "severe" congestion and cytoplasmic vacuolization of the epithelial cells of the proximal tubules were observed in the kidneys of rats at the two highest doses. Hydropic degeneration of hepatocytes was also noted at the highest dose. Reabsorption vacuoles were observed in the thyroid gland of animals in all groups, including controls, and increased in severity with increasing dose. However, quantitative incidence or severity data for the histopathologic observations were not reported. Based on moderate kidney vacuolization and congestion, a NOAEL of 0.36 mg/kg-day and a LOAEL of 1.2 mg/kg-day CN^- were identified from this study.

Kreutler et al. (1978) evaluated the short-term effects on the thyroid of oral exposure to cyanide. Male weanling albino rats (strain not specified; 10–24 animals/group) were fed diets containing either low protein (2% casein) or normal protein (20% casein) for 2 weeks; treated rats received the same diets supplemented with 0.2% KCN (equivalent to 99 mg/kg-day CN^-, using an average body weight of 95 g and average food consumption rates [U.S. EPA, 1988]). Additional groups were administered the low-protein diet with or without KCN and with or without iodide supplementation. Body weights and food consumption were recorded. Blood

was collected and evaluated for serum TSH levels. Thyroids were removed and weighed. No difference in body weight was observed among cyanide-treated rats and their respective control groups. Rats treated with cyanide on the low-protein diet had significantly elevated serum TSH levels and increased thyroid weights compared to the low-protein control group; supplementation with iodide in addition to cyanide eliminated these effects. There was no effect on serum TSH or thyroid weight in the cyanide-treated rats on the normal-protein diet. This study suggests that severely protein- and iodine-deficient diets are likely to increase the sensitivity of the thyroid gland to cyanide ingestion.

4.4.2. Acute Inhalation Studies

Relatively few inhalation studies providing quantitative data are available in animals exposed repeatedly to HCN. Some studies of acute exposure are available in rats, rabbits, and monkeys (Bhattacharya et al., 1994; Purser et al., 1984; Hugod, 1981). Inhalation LC_{50} values reported in animals range from 151 to 579 mg/m^3 HCN in various species (ATSDR, 2006). These studies provide limited information because sample sizes were either small (Purser et al., 1984) or only a single organ or endpoint was assessed (Bhattacharya et al., 1994; Hugod, 1981; Valade, 1952).

Purser et al. (1984) exposed cynomolgus monkeys individually to 100, 102, 123, 147, or 156 ppm HCN for up to 30 minutes. These concentrations correspond to 111, 113, 136, 163, or 172 mg/m^3 HCN. A single monkey was exposed per concentration, with one monkey exposed to both 100 and 147 ppm in separate experiments. There was no control group. The time to incapacitation decreased with increasing exposure levels and ranged from 8 to 19 minutes. The authors noted that three of the exposures (exposure levels not reported, presumably the three highest concentrations) were terminated within 30 minutes due to the severity of the symptoms. The observed symptoms included hyperventilation, decreased and arrhythmic heart rate, loss of muscle tone and reflexes, and convulsions. Blood cyanide levels reached steady state within 10 minutes. There was no correlation between air concentration and blood cyanide levels.

Bhattacharya et al. (1994) investigated the effects of inhalation of 55 ppm HCN (61 mg/m^3 HCN) for 30 minutes on the pulmonary mechanics of six male Wistar rats.[3] In treated animals, the airflow was increased (20%), accompanied by increased transthoracic pressure (40%) and tidal volume (50%). The respiratory rate, compliance, and minute volume decreased 50, 60, and 25%, respectively, accompanied by a decrease in pulmonary phospholipids (i.e., surfactant) of about 10–30%. Other effects of cyanide were not evaluated.

Extensive involvement of the CNS in cyanide toxicity was demonstrated by Valade (1952), who exposed groups of four dogs to 50 mg/m^3 (45 ppm) HCN for a varying number of 30-minute exposure periods conducted at 2-day intervals. Clinical signs included tremors,

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[3]Data for the investigated parameters were presented graphically and thus, the magnitudes of change were estimated for this review.

stiffness, ataxia, dyspnea, vomiting, and diarrhea. In the longest-term exposure of 36 days (consisting of 19 exposure periods), two of the four dogs died. Necropsies of the dead and surviving dogs all showed histopathology in the brain consisting of vasodilation, hemorrhages, and various cellular lesions.

Myocardial morphology in rabbits was investigated following inhalation of HCN as part of a study attempting to identify constituents of tobacco smoke responsible for the increased risk of cardiovascular disease observed in smokers (Hugod, 1981). Male rabbits (22/group) were exposed to 0 or 0.5 ppm HCN for a period of 4 weeks, after which the animals were sacrificed and examined for myocardial abnormalities. Following blinded morphologic examination, no significant effects of cyanide were detected on myocardial ultrastructure.

4.4.3. Neurotoxicity Studies

Crampton et al. (1979) reported on a study in which baboons (7–10/group) were fed a low cobalamin (vitamin B_{12}) diet that was either supplemented with hydroxocobalamin (control) or unsupplemented. Treated animals received 1 mg/kg-day KCN subcutaneously for 5 days. The body weight of treated animals (with and without hydroxycobalamin supplementation) did not differ from that of untreated animals. No neurological effects were evident from nerve conduction measurements or in extensive histopathologic examination of the nervous system, apparently the only organ system examined.

As described in Section 4.1.2, neurological symptoms have been reported in populations that traditionally consume foods with high concentrations of cyanogenic glycosides, such as cassava (ATSDR, 2006; Banea-Mayambu et al., 1997; Ministry of Health Mozambique, 1984; Osuntokun, 1973). Effects include spastic paraparesis, ataxic tropic neuropathy, optic atrophy, and decreased nerve conduction velocity. Osuntokun (1973) reported that the neurological effects correlated with blood thiocyanate levels, but other reports found no correlation between disease severity and thiocyanate level (Ministry of Health, Mozambique, 1984). Several studies (Oluwole et al., 2003; Banea-Mayambu et al., 1997; Kamalu, 1993; Olusi et al., 1979) indicated that constituents of cassava other than cyanide, such as the parental cyanogenic glycoside, linamarin, may directly contribute to the characteristic endemic neurotoxicity observed in these populations. Specifically, an ecological epidemiologic study conducted in Zaire (Banea-Mayambu et al., 1997) indicated that prevalence of this endemic neuropathy was more closely correlated with urinary linamarin than urinary thiocyanate.

Fechter et al. (2002) evaluated the effect of HCN exposure on hearing loss and its interaction with noise-induced hearing loss. Male Long-Evans rats were exposed to HCN for 3.5 hours/day at concentrations of 0, 10, 30, or 50 ppm (equivalent to 0, 11, 33, or 55 mg/m^3 HCN, respectively), to noise alone (i.e., 100 dB volume octave band noise for 2 hours/day unaccompanied by cyanide exposure), or to noise plus 0, 10, 30, or 50 ppm HCN. Groups of 16 animals were exposed to air alone (0 ppm HCN) or noise alone, and groups of 6–12 animals

were exposed to HCN or HCN plus noise. Hearing loss was assessed 4 weeks after exposure by evaluating pure tone compound action potential (CAP) thresholds at frequencies ranging from 2 to 64 kHz (i.e., measuring the response at low through high pitches). The CAP threshold is a measure of change in the electrochemical response of nerve cells in response to auditory stimulation, a response that is considered to be a measure of cochlear function. This approach was used in order to evaluate permanent hearing loss rather than the transient loss that occurs immediately after exposure. Histologic analysis was also conducted on unexposed rats and on rats exposed to noise alone or in combination with 10 or 30 ppm HCN (three to four rats per group).

CAP thresholds were not affected by exposure to 10 or 30 ppm HCN. At 50 ppm HCN (in the absence of noise), CAP thresholds were slightly elevated, but significant differences among treated groups relative to control were not observed (using ANOVA for repeated measures). As expected, noise alone did increase the CAP threshold, indicating hearing loss. In the groups exposed to noise and HCN, there was a concentration-related increase in the CAP threshold at frequencies of 12–40 kHz, with statistically significant differences at 30 and 50 ppm as compared with controls. These data indicate that HCN can potentiate noise-induced hearing loss, but they do not indicate an effect of HCN alone on hearing loss.

In a related study from the same laboratory, i.p. injection of rats with 7 mg/kg KCN (2.8 mg/kg CN^-) caused significant transient hearing loss (Tawackoli et al., 2001). The authors also found that, in the absence of noise, auditory function recovered as cyanide was eliminated from the blood. These studies together suggest that hearing loss from cyanide exposure is a potentially sensitive neurological marker of toxicity. The return of function with the elimination of cyanide from the blood raises the question of whether a permanent effect would occur under conditions of high noise exposure and prolonged elevation of blood cyanide levels.

4.4.4. Immune Endpoints

Studies specifically designed to evaluate immune endpoints have not been located in the HCN database. Additionally, no functional immune measures were identified in the database. Limited information on immune endpoints exists from human occupational studies and animal studies. El Ghawabi et al. (1975) found that the percentage of lymphocytes in peripheral blood was statistically significantly elevated over controls in workers occupationally exposed to HCN (7–12 mg/m^3) for 5–15 years. The percentage of lymphocytes in exposed workers was 42% (range 32–50%) compared to 30% in controls (range 26–40%). The total number of leucocytes did not differ between groups. The biological significance of this magnitude of change in the relative percentage of lymphocytes is unclear, as is the impact of other chemicals to which the workers were concomitantly exposed. Another occupational study (Blanc et al., 1985) examined workers an average of 11 months after cessation of exposure to average concentrations of

17 mg/m^3 (15 ppm) for a mean duration of 11 months. Analyses included a complete blood count and differential with no significant findings reported for these endpoints.

There are no animal inhalation studies that evaluate the immunotoxicity of HCN, but inhalation studies on related compounds are available. These studies have evaluated limited immune-relevant endpoints and are mostly negative. A 3-month inhalation study of ACH (HCN exposure equivalent of up to 18 mg/m^3 or 16 ppm) in rats examined spleen weight and gross and microscopic histopathology of the spleen, lymph nodes, and thymus. In addition, hematology was examined, including white blood cell (WBC) and differential WBC counts. No changes were seen in these endpoints (Monsanto Co., 1985a, b). Six-month inhalation studies of (CN)$_2$ inhalation in male rats and monkeys exist (HCN exposure equivalent of 28 mg/m^3 or 25 ppm). Gross necropsy was performed on the spleen and bone marrow. No changes were seen in these endpoints in either species (Lewis et al., 1984).

Oral studies of cyanide have examined limited immune endpoints. Three-month drinking water studies in rats and mice (NTP, 1993) with doses up to 12.5 mg/kg-day in rats and 24 mg/kg-day in mice examined immune organs (spleen, thymus, bone marrow, and lymph nodes) and conducted hematology, including WBC and differential WBC counts. NTP (1993) did not demonstrate significant changes in any of these endpoints. Additionally, a 2-year oral study in rats with doses up to 10.8 mg/kg-day examined spleen, thymus, and hematology endpoints and did not note immunological effects (Howard and Hanzal, 1955).

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genotoxicity

KCN was not mutagenic in *Salmonella typhimurium* strains TA82, TA97, TA102, TA98, TA100, TA1535, TA1537, or TA1538 in the reverse mutation assay with or without metabolic activation (De Flora et al., 1984; De Flora, 1981). NaCN was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 with or without metabolic activation (NTP, 1993). A positive response was reported, however, for HCN in *S. typhimurium* strain TA100 without metabolic activation; adding metabolic activation reduced the magnitude of the positive response to 40% of what it had been without metabolic activation (Kushi et al., 1983). Negative results were obtained in the DNA-repair test in *Escherichia coli* strains WP67, CM871, and WP2 (De Flora et al., 1984) and in a test for inhibition of DNA synthesis in HeLa cells (Painter and Howard, 1982).

Overall, cyanide has tested negative in bacterial mutagenicity studies with and without S9 activation (NTP, 1993; De Flora et al., 1984; De Flora, 1981), although a positive result was obtained in *S. typhimurium* strain TA100 with and without S9 activation (Kushi et al., 1983). Neither standard chromosome aberration assays nor mammalian gene mutation studies of

cyanide are available. Cyanide was negative in assays for the production of DNA damage and repair (De Flora et al., 1984; Painter and Howard, 1982).

4.5.2. Acute Neurotoxicity

The mode of action for the acute toxicity of cyanide is well understood (Klaassen, 2001; Hall and Rumack, 1990). Cyanide is considered a chemical asphyxiant because it impairs aerobic metabolism without affecting oxygen delivery to the tissues. It has a high affinity for iron in the ferric state, resulting in binding to and inactivation of tissue cytochrome c oxidase. Since cytochrome c oxidase normally accepts oxygen from the blood and functions as an electron acceptor in cellular energy production, this inactivation inhibits cellular respiration. As anaerobic metabolism proceeds, blood levels of pyruvic acid, lactic acid, and NADPH rise; the ATP/adenosine diphosphate (ADP) ratio decreases. The earliest effects of acute cyanide toxicity occur in organs with high aerobic energy demands, particularly the brain and heart. The inhibition of oxygen use by cells causes oxygen tension to rise in the peripheral tissues, which results in a decrease in the unloading gradient for oxyhemoglobin. Thus, oxyhemoglobin is present in the venous blood. In addition to cytochrome c oxidase, cyanide binds to other metalloproteins and other cellular molecules, including catalase, peroxidase, methemoglobin, and hydroxycobalamin; this binding also contributes to the symptoms of acute cyanide toxicity.

Cyanide also stimulates the release of secondary neurotransmitters and catecholamines from the adrenal glands and adrenergic nerves (Kiuchi et al., 1992; Kanthasamy et al., 1991). Thus, the cardiac effects and the peripheral autonomic responses observed following cyanide exposure appear to be due to the increase of plasma catecholamine levels. CNS necrosis and demyelination caused by cyanide may be due to vasoconstriction and low blood flow in the brain, resulting from low carbon dioxide levels (Brierley et al., 1976). Alternatively, the decreased ATP/ADP ratio may alter energy-dependent calcium homeostasis in nerve cells (Johnson et al., 1986). Thus, the acute effects of cyanide result primarily from the interruption of aerobic metabolism and from the release of secondary neurotransmitters and catecholamines; these effects include altered respiration, vomiting, nausea, and weakness and ultimately convulsions, coma, and death.

4.5.3. Thyroid Disruption

The primary cyanide metabolite, SCN^-, has the same ionic charge and is of similar size as iodide. SCN^- competitively inhibits iodide uptake in the thyroid by the sodium-iodide (Na^+/I^-) symporter (NIS). Iodine is essential for the normal production of the thyroid hormones T_3 and T_4. The NIS is a transmembrane protein that actively transports iodide from the bloodstream, against an electrical and chemical gradient, and concentrates it in the thyroid gland. The human NIS has been reported to have greater affinity for thiocyanate than for iodide (De Groef et al., 2006; Tonacchera et al., 2004; Wolff, 1998). In addition to reducing iodide uptake by the

thyroid, thiocyanate may also cause iodide already accumulated in the thyroid to be discharged (Wolff, 1998). Additional compounds that have a similar mode of action (i.e., competitive inhibition of the NIS) include perchlorate, nitrate, chlorate, and fluoroborate; however, each of these compounds has differing affinities for the NIS and thus different potencies of iodine uptake inhibition (Tonacchera et al., 2004; Van Sande et al., 2003; Greer et al., 1966). For example, perchlorate has been estimated to be 15–20 times more potent than thiocyanate in terms of iodine uptake inhibition (Tonacchera et al., 2004; Greer et al., 1966).

The effect of thiocyanate on the thyroid gland is dose dependent and controlled by homeostatic processes that tightly regulate and control the synthesis of essential thyroid hormones in order to ensure a constant systemic supply to meet physiological needs (NRC, 2005). If thiocyanate interference with iodide uptake is of sufficient magnitude to decrease the production and secretion rate of thyroid hormones (T_4 and T_3), then circulating levels of these hormones decrease. Homeostatic mechanisms mediated mainly via the hypothalamo-pituitary-thyroid feedback axis are rapidly activated to modulate thyroid hormone synthesis (NRC, 2005; Hill et al., 1989). As the blood levels of these hormones drop, the hypothalamus, through the release of thyrotropin-releasing hormone, stimulates the pituitary gland to produce TSH. TSH stimulates the thyroid gland to increase the rate at which it produces and secretes thyroid hormones. Elevated TSH levels stimulate histologic changes meant to increase thyroid secretion, such as increased size and number of thyroid cells (Guyton and Hall, 2000). Clinically, this increased size and number of thyroid cells manifests as an enlarged thyroid gland (goiter). It is only when thiocyanate intake levels are sustained and high enough to overwhelm homeostatic processes that decreased synthesis and secretion of thyroid hormones would be expected to occur and thus result in hypothyroidism and effects secondary to hypothyroidism. This mode of action may be relevant to the thyroid effects observed in both humans and animals, including thyroid gland enlargement, decreased thyroid hormones, and increased TSH (Manzano et al., 2007; Banerjee et al., 1997; Kamalu and Agharanya, 1991; Jackson, 1988; Blanc et al., 1985; Philbrick et al., 1979; El Ghawabi et al., 1975).

4.5.4. Reproductive Effects

The NTP (1993) observed a suite of reproductive effects in rats and mice, including decreased epididymis weight, testis weight, and testicular spermatid count in rats and mice treated for 3 months with NaCN in drinking water. The mode of action of these reproductive effects is not well established. However, some data exist in hypothyroid animals, suggesting that disruptions in thyroid hormone levels may affect the male reproductive system. Studies in humans and animals have demonstrated that cyanide exposure can result in decreased thyroid hormone levels (Jackson, 1988; Philbrick et al., 1979; El Ghawabi et al., 1975). Therefore, it is possible that the observed reproductive effects following exposure to cyanide may be mediated through decreases in thyroid hormones mediated through the cyanide metabolite thiocyanate.

Thyroid hormones are important in the growth and development of a wide range of tissues, including the male reproductive tract (Kobayashi et al., 2007; Wistuba et al., 2007). Some reports investigating the developmental effects of hypothyroidism in animal models have indicated male reproductive effects, including altered maturation of male reproductive organs and impaired spermatogenesis (Wistuba et al., 2007; Del Rio et al., 2003; Maran and Aruldhas, 2002). Persistent neonatal hypothyroidism in animal models has been shown to result in reduced reproductive organ weight and decreased sperm count and motility (Sahoo et al., 2008; Hamouli-Said et al., 2007; Del Rio et al., 1998; Kumar et al., 1994). Conversely, transient neonatal hypothyroidism has been shown to cause increased testis size and increased sperm production (Sahoo et al., 2008; Joyce et al., 1993; Cooke, 1991). These experimental observations indicate that reproductive tissues are sensitive to thyroid hormone levels during development.

In addition to thyroid effects on the growth and development of the reproductive tissues, some research has suggested that the adult reproductive system is also modulated by thyroid hormones. In adults, proper thyroid function has also been shown to be important for maintenance of fertility in adult males and females (Trokoudes et al., 2006; Poppe and Velkeniers, 2004). Hypothyroidism in adult males has been noted to cause alterations in sex steroid hormone metabolism, spermatogenesis, and fertility (Krassas and Pontikides, 2004).

Mechanistic studies in adult animals have indicated that reproductive organs, including the testis and epididymis, may be sensitive to alterations in thyroid hormone levels. A study by De Paul et al. (2008) demonstrated staining for thyroid receptor protein and mRNA in the adult rat epididymis, which was shown to be increased in hypothyroid rats showing responsiveness of adult epididymis tissue in response to decreased thyroid hormone. Additional studies have found cellular and ultrastructural changes in the adult rat epididymis following induced hypothyroidism (following thyroidectomy) (Del Rio et al., 2003, 2001, 1979), some of which were reversible following supplementation with thyroid hormone (Del Rio et al., 1979). Similarly, another study found reduced epididymis weight in thyroidectomized rats, which was reversible following T_4 supplementation (Kala et al., 2002).

In summary, research exists to suggest that reproductive tissues in developing and adult animals are responsive to alterations in thyroid hormone levels. Additional evidence also exists to suggest that specific structural changes and decreased epididymis weight can be mediated through hypothyroidism in the adult animal. Though some information supports this hypothetical mode of action that reproductive effects observed in the NTP (1993) study may be due to alterations in thyroid function due to exposure to cyanide, specifically the cyanide metabolite thiocyanate, uncertainty exists due to the lack of any measurement of indicators of thyroid function, such as thyroid hormones (TSH, T_3, T_4) or thyroid weight (NTP, 1993).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

Tables 4-6 and 4-7 present summaries of noncancer effects from repeated oral and inhalation exposure to cyanide. Chronic and subchronic cyanide oral exposure studies in experimental animals indicate that the thyroid, CNS, and male reproductive organs are sensitive targets of toxicity (Manzano et al., 2007; Soto-Blanco et al., 2002a, b; NTP, 1993; Jackson, 1988). Information from human occupational studies suggests that subchronic and chronic inhalation exposure to cyanide may be associated with CNS symptoms (including headache, weakness, and changes in taste and smell) and thyroid alterations (enlargement, altered iodine uptake, increased TSH, and decreased T_3 and T_4) (Banerjee et al., 1997; Leeser et al., 1990; Blanc et al., 1985; El Ghawabi et al., 1975). Another study also suggests that chronic exposure to HCN in a metal-tempering plant may reduce pulmonary function in chronically exposed workers (Chatgtopadhyay et al., 2000).

Table 4-6. Summary of subchronic and chronic oral toxicity studies for cyanide in animals

Species, strain, sex	Reference	Dose (mg/kg-d CN⁻)	Route	Duration	Response at LOAEL	NOAEL	LOAEL	Comments
					**Subchronic and chronic**			
Pig (6 or 10/group; sex not specified)	Manzano et al. (2007)	0, 0.8, 1.6, 2.4	Diet; KCN	10 wk	Increased thyroid weight	1.6	2.4	All doses showed altered histology in thyroid, liver, kidney, and CNS (no incidences given for histologic lesions).
Rat, Wistar (6–7 males/ group)	Soto-Blanco et al. (2002a)	0, 0.06, 0.12, 0.24	Gavage	12 wk	Histopathologic changes in CNS	Not determined	Not determined	No incidences given; no changes in T_3 and T_4.
Rat, SD (26–40/group)	Leuschner and Neumann (1989)	0, 16, 32, 64	Drinking water; KCN	13 wk	Decreased body weight	16	32	Unpublished study.
Rat, F344 (10/sex/ group)	NTP (1993)	Males: 0, 0.16, 0.48, 1.4, 4.5, 12.5 Females: 0, 0.16, 0.53, 1.7, 4.9, 12.5	Drinking water; NaCN	13 wk	Decrease in cauda epididymis weight and sperm motility	Not determined	1.4	Thyroid hormones and thyroid weight not measured.
Mouse, B6C3F₁ (10/sex/ group)	NTP (1993)	Males: 0, 0.26, 0.96, 2.7, 8.6, 24.4 Females: 0, 0.32, 1.1, 3.3, 10.1, 28.8	Drinking water; NaCN	13 wk	Decrease in cauda epididymis and epididymis weight	8.6	24.3	Thyroid hormones and thyroid weight not measured.
Dog, mongrel (6 males/ group)	Kamalu (1993); Kamalu and Agharanya (1991)	0, 1.04	Diet; NaCN	14 wk	Casts in renal tubules, adrenal gland hypertrophy and hyperplasia, and decreased spermatids in stage VIII of the spermatogenic cycle; decreased T_3; increased thyroid weight	None	1.04	Dogs suffered from parasitic infections.

Table 4-6. Summary of subchronic and chronic oral toxicity studies for cyanide in animals

Species, strain, sex	Reference	Dose (mg/kg-d CN⁻)	Route	Duration	Response at LOAEL	NOAEL	LOAEL	Comments
Goat (6–8 males/group)	Soto-Blanco et al. (2002b)	0, 0.12, 0.24, 0.48, 1.2	Milk, drinking water	5 mo	Histopathologic changes in CNS	Not determined	Not determined	Inadequate dose-response characterization to identify NOAEL/LOAEL.
Pig (3/group, mixed sexes)	Jackson (1988)	0, 0.4, 0.7, 1.2	Gavage in water; KCN	6 mo	Decreased T_3 and T_4; behavioral changes	0.7	1.2	Single daily bolus dose; no other endpoints evaluated.
Rabbit (6 males/group)	Okolie and Osagie (2000, 1999)	0.2, 20	Diet; KCN	10 mo	Decreased body weight, focal liver necrosis, tubular and glomerular necrosis of kidneys, pulmonary edema and necrosis	0.2	20	Cyanide in control group from determination of basal amount in feed.
Rat, strain not specified (10 males/group)	Philbrick et al. (1979)	0, 44	Diet	11.5 mo	Vacuolation of spinal cord white matter; decreased T_4 at 4 mo and increased thyroid weight at 11.5 mo	None	44	
Rat (10/sex/group)	Howard and Hanzal (1955)	0, 4.3, 10.8	Diet	2 yr	None	10.8	None	
Reproductive and developmental								
Rat, Wistar (20 dams, 5 pups/ group)	Imosemi et al. (2005)	0, 20	Diet	Gestation, up to PND 50	Decreased body weight, brain, and cerebellar weight; altered cerebellar dimensions	None	20	Same study as Malomo et al. (2004); aggressive and restless behavior noted in treated dams.
Rat, Wistar (20 dams, 5 pups/ group)	Malomo et al. (2004)	0, 20	Diet	Gestation, up to PND 50	Reduced thickness of ML and increased thickness of EGL of cerebellum	None	20	Indicative of delayed maturation and migration of cerebellar cells.
Goat (5–8/group)	Soto-Blanco and Gorniak (2004)	0, 0.4, 0.8, 1.2	Gavage in water; KCN	D 24–150 (birth)	Increased T_3 in dams and offspring at birth	0.8	1.2	Some of the dams in the highest dose group experienced tremors and ataxia.
Goat (7/group)	Soto-Blanco and Gorniak (2003)	0, 0.4, 0.8, 1.2	Gavage in water	Lactation d 0–90	Vacuolation of thyroid, kidney epithelial cells, and hepatocytes in offspring and dams	Not determined	Not determined	Histological lesions reported in treated dams and offspring but incidence not given.

Table 4-6. Summary of subchronic and chronic oral toxicity studies for cyanide in animals

Species, strain, sex	Reference	Dose (mg/kg-d CN⁻)	Route	Duration	Response at LOAEL	NOAEL	LOAEL	Comments
Rat, strain not specified (20 females/ group)	Tewe and Maner (1981)	1.2, 21.6 (adults) 1.9, 34.3 (weanlings)	Diet; cassava and KCN	Throughout mating, gestation, and lactation	Decreased food consumption, growth rate, liver weight in weanlings	1.9	34.3	Control animals fed basal diet containing low-HCN cassava; treated animals fed basal diet supplemented with KCN.

Table 4-7. Summary of subchronic and chronic inhalation toxicity studies for cyanide in humans

Study population	Reference	Exposure	Duration	Response	NOAEL mg HCN/m^3	LOAEL mg HCN/m^3	Comments
Males n = 36 exposed workers n = 20 unexposed controls Electroplating workers across 3 factories	El Ghawabi et al. (1975)	7.07, 8.9, 11.5 mg/m^3 HCN (6.4, 8.1, 10.4 ppm)	5–15 yr	Thyroid enlargement, altered iodide uptake, and CNS symptoms; increased lymphocytes	None	7.07	Urinary thiocyanate correlated with HCN air concentration.
Males n = 63 HCN exposed workers n = 100 DPO plant workers	Leeser et al. (1990)	0.03–1.03 mg/m^3 HCN	1–32 yr mean: 12.6 yr	Increased self-reported symptoms, increased lymphocytes, decreased hemoglobin	None	1.03	Unpublished study; no change in T$_4$.
Males, n = 36 exposed workers, divided into low (n = 13) medium (n = 14) or high (n = 9) exposure Silver reclaiming facility	Blanc et al. (1985)	16.6 mg/m^3 HCN (15 ppm) 24-hr TWA taken 1 d after plant closed	0.5–21 mo; median: 8.5 mo; mean: 11 mo	Increased TSH, increased T$_3$ uptake, CNS symptoms	None	16.6	No thyroid enlargement seen; study conducted 11 months postexposure.

The CNS is a target of both acute and chronic cyanide exposure. Symptoms of severe CNS toxicity following acute cyanide exposure include respiratory depression, convulsions, coma, and death. Chronic and subchronic inhalation exposure in workers has been reported to result in symptoms, including headaches, weakness, nausea, and changes in taste and smell, at doses ranging from 1 to 17 mg/m^3 (Leeser et al., 1990; Blanc et al., 1985; El Ghawabi et al., 1975). Behavioral changes and CNS lesions have been reported in animals exposed orally to cyanide. Histopathologic effects on various CNS structures also have been observed following subchronic exposure in rats (Soto-Blanco et al., 2002a; Philbrick et al., 1979) and goats (Soto-Blanco et al., 2002b). Philbrick et al. (1979) reported that vacuolation was observed in the spinal cord white matter of rats treated with cyanide or thiocyanate for 1 year with 44 mg/kg-day. Studies by Soto-Blanco et al. (2002a, b) in rats and goats at doses of 0.24–1.2 mg/kg-day reported effects, including neuron loss in the hippocampus, spheroids on white and gray matter of the spinal cord, damaged Purkinje cells, and loss of white matter in the cerebellum; however, no quantitative data or statistical analyses were presented for these effects. Behavioral changes in pigs and rats have also been observed with oral exposure to cyanide. Jackson (1988) observed that pigs administered KCN equivalent to 0.4–1.2 mg/kg-day cyanide in the drinking water for 6 months exhibited increased flight response, a decrease in fighting, and a decrease in exploratory behavior. Additionally, pregnant rats treated with 20 mg/kg-day cyanide by gavage demonstrated aggressive and restless behavior (Imosemi et al., 2005). Though the mode of action of acute cyanide toxicity is well understood (Klaassen, 2001; Hall and Rumack, 1990), the mode of action of CNS changes observed with chronic cyanide exposure is unclear. It is plausible, due to the mode of action of cyanide of inhibition of ATP synthesis, that CNS changes upon chronic CN^- exposure may also be due to energy deprivation in areas of high metabolic activity in the brain. Conversely, a chronic study in rats found similarly increased vacuolation of spinal cord white matter, astrogliosis, and fluid accumulation compared to controls regardless of whether animals were treated with KCN or KSCN, indicating that these histologic lesions may be due to SCN^- (Philbrick et al., 1979).

The thyroid is also an organ sensitive to cyanide, particularly following long-term exposure. Cyanide's effects on the thyroid are mediated by its metabolite, thiocyanate. Thyroid enlargement and altered iodine uptake have been seen in workers exposed for 5–15 years to HCN at concentrations ranging from 7 to 12 mg/m^3 (El Ghawabi et al., 1975). A retrospective study of a group of 36 male former workers who had been exposed to HCN fumes in a silver-reclaiming facility for a mean duration of 11 months found that TSH levels, although still within normal levels, were statistically significantly elevated compared with those of controls (Blanc et al., 1985). Thyroid effects, including enlargement, decreased hormone levels, and altered histology have also been seen in experimental animals orally treated with cyanide (Manzano et al., 2007; Soto-Blanco and Gorniak, 2003; Jackson, 1988; Philbrick et al., 1979). Rats treated for 4 months at 44 mg/kg-day had significantly decreased plasma T_4 levels (53%) and decreased

T_4 secretion rates (68%) compared with those of controls; however, after 11 months of cyanide treatment, T_4 levels no longer differed from controls, although T_4 secretion rates were depressed 27%. At the termination of the study, thyroid weights were significantly increased by 43% in treated animals (Philbrick et al., 1979). A study in pigs found a dose-related decrease in T_3 and T_4 (23 and 13%, respectively) in animals administered 1.2 mg/kg-day CN^- (Jackson, 1988). Another study in pigs found increased thyroid weight (24%) in animals administered 4.3 mg/kg-day CN^- for 10 weeks, although significant alterations in thyroid hormones were not observed (Manzano et al., 2007). Histologic changes in the thyroid, characterized by an increased number of reabsorption vacuoles on the colloid of the thyroidal follicles, were observed in dams and offspring treated for 90 days with 0.4–1.2 mg/kg-day CN^- (Soto-Blanco and Gorniak, 2003). However, this study found significantly *increased* T_4 levels in dams treated with 1.2 mg/kg-day, which adds uncertainty to the interpretation of effects observed in this study. It is well established that the anti-thyroid effects of cyanide are due to its primary metabolite, SCN^-. Thiocyanate competitively inhibits the active transport of iodide into the thyroid gland, and thus, if homeostatic mechanisms of the thyroid are overwhelmed, the available concentration of the iodine-based thyroid hormones T_3 and T_4 can be decreased (Crump and Gibbs, 2005; NRC, 2005; Guyton and Hall, 2000).

Alterations in the male reproductive system have also been observed in some studies. Male reproductive effects were reported in rats and mice following exposure in drinking water (NTP, 1993) and in dogs following exposure in feed (Kamalu, 1993). Decreased epididymis, cauda epididymis, and testis weight, and decreased sperm counts were seen in rats treated with $\geq$12.5 mg/kg-day for 13 weeks (NTP, 1993). Decreased relative epididymis and cauda epididymis weights were also observed in mice treated with $\geq$24.3 mg/kg-day for 13 weeks (NTP, 1993). In the Kamalu (1993) study, dogs treated with 1.04 mg/kg-day for 14 weeks exhibited histologic changes in the testis, including a significantly decreased percentage of tubules in stage VIII of the spermatogenic cycle. The NTP (1993) authors suggested that the male reproductive effects may be related to perturbations in hormonal balance, although thyroid hormones or thyroid weight were not evaluated as part of the study. However, some data from hypothyroid animals suggest that thyroid disruption can result in reproductive changes, including sperm decrements and reproductive organ weight decreases (see Section 4.5.4). It is not known whether workers occupationally exposed to cyanide would be expected to suffer reproductive effects, since it does not appear that sperm or other reproductive parameters were investigated in the available occupational studies (Blanc et al., 1985; El Ghawabi et al., 1975).

Cyanide affects the kidneys and liver at doses similar to those that result in male reproductive toxicity, depending on the species tested. Kidney effects, characterized by casts in renal tubules, were reported in dogs following subchronic oral dosing at 1.04 mg/kg-day for 14 weeks (Kamalu, 1993). Rabbits dosed at 20 mg/kg-day for 40 weeks had evidence of tubular and glomerular necrosis (Okolie and Osagie, 1999). Additionally, goat dams and offspring

treated with 0.4–1.2 mg/kg-day throughout lactation were reported to have vacuolation of the kidney epithelial cells. Similarly, liver effects with cyanide exposure have been seen in rats, mice, rabbits, and goats. Goats treated with 0.4–1.2 mg/kg-day for 90 days were reported to have vacuolation of hepatocytes (Soto-Blanco and Gorniak, 2003). Focal necrosis was seen in rabbits treated with 20 mg/kg-day in the diet for 10 months (Okolie and Osagie, 1999), and increased liver weight was observed in female rats and mice administered 12.5 or 23.4 mg/kg-day, respectively, in drinking water for 13 weeks (NTP, 1993). The mode of action for the effects of cyanide on the liver and kidneys has not been identified. One hypothesis suggested by Okolie and Osagie (1999) is that interference with thyroid function by thiocyanate can lead to decreased tissue protein turnover, resulting in depressed growth, as well as liver and kidney effects. Additionally, high-dose exposure of cyanide in heavily oxygen-dependant tissues, such as the liver, may result in ATP deprivation of the tissues.

4.7. EVALUATION OF CARCINOGENICITY

Under the U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, there is "inadequate information to assess the carcinogenic potential" of cyanide. Studies examining cancer incidence in occupationally exposed cyanide workers are not available. Studies of cancer in populations exposed to thiocyanate via diet were limited to examinations of thyroid cancer, and the results are generally not positive (Bosetti et al., 2002; Kolonel et al., 1990), although one recent case-control study has associated high consumption of goitrogenic food and low iodine intake with increased incidence of thyroid cancer in women (Truong et al., 2010). The currently available data indicate that cyanide is not genotoxic. Bacterial mutagenicity assays, with and without activation, are predominantly negative (NTP, 1993; De Flora et al., 1984; De Flora, 1981). The only available chronic animal study of cyanide that analyzed a wide variety of tissues is an oral study in rats (Howard and Hanzal, 1955), in which tumors or lesions were not associated with either dose group following dietary administration of cyanide at doses up to 10.8 mg/kg-day for 2 years. This study is limited by small sample sizes (n = 10), histopathologic assessment of only a subset of potential target organs of carcinogenicity, and uncertainty regarding dose due to HCN volatility issues. Therefore, the available data for cyanide are inadequate to assess the carcinogenic potential of cyanide.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES
4.8.1. Possible Childhood Susceptibility

Due to the mode of action of the primary cyanide metabolite, thiocyanate, fetuses exposed in utero may be most susceptible to the effects of cyanide exposure, leading to potentially reduced thyroid hormone production during critical periods of brain development. The effects of significantly reduced thyroid hormone levels that result from untreated subclinical or clinical hypothyroidism are much more severe in the developing young than in adults (NRC,

2005; Guyton and Hall, 2000). While hypothyroidism in adults typically results in goiter (an enlarged thyroid), congenital hypothyroidism has been associated with stunted bodily growth and impaired mental development in fetuses, infants, and young children. Hypothyroidism has been associated with neurodevelopmental delay and functional and structural neurological deficits in young humans and/or rats (NRC, 2005). In human fetuses and neonates, the effects of severe congenital hypothyroidism associated with severe maternal hypothyroidism during pregnancy are irreversible and are characterized by long-term impairment of behavior, locomotor ability, speech, hearing, and cognition (Chan and Kilby, 2000). In moderately hypothyroid neonates born to mothers who were hypothyroid during pregnancy, prompt supplementation with thyroid hormone may restore neurodevelopmental function to some degree (NRC, 2005). A retrospective study of over 25,000 pregnant women indicated that mild subclinical maternal hypothyroidism can adversely affect neurological development. Children (aged 7–9 years) whose mothers had subclinical hypothyroidism during pregnancy were found to have IQ scores 7 points lower than the matched control children (Haddow et al., 1999). Another study observed that pregnant women with subclinical hypothyroidism were 3 times more likely to have a placental abruption (relative risk [RR] 3.0, 95% CI 1.1–8.2), a serious pregnancy complication associated with high perinatal mortality rates. This study also observed that pregnant women with subclinical hypothyroidism were twice as likely to have a preterm birth (RR 1.8, 95% CI 1.1–2.9) than controls (Casey et al., 2005). These authors speculated that the reduced IQ of children born to mothers with subclinical hypothyroidism may be related to the effects of prematurity; however, the mean gestational week at delivery in hypothyroid women in Haddow et al. (1999) was no different from controls. Additional studies support the indication of lower neurodevelopmental scores in offspring of women with free T_4 levels in the lowest 10th percentile and normal TSH values during early gestation (Kooistra et al. 2006; Pop et al., 2003).

In rats, hypothyroidism during development has been associated with anatomical alterations in the cerebellum, including reduction of growth and branching of Purkinje cells, delayed proliferation and migration of granule cells, delayed myelination, and changes in synaptic connections among cerebellar neurons (Koibuchi and Chin, 2000). Although animal models may provide information on the potential neuroanatomical and neurophysiological effects of highly reduced maternal thyroid hormone levels during gestation, they are limited in the ability to assess subtle changes in neurodevelopment, cognition, and behavior that may occur in humans. Further, the homeostatic system in humans is regarded as more robust and elastic than that in rodents (NRC, 2005). Thus, animal models provide qualitative, but not quantitative, information on the effects of low human fetal availability of thyroid hormones during gestation and early development (Jahnke et al., 2004). The magnitude of decrease in serum T_4 levels that might result in neurodevelopmental effects during gestation and early childhood is not well characterized and would depend on many factors, including whether T_4 levels are reduced during critical windows of development, the extent of the ability of the fetus to produce its own thyroid

hormones to compensate for decreased maternal thyroid hormone availability, and the nature and extent of other nutritional deficiencies associated with thyroid hormone production (e.g., iodine, selenium).

Although cyanide is a known neurotoxicant, a dose-response characterization of neurodevelopmental toxicity resulting from competitive inhibition of iodide uptake in the thyroid gland by its thiocyanate metabolite has not been demonstrated. This relationship is complicated by the interdependence of several factors including the intake of iodine, protein, and selenium (and likely additional micronutrients), exposure to other chemicals that modify thyroid function, and pre-existing thyroid conditions (Pearce and Braveman, 2009; Triggiani et al., 2009). However, based upon the mode of action of thyroid disruption and studies following offspring neurodevelopment and pregnancy outcomes of hypothyroid mothers, it is clear that the developing fetus and infant are at a disproportionately high risk from chemicals such as cyanide, which antagonize thyroid function.

4.8.2. Possible Gender Differences

Experimental animal studies have indicated that male reproductive toxicity is a target of chronic cyanide toxicity in rats, mice, and dogs (Kamalu, 1993; NTP, 1993). NTP (1993) found reduced testicular spermatid count in male rats and decreased reproductive organ weights, including the epididymis and testes, in both rats and mice. However, effects on female reproductive organs were not reported in rats or mice at any dose tested (NTP, 1993). Few studies identifying effects from cyanide exposure studied both sexes; therefore, information suitable to assess gender differences is lacking.

Population-based studies of thyroid disorders indicate gender-related trends, with women being more likely to develop goiter and hypothyroidism (NLM, 2008b; Knudsen et al., 2002). Additionally, analysis of a large data set from the 2001–2002 National Health and Nutrition Examination Survey (NHANES) indicated statistically significant associations between urinary levels of perchlorate (which shares a common mode of action of competitive iodine inhibition with SCN^-) and changes in TSH and T_4 levels in women but not in men, with the association strongest in women with low iodine intake (Steinmaus et al., 2007; Blount et al., 2006). Therefore, women, especially those with low iodine intake, may be more susceptible to thyroid disruption compared to men.

4.8.3. Other Susceptible Populations

Due to the ability of thiocyanate to competitively inhibit iodine uptake, people with preexisting hypothyroidism or low iodine intake, especially pregnant and lactating women, may represent a susceptible population due to an increased need for iodine during these periods (WHO, 1994). Kreutler et al. (1978) observed that thyroid effects in rats induced by exposure to KCN could be attenuated by administering iodine concurrently with cyanide. Additionally, an

epidemiologic study by Brauer et al. (2006) demonstrates that populations with low ratios of urinary iodine to urinary thiocyanate are at increased risk of developing enlarged thyroid.

Populations with low iodine intake exposed to additional chemicals that operate by a similar mode of action as thiocyanate also represent an additional sensitive population because of expected additive effects. In addition to thiocyanate, other chemicals, such as perchlorate and nitrate, can act as competitive inhibitors of NIS, the membrane protein that actively transports iodine into the thyroid follicular cells (De Groef et al., 2006; Van Sande et al., 2003). A recent study by Steinmaus et al. (2007) investigated the relationship between urinary levels of thiocyanate and perchlorate and thyroid hormone levels in women with low iodine intake (categorized as <100 µg/L urinary iodine) by using cross-sectional human data gathered as part of NHANES. The authors found no association between urinary thiocyanate levels and T_4 or TSH levels in women with low iodine intake. However, an association was seen between exposure to perchlorate and decreased serum T_4 levels. The authors found that this observed association was strengthened when thiocyanate exposure was considered together with perchlorate.

The effect of co-exposures of cyanide with other chemicals is not completely understood. For example, carbon monoxide co-exposure with hydrogen cyanide in acute exposure scenarios, such as structural fires, is thought to potentiate effects including hypoxia and mortality (Eckstein and Maniscalco 2006; Moore et al., 1991; Norris et al., 1986); however, the impact of chronic, low level co-exposure of these two chemicals, such as through vehicle exhaust and tobacco smoke, is not known.

People with protein deficiency may also be more sensitive to the cyanide-induced thyroid effects. Kreutler et al. (1978) observed that rats on a low-protein diet (2% casein) demonstrated increased plasma TSH and thyroid weights following KCN administration, whereas rats on a normal-protein diet (20% casein) exposed to the same concentrations of cyanide did not develop these effects. Reduced availability of sulfur-containing amino acids in protein-deficient diets may impact the concentration of sulfur donors available for the detoxification of cyanide (Frakes et al., 1986). Studies in human populations ingesting large quantities of cyanide-containing foods, such as those made from cassava flour, also suggest that increased susceptibility to thyrotoxic effects are also associated with dietary deficiencies of protein, iodide, vitamin B_{12}, or other vitamins (Makene and Wilson, 1972; Osuntokun et al., 1969).

5. DOSE RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)
5.1.1. Choice of Principal Study and Critical Effect

The dose-response data available on subchronic or chronic oral exposure to cyanide are limited to experimental studies in animals. Though clinical data from several acute human exposures are available, no chronic or subchronic studies which provide dose-response information following oral exposure to cyanide in humans exist. Two chronic oral exposure studies exist for cyanide, Philbrick et al. (1979) and Howard and Hanzal (1955), both in rats, though they are limited by the use of only one high dose (Philbrick et al., 1979) or the failure to detect effects (Howard and Hanzal, 1955). Additionally, there are two well-designed subchronic studies in rats and mice that tested multiple dose levels and examined an array of endpoints and tissues (NTP, 1993), and other subchronic studies in a variety of animal models assessing more limited endpoints and tissues (Manzano et al., 2007; Soto-Blanco et al., 2002a, b; Okolie and Osagie, 2000, 1999; Kamalu, 1993; Jackson, 1988). Furthermore, several developmental studies on oral cyanide exposure in rats and goats exist (Imosemi et al., 2005; Malomo et al., 2004; Soto-Blanco and Gorniak, 2004; Tewe and Maner, 1981).

Manzano et al. (2007) treated 6 or 10 pigs per group (sex not specified and number of animals unclear due to inconsistencies in reporting) with potassium cyanide (KCN) administered in the diet at 0, 1.4, 2.8, or 4.3 mg/kg-day for 10 weeks. An increase of 24% in thyroid weight was seen at 4.3 mg/kg-day. Histologic alterations were reported in the thyroid, liver, kidney, and CNS in all dosed animals. However, no incidence data or statistical analyses were provided for these histologic findings, precluding a characterization of the dose response for these effects. This study identified a LOAEL of 4.3 mg/kg-day and a NOAEL of 2.8 mg/kg-day for a statistically significant increase in thyroid weight. This study is limited by poor reporting of study design and observed histologic effects. Due to these limitations and the availability of studies demonstrating effects at lower levels, this study was not selected as the principal study.

Jackson (1988) evaluated the effects of gavage administration of KCN on behavior and thyroid function in miniature pigs. Doses of 0, 0.4, 0.7, or 1.2 mg/kg-day KCN were administered by gavage to three pigs per group (mixed sexes) for 24 weeks. Both T_3 and T_4 demonstrated a dose-related decrease (23 and 13%, respectively) that was statistically significant by week 18 of the study. Changes in thyroid hormones were portrayed graphically as means, without reporting variance or data for individual animals. The author concluded that the overall pattern of behavioral changes, characterized as an increased ambivalence and slower response to stimuli, was different in the highest dose group compared to control animals. Based on behavioral changes and decreased thyroid hormones, the LOAEL and NOAEL values are 1.2 and 0.7 mg/kg-day cyanide ion (CN^-), respectively. The biological significance of the behavioral

changes observed in this study is unclear. In addition, the utility of this study is limited by the use of bolus dosing. In comparison to relatively steady intake throughout the day via dietary administration, bolus dosing produces higher peak blood levels as the entire daily dose is rapidly absorbed. This difference is especially important considering that the toxicity of cyanide is highly rate dependent. Thus, the findings from bolus exposure to cyanide are considered less relevant to subchronic or chronic exposure conditions in humans. Due to the use of a bolus regimen, this study was not considered appropriate for selection as the principal study.

Soto-Blanco et al. (2002a) treated Wistar rats (six to seven per group) with 0, 0.06, 0.12, or 0.24 mg/kg-day by gavage for 12 weeks and reported histopathologic changes in the CNS. The same authors also conducted a 5-month drinking water study in female goats (six to eight per group) with concentrations ranging from 0 to 1.2 mg/kg-day (Soto-Blanco et al., 2002b). In these studies, the authors reported a variety of histopathologic changes in the CNS described as spheroids on the spinal cord, neuronal loss in the hippocampus, damaged Purkinje cells, gliosis, and loss of cerebellar white matter. However, the authors provided no quantitative data, precluding a dose-response characterization of the reported effects. The lack of quantitation of the observed histologic effects and the use of bolus dosing precluded further consideration as the principal study.

Kamalu (1993) evaluated the toxicity of sodium cyanide (NaCN) administered to 22-week-old mongrel dogs (six males per group) for 14 weeks. The diet was supplemented with NaCN corresponding to 0 or 1.04 mg/kg-day CN^-. Lesions in the kidneys and adrenal gland were reported at the only dose tested; however, no quantitation of these lesions was provided. In the testes, a specialized morphologic analysis indicated that the treated dogs had a significantly decreased percentage of tubules in stage VIII of the spermatogenic cycle, as compared with controls ($p < 0.01$). An evaluation of the thyroid of animals in this study was published by Kamalu and Agharanya (1991). Serum T_3 was significantly decreased (55%) and thyroid weight was significantly increased (23%) in the cyanide-exposed group. Based on thyroid enlargement and histopathologic changes in the kidneys, testes, and adrenal glands, the only dose tested, 1.04 mg/kg-day, was considered to be the LOAEL. The authors reported that the animals suffered from recurring parasitic infestations that required treatment with pharmaceuticals throughout the study. Therefore, the use of the data from the Kamalu (1993) and Kamalu and Agharanya (1991) studies are limited by the use of dogs of compromised health status and were not selected as the principal study for the derivation of the RfD.

An unpublished study by Leuschner and Neumann (1989) administered KCN to male Sprague-Dawley rats (26–40/group) in drinking water for 13 weeks. Administered doses were 0, 40, 80, or 160 mg KCN/kg-day or 0, 16, 32, or 64 mg CN^-/kg-day. Early mortality was observed at the high dose with 11 animals dying prematurely. Body weight was statistically significantly decreased 42% at the high dose and 15% in the mid dose. Organ weight changes were not observed at the lowest dose level tested (16 mg CN^-/day). Absolute thymus weight was

statistically significantly decreased (20%) at the mid-dose level (32 mg CN⁻/day). Statistically significant relative and absolute organ weight changes were seen at the highest dose level (64 mg CN⁻/kg-day), although these changes were inconsistent. At the highest dose, absolute heart, liver, spleen, kidney, and brain weights were statistically significantly decreased compared to the controls. However, when relative weights were calculated, all organs showed increased weight compared to controls (except for the thymus, which was decreased). A LOAEL of 32 mg/kg-day was identified based on decreased body weight. Due to the availability of studies demonstrating effects at lower doses, this study was not selected as the principal study.

Studies observing low-level developmental effects were also considered in the selection of the principal study and critical effect (Soto-Blanco and Gorniak, 2004, 2003). Soto-Blanco and Gorniak (2004) administered gavage doses of CN⁻ equivalent to 0, 0.4, 0.8, or 1.2 mg/kg-day throughout gestation (GD 24 to birth) to pregnant goats (six per group) and observed elevated T_3 (but not T_4) levels in dams and offspring tested at birth in the highest dose group. Another publication by the same authors (Soto-Blanco and Gorniak, 2003) treated goats with 0, 0.4, 0.8, or 1.2 mg/kg-day during lactation (PNDs 0–90) and identified vacuolation of kidney epithelial cells and hepatocytes in offspring and dams at unspecified doses. Incidence or severity of the reported histologic lesions was not provided, precluding any characterization of dose response. These studies are limited by the use of bolus doses and the lack of dose-response characterization, which precludes their selection as principal studies.

A study by NTP (1993) examined the toxicity of cyanide over a wide range of doses. NTP administered NaCN in drinking water to rats and mice (10/sex/group) at concentrations of 0, 0.16, 0.48, 1.4, 4.5, and 12.5 mg/kg-day CN⁻ in male rats; 0, 0.16, 0.53, 1.7, 4.9, and 12.5 mg/kg-day in female rats; 0, 0.26, 0.96, 2.7, 8.6, and 24.4 mg/kg-day CN⁻ in male mice; and 0, 0.32, 1.1, 3.3, 10.1, and 28.8 mg/kg-day in female mice. Reproductive effects were observed in male animals of both species, although rats appeared to be the more sensitive species. In rats, a statistically significant decrease in cauda epididymis weight (7%) was seen at doses ≥1.4 mg/kg-day. A 7% decrease in whole epididymis weight (as compared to cauda epididymis weight) was seen at 12.5 mg/kg-day. At the highest dose tested, 12.5 mg/kg-day, epididymis and cauda epididymis weights were decreased by 7 and 13%, respectively. Dose-related decreases in testis weight (8%), number of spermatid heads (14%), and spermatid concentration (14%) were also found to be significant at doses ≥12.5 mg/kg-day. No change in epididymal sperm count was observed at any dose; however, a statistically significant decrease in epididymal sperm motility was observed at doses ≥1.4 mg/kg-day CN⁻, although it did not appear to increase in severity with dose.

In consideration of the available studies reporting low-dose effects of chronic and subchronic oral exposure to cyanide in animals, the NTP (1993) study was chosen as the principal study. This study was well designed, with five dose groups of 10 animals per group per sex and species. Numerous tissues and endpoints were assessed, and methods and observed

effects were thoroughly reported. This study identified statistically significant male reproductive effects in rats and mice that increased in severity in a dose-dependent manner. The observed effects included decreased cauda and whole epididymis weights, decreased testes weight, and altered sperm parameters.

The reproductive effects observed by NTP (1993) are consistent with an effect on male reproductive endpoints, including organ weights and sperm parameters, although the magnitude of the effects alone may be insufficient to decrease fertility in rats. However, human males have markedly lower rates of sperm production and sperm counts compared with rats; thus, the potential impact of decrements in sperm quality in humans is considered to be greater than that of rats (U.S. EPA, 1996; Working, 1988). Furthermore, the cyanide database contains limited additional support for the specific endpoint of reproductive toxicity (Kamalu, 1993). Therefore, for the above reasons, NTP (1993) was chosen as the principal study, and all statistically significantly altered reproductive endpoints in rats and mice were benchmark dose (BMD) modeled and are presented in Section 5.1.2 and Appendix B.

EPA has selected decreased cauda epididymis weight as the critical effect because it was determined that this effect represents the most sensitive endpoint indicative of male reproductive toxicity. The cauda epididymis is one of the three primary subsections of the epididymis (along with the caput and corpus) and functions as the site of sperm storage and maturation. Because the cauda is part of the epididymis, these weights are not independent endpoints. BMD analysis of the observed reproductive data from rats and mice indicated decreased (absolute) whole epididymis and cauda epididymis weights to be the most sensitive reproductive effects observed. Points of departure (PODs) for these endpoints identified through BMD modeling were virtually identical. However, examination of the organ weight data from the principal study (NTP, 1993) indicated that data for decreased cauda epididymis weight was statistically significantly decreased (7%) at the lowest dose tested, whereas the decrease in whole epididymis weight (2%) was not. It is possible that use of whole epididymis weight may mask the effect first observed in the cauda region of the epididymis. Thus, decreased cauda epididymis weight was considered a more sensitive effect.

Altered sperm parameters support the observed decreases in reproductive organ weight seen in the NTP (1993) study. At the lowest dose examined, 1.4 mg/kg-day CN^-, a modest, but statistically significant decrease in epididymal sperm motility (4%) was observed, although its severity did not increase with dose. Additionally, at the highest dose tested, testicular spermatid count was statistically significantly decreased (14%). Epididymal sperm count was not affected at any dose tested.

Human male fertility is established to be lower than that of rodent test species; thus, human fertility may be more susceptible to damage from toxic agents (Working, 1998; U.S. EPA, 1996). Therefore, according to the U.S. EPA *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), statistically significant changes to measures in sperm parameters

including sperm count, morphology, or motility are considered adverse. However, no decrease in epididymal sperm count and only a modest decrease in sperm motility were observed at doses at which the cauda epididymis weight was statistically significantly decreased. The data from NTP (1993) suggest that cauda epididymis weight is an effect that precedes more severe decrements in sperm parameters, such as decreased testicular spermatid count, seen at the highest dose. Therefore, decreased cauda epididymis weight, the most sensitive effect observed in this study, was chosen as the critical effect.

Several studies in a variety of experimental models described above have reported LOAELs in the same range or lower than the reproductive effects identified by NTP (1993). However, interpretations of these studies are complicated by various issues, including limited reporting of methods, incidences, severity, and statistical significance of observed effects in addition to the use of bolus dosing regimens or animals of compromised health status (Manzano et al., 2007; Soto-Blanco and Gorniak, 2004, 2003; Soto-Blanco et al., 2002a, b; Kamalu, 1993; Jackson, 1988). Nevertheless, possible reference values based on the observed effects from Jackson (1988), Manzano et al. (2007), and Kamalu (1993) are presented for comparison in Section 5.1.4.

5.1.2. Method of Analysis

Statistically significantly altered reproductive endpoints in rats and mice observed in the NTP (1993) study were BMD modeled, including decreased cauda and whole epididymis weights, decreased testes weight, and altered sperm parameters (Table 5-1). Epididymal sperm motility, although statistically significantly decreased in all treated groups, did not exhibit a dose-response relationship, and thus, was not amenable to BMD modeling. For reproductive organ weight changes, absolute reproductive organ weights, as opposed to relative organ weights, were modeled. The absolute reproductive organ weight data presented by NTP (1993) showed dose-related decreases in rats and mice. Relative organ weights did not show a stronger dose-response than absolute organ weights. The study found body weight decreases in the highest dose group of male rats (6%, $p < 0.05$) and in the highest dose of male mice (4%, not statistically significant). Given the lack of substantive body weight changes in rats and mice, especially at lower doses that showed organ weight changes, relative organ weights were not analyzed further.

Table 5-1. Reproductive endpoints in male rats and mice observed following administration of NaCN in drinking water for 13 weeks

Concentration (ppm)	0	30	100	300
Rats				
Dose (mg/kg-d)	0	1.4	4.5	12.5
Weights (g)[a]				
Testis, absolute	1.58 ± 0.094	1.56 ± 0.063	1.52 ± 0.063	1.46 ± 0.063[c]
Epididymis, absolute	0.448 ± 0.019	0.437 ± 0.016	0.425 ± 0.022[b]	0.417 ± 0.016[c]
Cauda epididymis, absolute	0.162 ± 0.009	0.150 ± 0.013[b]	0.148 ± 0.013[b]	0.141 ± 0.009[c]
Spermatid measurements[a]				
Spermatid count (/10^{-4} mL)	89.28 ± 9.64	84.68 ± 12.74	82.90 ± 9.99	77.10 ± 6.96[b]
Mice				
Dose (mg/kg-d)	0	2.7	8.6	24.3
Weights (g)[a]				
Epididymis, absolute	0.049 ± 0.003	0.047 ± 0.006	0.047 ± 0.003	0.044 ± 0.003[b]
Cauda epididymis, absolute	0.017 ± 0.003	0.016 ± 0.000	0.015 ± 0.003[b]	0.014 ± 0.003[b]

[a]Values are mean ± SD.
[b]Significantly different from control at $p \leq 0.05$ using Shirley's test.
[c]Significantly different from control at $p \leq 0.01$ using Shirley's test.

Source: NTP (1993).

Continuous models (i.e., linear, polynomial, and power) with constant variance were fit to the data by using U.S. EPA BMD software (BMDS) (version 1.4.1). The other continuous model available in BMDS, the Hill model, was not fit to these data because fitting of the Hill model requires the estimation of four parameters (i.e., intercept, v, n, and k), which necessitates having a minimum of five dose groups in order to have adequate degrees of freedom for testing model fit. The NTP (1993) study employed only four dose groups, and thus, the Hill model could not be fit to these data.

A benchmark response (BMR) level was selected corresponding to a change in the mean response equal to 1 SD from the control mean for cauda epididymis weight. Information regarding the degree of change in this endpoint that is considered biologically significant was not available in the literature. Therefore, the BMR for continuous data of 1 SD change in the control mean was selected under the assumption that it represents a minimally biologically significant response level. By using the best fitting model for this data set, a 1 SD change was equivalent to a 7% decrease in cauda epididymis weight. The BMD modeling reports generated from modeling the reproductive endpoints from the NTP (1993) study are summarized below in Table 5-2.

Table 5-2. BMD modeling results for observed reproductive endpoints

Endpoint	Fitted model	Goodness-of-fit *p*-value	AIC	BMD	BMDL
Rats					
Cauda epididymis weight (absolute)	Linear	0.08	-312.75	8.4	5.6
	Polynomial	**0.11**	**-313.10**	**3.5**	**1.9**
	Power	0.08	-312.75	8.4	5.6
Epididymis weight (absolute)	Linear	0.22	-274.73	8.2	5.6
	Polynomial	**0.73**	**-275.64**	**3.2**	**1.8**
	Power	0.22	-274.73	8.2	5.6
Testis weight (absolute)	**Linear**	**0.82**	**-167.94**	**7.4**	**5.1**
	Polynomial	0.98	-166.32	5.3	2.4
	Power	**0.82**	**-167.94**	**7.4**	**5.1**
Spermatid concentration	**Linear**	**0.70**	**227.04**	**11.2**	**6.9**
	Polynomial	0.53	228.73	8.5	2.9
	Power	**0.70**	**227.04**	**11.2**	**6.9**
Mice					
Epididymis weight (absolute)	**Linear**	**0.67**	**-378.71**	**21.5**	**13.0**
	Polynomial	0.38	-376.73	20.5	6.6
	Power	**0.67**	**-378.71**	**21.5**	**13.0**
Cauda epididymis weight (absolute)	**Linear**	**0.87**	**-402.99**	**25.9**	**14.6**
	Polynomial	0.82	-399.67	16.3	5.2
	Power	0.69	-400.99	16.3	14.6

AIC = Akaike's Information Criterion; BMDL = 95% lower confidence limit on the BMD

Data source: NTP (1993).

All three models provided an adequate fit to this data set based on the goodness-of-fit statistic ($p \geq 0.1$). Of these three models, the polynomial model provided the best fit to the data based on this model's exhibiting the lowest Akaike's Information Criterion (AIC) and visual inspection of the plot of observed versus expected values across the three models. The detailed BMD modeling output for the selected polynomial model is presented in Appendix B. The BMD associated with a 1 SD decrease in cauda epididymis weight in rats is 3.5 mg/kg-day, and its 95% lower confidence limit (BMDL) is 1.9 mg/kg-day.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The BMDL of 1.9 mg/kg-day based on decreased cauda epididymis weight in rats was used as the POD for the derivation of the RfD. A total UF of 3,000 was applied to the POD of 1.9 mg/kg-day: 10 for interspecies extrapolation from animals to humans (UF_A), 10 for human

intraspecies variability (UF$_H$), 10 to account for the use of a subchronic study (UF$_S$), and 3 to account for database deficiencies (UF$_D$).

A default 10-fold UF was applied to account for uncertainties in extrapolating from laboratory animals to humans. Humans and laboratory animals have qualitatively similar absorption, distribution, metabolism, and excretion of cyanide. However, quantitative comparisons of toxicokinetic parameters are lacking. Additionally, a wide range of sensitivity to effects of cyanide has been observed between different species of experimental animals. The available data do not provide quantitative information on the difference in susceptibility to cyanide between rats and humans.

A default 10-fold UF was applied to account for variation in susceptibility among members of the human population (i.e., interindividual variability). Insufficient information is available to quantitatively estimate variability in human susceptibility to cyanide.

A 10-fold UF was applied for the extrapolation of subchronic-to-chronic exposure duration. The 91-day study by NTP (1993) falls well short of a lifetime duration. In addition, there is a lack of data on male reproductive parameters following chronic administration of cyanide, and the mode of action of the reproductive effects observed in this study is unclear. Therefore, it is unknown whether effects would be more severe or would be observed at lower doses with a longer exposure duration. For these reasons, the UF of 10 to extrapolate from a study with a subchronic duration was applied.

A 3-fold UF was applied to account for deficiencies in the cyanide toxicity database, including the lack of a multigenerational reproductive toxicity study and a sensitive neurodevelopmental toxicity study. The database includes limited human data from epidemiological studies of workers exposed by inhalation to hydrogen cyanide (HCN) (Chatgtopadhyay et al., 2000; Banerjee et al., 1997; Blanc et al., 1985; El Ghawabi et al., 1975). The database also includes studies in laboratory animals, including chronic and subchronic dietary exposure studies and developmental studies. The database includes oral toxicity studies in various animal species, including rats, mice, rabbits, dogs, pigs, and goats. A developmental study with skeletal and visceral examination has not been conducted for cyanide; however, developmental studies exist in rats and goats evaluating the thyroid, kidney, liver, pancreas, brain, and CNS of gestationally and/or lactationally exposed offspring (Imosemi et al., 2005; Malomo et al., 2004; Soto-Blanco and Gorniak, 2004, 2003; Tewe and Maner, 1981). External or overt developmental effects with cyanide exposure have not been noted at doses up to 1.2 mg/kg-day in goats (Soto-Blanco and Gorniak, 2004, 2003) and 21.6 mg/kg-day in rats (Imosemi et al., 2005; Malomo et al., 2004; Tewe and Maner, 1981). However, due to the mode of action of thiocyanate involving competitive iodine uptake inhibition and implications for neurotoxicity in the developing animal, the lack of a sensitive neurodevelopmental toxicity study to assess endpoints sensitive to thyroid disruption is an additional weakness in this database. The cyanide database is also lacking an appropriately designed multigenerational reproductive

toxicity study, although an assessment of reproductive organs was included as a component of the 13-week NTP (1993) studies in rats and mice, and testicular histology was also assessed in dogs (Kamalu, 1993). These studies in adult animals demonstrated low-dose reproductive effects. The observance of these effects reinforces the need for a multigenerational assessment of reproductive endpoints. Therefore, in consideration of the above data gaps, a 3-fold UF to account for deficiencies in the database was applied.

The UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 1 SD change from the control mean in epididymis weight was selected under an assumption that it represents a minimal biologically significant response level.

The oral RfD for CN^- was calculated as follows:

$$RfD = BMDL_{10} \div UF$$
$$= 1.9 \text{ mg/kg-day} \div 3,000$$
$$= 6.3 \times 10^{-4} \text{ mg/kg-day (rounded to } 6 \times 10^{-4} \text{ mg/kg-day)}$$

The RfDs for simple cyanide salts like NaCN and KCN, which freely dissociate into cyanide, are calculated from the RfD for CN^- by adjusting for molecular weight (i.e., the RfD is multiplied by the ratio of the total molecular weight of the compound to the molecular weight of the CN^-):

RfD for aqueous HCN [HCN(aq)] = $6.3 \times 10^{-4} \times 27/26 = 7 \times 10^{-4}$ mg/kg-day
RfD for NaCN = $6.3 \times 10^{-4} \times 49/26 = 1 \times 10^{-3}$ mg/kg-day
RfD for KCN = $6.3 \times 10^{-4} \times 65/26 = 2 \times 10^{-3}$ mg/kg-day
RfD for calcium cyanide[4] [Ca(CN)$_2$] = $6.3 \times 10^{-4} \times 92/(2 \times 26) = 1 \times 10^{-3}$ mg/kg-day
RfD for potassium silver cyanide[5] [KAg(CN)$_2$] = $6.3 \times 10^{-4} \times 199/26 = 5 \times 10^{-3}$ mg/kg-day
RfD for cyanogen[5] (CN)$_2$ = $6.3 \times 10^{-4} \times 52/26 = 1 \times 10^{-3}$ mg/kg-day

Use of the RfD for free cyanide to calculate RfDs of other cyanide compounds may be merited, but the ability of the individual cyanogenic species to dissociate and release free cyanide in aqueous solution (and at physiological pHs) should be taken into consideration. If dissociation of the compound is expected, then liberated cations should be considered for potential toxicity independent of CN^-. Also, some metallocyanides, such as copper cyanide, have chemical-specific data and are not included in this analysis.

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[4]Two molar equivalents of free CN^- released in water.
[5]One molar equivalent of free CN^- released in water.

5.1.4. RfD Comparison Information

Reduced reproductive organ weight, altered sperm parameters, increased thyroid weight, altered thyroid hormones, and altered testicular, kidney, and adrenal histopathology are observed low-level effects following subchronic oral exposure to cyanide (Manzano et al, 2007; Kamalu, 1993; NTP, 1993; Jackson, 1988). Table 5-3 provides a summary of potential PODs and resulting potential reference values derived from these endpoints. Additionally, Figure 5-1 provides a graphical representation of this information. This figure should be interpreted with caution since the PODs across studies are not necessarily comparable, nor is the confidence the same in the data sets from which the PODs were derived. The PODs presented in this figure are based on either a $BMDL_{1SD}$, NOAEL, or LOAEL (if no NOAEL was available).

Table 5-3. Potential PODs with applied UFs and resulting potential reference values

Effect	Potential POD	Species	UF[a]						Potential reference value
			Total	A	H	L	S	D	
Decreased testis weight	5.1[b]	Rat	3,000	10	10	NA	10	3	2×10^{-3}
Decreased epididymis weight	1.8[b]	Rat	3,000	10	10	NA	10	3	6×10^{-4}
Decreased cauda epididymis weight	1.9[b]	Rat	3,000	10	10	NA	10	3	6×10^{-4}
Decreased testicular spermatid concentration	6.9[b]	Rat	3,000	10	10	NA	10	3	2×10^{-3}
Decreased epididymis weight	13[b]	Mouse	3,000	10	10	NA	10	3	4×10^{-3}
Decreased cauda epididymis weight	14.6[b]	Mouse	3,000	10	10	NA	10	3	5×10^{-3}
Altered thyroid hormones, behavioral changes	0.7[c]	Pig	3,000	10	10	1	10	3	2×10^{-4}
Increased thyroid weight	1.6[d]	Pig	3,000	10	10	1	10	3	5×10^{-4}
Kidney, testes, and adrenal gland effects	1.04[e]	Dog	30,000	10	10	10	10	3	4×10^{-5}

[a]UFs: A = animal to human (interspecies); H = interindividual (intraspecies); L = LOAEL-to-NOAEL; S = subchronic to chronic duration; D = database deficiency.
[b]BMDL based on BMD modeling of a 1 SD change. Source: NTP (1993); NA, not applicable.
[c]POD based on NOAEL. Sources: Jackson (1988);
[d]POD based on NOAEL. Source: Manzano et al. (2007).
[e]POD based on LOAEL. Source: Kamalu (1993).
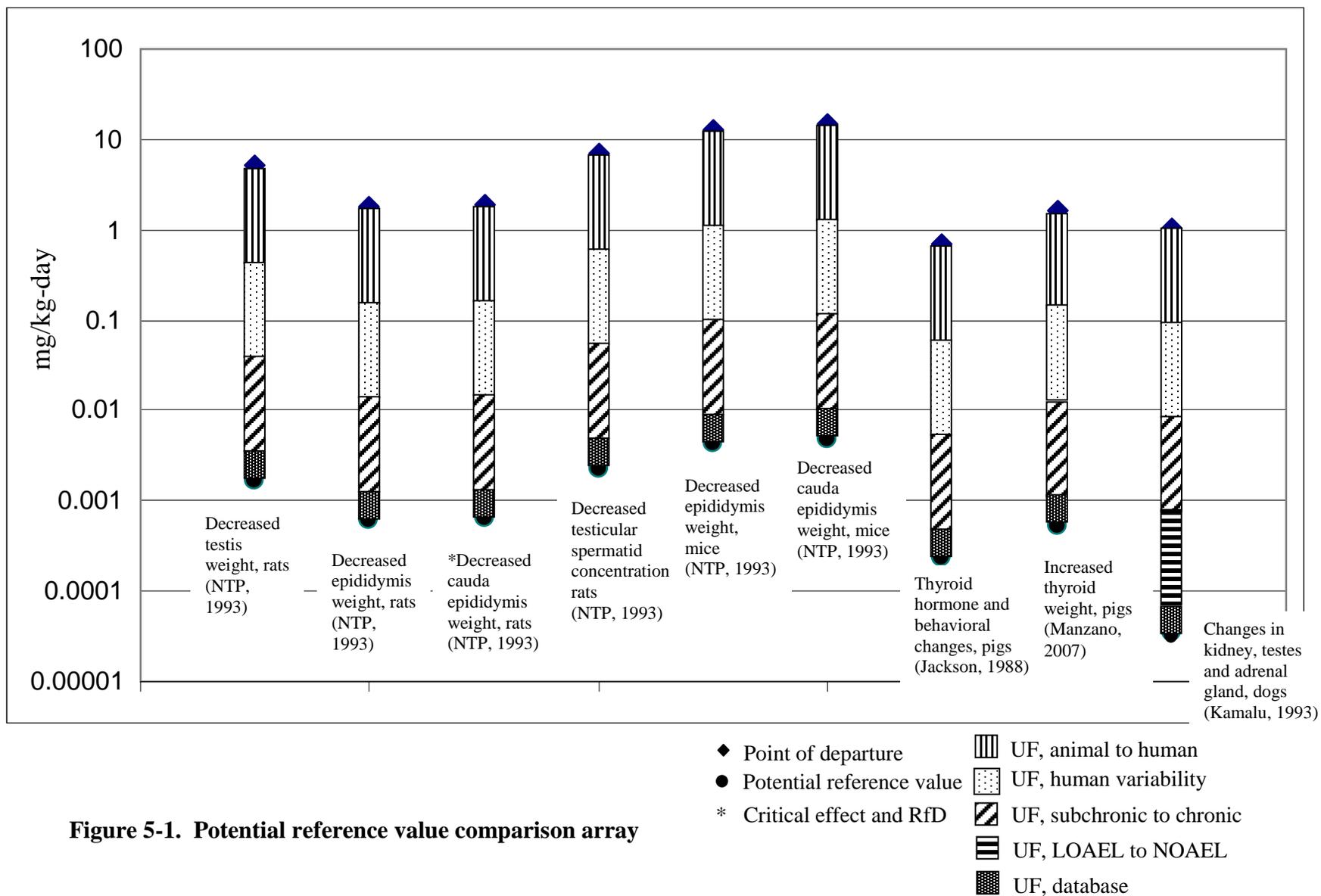
Figure 5-1. Potential reference value comparison array

Legend:
- ◆ Point of departure
- ● Potential reference value
- * Critical effect and RfD
- ⊞ UF, animal to human
- ⊡ UF, human variability
- ⊘ UF, subchronic to chronic
- ⊟ UF, LOAEL to NOAEL
- ▦ UF, database

Chart axis label: mg/kg-day

Bar labels:
- Decreased testis weight, rats (NTP, 1993)
- Decreased epididymis weight, rats (NTP, 1993)
- *Decreased cauda epididymis weight, rats (NTP, 1993)
- Decreased testicular spermatid concentration rats (NTP, 1993)
- Decreased epididymis weight, mice (NTP, 1993)
- Decreased cauda epididymis weight, mice (NTP, 1993)
- Thyroid hormone and behavioral changes, pigs (Jackson, 1988)
- Increased thyroid weight, pigs (Manzano, 2007)
- Changes in kidney, testes and adrenal gland, dogs (Kamalu, 1993)

A composite UF of 30,000 was applied to the LOAEL for the endpoints identified in the Kamalu (1993) study, which is generally considered too large to support derivation of a reference value. In the report, A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002), the RfD/RfC Technical Panel concluded that, in cases where the total UF is more than 3,000, it is unlikely that the study or database is sufficient to derive a reference value. Thus, the magnitude of the uncertainty associated with this specific study indicates it is insufficient to support derivation of a potential reference value.

Some indication of the confidence associated with the resulting potential reference values is reflected in the magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues associated with each data set, the rationale for the selection of the principal study, and the critical effect used to derive the RfD. As discussed in Section 5.1.1, among the studies considered, the subchronic study by NTP (1993) provided the data set for the derivation of the RfD.

5.1.5. Previous RfD Assessment

An RfD for cyanide of 2×10^{-2} mg/kg-day was posted on the IRIS database in 1985 and was based on co-principal studies (Philbrick et al., 1979; Howard and Hanzal, 1955). Howard and Hanzal (1955) fed food fumigated with HCN to rats for 2 years and identified the high dose of 10.8 mg/kg-day as the NOAEL. Philbrick et al. (1979) evaluated the thyroid and nervous system in rats fed KCN for 11.5 months. The single dose tested, 44 mg/kg-day, was identified as a LOAEL, based on myelin degeneration in the CNS and increased thyroid gland weight. These two studies were considered together to identify the critical effect and the POD. The previous RfD was based on a NOAEL of 10.8 mg/kg-day. The NOAEL was divided by a total UF of 500, including a factor of 10 each for extrapolation from animals to humans and intraspecies variability. A modifying factor of 5 was used to account for the apparent tolerance to cyanide when it is ingested with food compared with administration by gavage or by drinking water (U.S. EPA, 1992). However the apparent difference may have been due to instability of the cyanide concentrations in the feed rather than differences in bioavailability (Palmer and Olson, 1979).

Since the posting of the 1985 IRIS RfD for cyanide, several new subchronic studies by the oral route have been published. In addition, new data are available that evaluate potential health effects following perturbations of thyroid function, in general, in pregnant women and their offspring (see Section 4.8.1). Specifically, these studies show increased pregnancy complications and decrements in learning and memory in offspring of women with subclinical hypothyroidism (Kooistra et al., 2006; Casey et al., 2005; Pop et al., 2003; Haddow et al., 1999). The revised RfD was derived based on a subchronic study by NTP (1993), which indicated sensitive reproductive effects in rats and mice following a 91-day exposure to NaCN. In

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summary, the current assessment includes new chemical-specific data for cyanide and new information regarding the severity of low level thyroid perturbations in sensitive populations.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)
5.2.1. Choice of Principal Study and Critical Effect

Limited data are available on the effects of long-term inhalation exposure to cyanide. Several occupational studies investigated the effects of inhalation exposure to HCN, and three of these studies provide evidence of effects on the thyroid. One of these studies included environmental exposure data based on breathing zone samples for the individual study participants. There are no subchronic or chronic inhalation exposure studies of HCN in animals.

El Ghawabi et al. (1975) reported statistically significantly altered rates of iodide uptake by the thyroid, thyroid enlargement, and CNS symptoms (e.g., self-reported increased incidence of headache, weakness, and sensory changes for taste and smell) in workers (n = 36) exposed to HCN for 5–15 years in three electroplating factories. Individual breathing zone measurements of HCN were collected from each worker. The mean concentrations across factories ranged from 7.07 to 11.5 mg/m^3 HCN and the values for individual workers ranged from 4.6 to 13.7 mg/m^3 HCN. Urine SCN$^-$ levels, an indicator of internal dose, collected from workers were highly correlated with individual HCN exposure concentrations (see Figure 4-1). Twenty of the exposed workers (56%) were identified with mild to moderate thyroid enlargement. Radioactive iodine uptake measured following a 2-day break in HCN exposure indicated statistically significantly elevated iodide uptake after 4 hours (38.7 compared to 22.4%) and 24 hours (49.3 compared to 39.9%) as compared to controls.

Increased 24-hour uptake of radioactive iodide by the thyroid has been reported to occur in hyperthyroidism, iodine deficiency, and goiter (NLM, 2008a; Ravel, 1995). The study authors concluded that the increased iodine uptake observed in the workers following the 2-day cessation in exposure was a postexposure response to depletion of iodine in the thyroid. A similar increase in iodide uptake has been seen with perchlorate (ClO$_4^-$), another competitive inhibitor of iodide uptake, following cessation of exposure. Lawrence et al. (2000) measured iodine uptake in volunteers administered doses of ClO$_4^-$ at baseline, at 2 weeks of dosing, and then 2 weeks postexposure cessation. The authors reported that iodide uptake decreased 10–38% in the low- and high-dose groups (compared to baseline) at 2 weeks of dosing. Two weeks after exposure was discontinued, iodide uptake was statistically significantly increased 22 and 25%, indicating a rebound effect in iodide accumulation postexposure.

The lowest mean concentration of HCN recorded in the three factories, 7.07 mg/m^3, is designated as a LOAEL for thyroid enlargement and altered iodide uptake. The study authors also indicated some coexposure of the workers to gasoline, alkali, and acid during the electroplating process, although the magnitudes of these exposures were not quantified and it is unclear if these exposures would impact the observed thyroid effects.

Blanc et al. (1985) conducted a study of silver-reclamation workers (n = 36) examined an average of 11 months following exposure. The median length of employment was 8.5 months and mean exposure duration was 11 months. Workers were categorized into low-, moderate-, or high-exposure groups based on their primary job activities. Information on exposure was limited, as the plant had been shut down following the death of one worker from cyanide overexposure. Environmental monitoring conducted the day after the plant was shut down found that the 24-hour TWA HCN exposure was 16.6 mg/m^3. Serum TSH levels in workers were significantly elevated relative to laboratory controls. The authors noted a significant positive trend with increasing exposure level for self-reported weight loss and several symptoms, including dizziness, syncope, and nausea and vomiting. Serum TSH levels in workers were reported as being significantly elevated in workers relative to laboratory controls. T_3 uptake in the highest exposed workers (n = 9) was statistically significantly elevated compared to laboratory controls. The authors reported that this elevation may reflect a post-inhibitory response. Because there were multiple possible routes of cyanide exposure, including dermal exposure and contamination of food, and because earlier air levels were likely higher than the measured TWA concentration, the environmental monitoring data do not allow for the selection of a LOAEL. Additionally, this study examined workers an average of 11 months post-occupational HCN exposure and may therefore have missed effects that have the potential to regress following cessation of exposure. The observation of significant effects on the thyroid almost 1 year after cessation of exposure indicates that these observed thyroid effects are not transient. Due to limitations of this study based on its retrospective design and because El Ghawabi et al. (1975) reported significant effects at lower levels, this study was not selected for the derivation of the RfC.

An unpublished study by Leeser et al. (1990) compared the health of 63 male cyanide salt production workers with a control group of 100 British workers in a cross-sectional study. Cyanide workers were exposed for periods ranging from 1 to 32 years with a mean exposure duration of 12.6 years and mean breathing zone concentrations of cyanide up to 1 mg/m^3. Several hematological parameters in cyanide workers were statistically significantly elevated compared to controls, including hemoglobin (15.57 vs. 15.08 g/dL), ratios associated with hemoglobin, such as MCH and MCHC, and lymphocyte count (2.87 compared to 2.55×10^9/L). However, the biological significance of these slight elevations in hematological parameters is unclear. Serum T_4 levels in cyanide-exposed workers were decreased in controls, but the difference was not reported as statistically significant by the study authors (85.13 ± 2.51 vs. 89.04 ± 1.81 nmol/L). Additionally, serum T_4 was below the clinical reference range (60–160 nmol/L) in 3 cyanide-exposed workers. Other commonly administered, and more sensitive, tests for thyroid function, including TSH, free T_4, or iodide uptake, were not measured. It is unclear whether a NOAEL for thyroid effects can be established by this study as only one, relatively insensitive indicator of thyroid function was measured. A LOAEL of 1 mg/m^3 cyanide for

increased lymphocyte count and increased hemoglobin concentration was identified. A NOAEL for thyroid effects was not identified from this study based on the lack of measurement of sensitive thyroid parameters, although overt hypothyroidism was not observed. Due to the failure of the study authors to include additional, more sensitive thyroid function tests, as well as the unclear biological significance of the hematological effects observed, this study was not selected as the principal study.

In another occupational study of electroplating workers exposed to HCN, workers ($n = 35$) exposed for 5 years had significantly decreased T_3 (48%) and T_4 (37%) and significantly increased TSH (142%) as compared to controls (Banerjee et al., 1997). Serum SCN^- was elevated in workers compared to controls. A significant negative correlation between serum T_4 and SCN^- concentrations and a significant positive correlation between TSH and SCN^- concentrations were observed. However, no information was provided on exposure levels; therefore, no NOAEL or LOAEL could be identified from this study.

Chandra et al. (1980) reported on a group of 23 electroplating workers chronically exposed to average breathing zone concentrations of 0.15 mg/m^3 HCN. The authors noted that the workers complained of symptoms typical of cyanide poisoning, but provided no additional information on specific symptoms or further analysis. In the absence of information on measured effects, no NOAEL or LOAEL could be identified from this study, precluding its use in a quantitative risk assessment.

Chatgtopadhyay et al. (2000) found some indication of decreased pulmonary function in workers at a metal-tempering plant. Specifically, the authors observed decreased pulmonary function in 24 workers exposed for a mean duration of 24 years. This study provided no information regarding the environmental exposure levels of the workers, and thus, no NOAEL or LOAEL could be identified, limiting this study's utility for risk assessment.

Considering the availability of studies in the HCN database, El Ghawabi et al. (1975) was chosen as the principal study. The results of this study indicate that chronic, low-level exposure to cyanide was associated with thyroid enlargement and altered iodine uptake in humans. This study examined workers exposed to HCN for extended durations (5–15 years). Although this study is limited by small sample size, it used matched controls and is not confounded by smoking since all workers and controls were nonsmokers. The authors collected individual breathing zone measurements of HCN exposure, which were strongly correlated with urinary SCN^-, a measure of internal exposure. The range of mean individual HCN concentrations reported from all three plants was 6.4–10.4 ppm and the range among the 36 individuals was 4.2–12.4 ppm, indicating a similar magnitude of exposure for exposed workers. Thyroid enlargement was strongly associated with HCN exposure, with 56% of the exposed workers diagnosed with mild to moderate thyroid enlargement. This observation is supported by an increased radioactive iodide uptake in workers ($p < 0.001$). Increased uptake of radioactive iodide has been reported to occur in hyperthyroidism, iodine deficiency, recovery from thyroid

suppression, and goiter (NLM, 2008a; Spencer, 2008; Ravel, 1995). The increase in iodide uptake may have resulted from temporary weekend cessation of exposure. A similar phenomenon of post-inhibitory response was also seen in the occupational study by Blanc et al. (1985), which noted significantly increased T_3 uptake observed in workers several months following HCN exposure.

The thyroid alterations reported in El Ghawabi et al. (1975) are believed to be biologically significant effects. These effects, particularly thyroid enlargement, are consistent with those observed in oral exposure animal studies (Manzano et al., 2007; Jackson, 1988; Philbrick et al., 1979). Additionally, other human inhalation studies have indicated thyroid effects in exposed workers (Banerjee et al., 1997; Blanc et al., 1985). The thyroid effects observed in El Ghawabi et al. (1975) are also supported by mode-of-action data for cyanide, indicating competitive iodide uptake (see Section 4.6). The thyroid enlargement observed in the HCN-exposed workers likely indicates antagonism of iodine uptake by the cyanide metabolite, SCN^-. This biological response indicates a stress on the homeostatic mechanisms of the thyroid, which is of special concern to populations that include individuals with iodine deficiency, individuals with clinical or subclinical hypothyroidism, and the developing fetus.

The HCN inhalation database contains limited exposure-response data. Information on acute human occupational inhalation exposure to HCN does exist, but is limited to case reports of accidental overexposures with unclear exposure concentrations and/or durations. Additionally, no chronic or subchronic HCN inhalation studies exist in animals. The only available studies that report exposure data and potential health effects of inhalation of HCN are occupational exposure studies. In consideration of this limited inhalation database, the El Ghawabi et al. (1975) study was chosen as the principal study. This study of electroplating workers at three factories in Egypt included individual breathing zone measurements from the study participants and reported a strong correlation between these measurements and urinary levels of SCN^-. However, the exposure assessment was based on a single 15-minute breathing zone sample for each worker and the potential for dermal exposure was not explicitly discussed. Despite the weakness in the available exposure information, the El Ghawabi et al. (1975) study was selected as the principal study, and thyroid enlargement and altered iodide uptake were designated as the critical effects. The lowest mean concentration of HCN reported, 6.4 ppm HCN, is designated as the LOAEL. The choice of thyroid dysfunction as a critical effect is supported by other epidemiologic studies in a silver-reclaiming factory (Blanc et al., 1985) and in electroplating workers (Banerjee et al., 1997).

5.2.2. Method of Analysis

A LOAEL was available from the principal study. Because quantitative concentration-response data were available only for one concentration, benchmark concentration modeling could not be conducted.

The lowest mean concentration recorded among the three factories evaluated by El Ghawabi et al. (1975) was 6.4 ppm. Assuming a temperature and pressure of 25ºC and 760 mm Hg, this LOAEL in ppm was multiplied by the molecular weight of HCN and divided by 24.45 to determine the LOAEL in mg/m^3 HCN.

$$\text{LOAEL (ppm)} \times 27/24.45 = 6.4 \text{ ppm} \times 27/24.45 = 7.07 \text{ mg/m}^3 \text{ HCN}$$

The standard method for adjustment of LOAEL or NOAEL values from occupational studies was employed as described in U.S. EPA (2002). Because El Ghawabi et al. (1975) did not report daily exposure durations for exposed workers, an 8-hour/day, 5-day/week exposure scenario was assumed. A default occupational ventilation rate of 10 m^3/8-hour day and a default ventilation rate for continuous ambient exposure of 20 m^3/24-hour day were used. The exposure was also adjusted to account for the difference between occupational exposure for 5 days/week versus continuous ambient exposure for 7 days/week:

$$\text{LOAEL}_{(ADJ)} = 7.07 \text{ mg/m}^3 \text{ HCN} \times 10/20 \times 5 \text{ days}/7 \text{ days} = 2.5 \text{ mg/m}^3 \text{ HCN}$$

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The RfC is based on thyroid enlargement and altered iodide uptake reported in an occupational study of electroplating workers and is supported by other occupational studies and oral animal studies reporting similar thyroid effects. The LOAEL from this study (adjusted for continuous exposure) of 2.5 mg/m^3 HCN was used as the POD.

A total UF of 3,000 was applied to the POD: 10 for the extrapolation of a LOAEL to a NOAEL (UF$_L$), 3 for the extrapolation from a subchronic to chronic exposure duration (UF$_S$), 10 for human intraspecies variability (UF$_H$), and 10 to account for database deficiencies (UF$_D$).

A 1-fold UF for extrapolation across species was applied because the RfC is based on thyroid enlargement and altered iodide uptake reported in an occupational study.

A 10-fold UF was used to account for variation in susceptibility to cyanide among members of the human population. Although some information is available on potential sensitive populations, as described in Section 4.8, there are insufficient quantitative data to inform the UF for human variability with chemical-specific data.

A 10-fold UF was used for extrapolating from a LOAEL to a NOAEL (UF$_L$) because the POD was a LOAEL.

A 3-fold UF was applied to account for extrapolation from what is assumed to be a largely subchronic exposure to chronic exposure duration. The workers in the principal study were exposed to cyanide for 5–15 years. Of the 36 workers, 14 had been exposed for 5 years, 14 for 5–10 years, 7 for 10–15 years, and 1 for >15 years. The mean and median exposure times for the worker population were not reported. Twenty of the 36 exposed workers had thyroid

enlargement; however, the authors found no correlation between duration of exposure and either incidence or magnitude of thyroid enlargement in the workers. A lack of an association could be related to the low sample size and/or the failure of the authors to consider iodine status of the workers. In addition, following continued administration of cyanide in rats, thyroid effects were less prominent at 11 months of exposure compared to 4 months of exposure (Philbrick et al., 1979), which provides some indication (although limited), that increased duration of exposure may not lead to an increase in thyroid effects. Therefore, it is unknown whether greater alteration in thyroid function or increased incidence of the effect would be observed with longer exposure duration. In the absence of information indicating the effects of HCN would not progress in incidence or severity, a subchronic to chronic UF of 3 was applied.

A 10-fold UF was applied to account for deficiencies in the cyanide inhalation database. The database includes limited human data from epidemiologic studies of inhalationally exposed workers (Blanc et al., 1985; El Ghawabi et al., 1975). Inhalation studies on ACH evaluated limited male and female reproductive endpoints and were negative for impacts on fertility (Monsanto Co., 1985a, b). Oral studies of cyanide exposure in rodents have suggested that the male reproductive tract is a sensitive target of cyanide toxicity following subchronic exposure (NTP, 1993). However, the database lacks developmental and multigenerational reproductive toxicity studies. Data are available which evaluate potential health effects following perturbations of thyroid function in pregnant women and their offspring (see Section 4.8.1). These studies indicate increased pregnancy complications and decrements in learning and memory in offspring of women with subclinical hypothyroidism (Kooistra et al., 2006; Casey et al., 2005; Pop et al., 2003; Haddow et al., 1999). Due to the proposed cyanide mode of action of thyroid disruption (through the metabolite thiocyanate), developmental neurotoxicity studies or developmental studies assessing maternal and fetal thyroid function are also considered data insufficiencies. Thus, a database UF of 10 was applied in this assessment to account for the lack of developmental and multigenerational reproductive toxicity studies.

The RfC for HCN was calculated as follows:

$$\text{RfC} = \text{LOAEL}_{(ADJ)} \div \text{UF}$$
$$= 2.5 \text{ mg/m}^3 \div 3{,}000$$
$$= 0.00083 \text{ mg/m}^3 \text{ (rounded to } 8 \times 10^{-4} \text{ mg/m}^3)$$

It is recommended that the RfC for HCN should not be used to estimate an RfC for cyanide salts due to inhalation considerations. Specifically, exposure to HCN occurs as a gas, whereas the extremely high boiling points and vapor pressures of cyanide salts predict that inhalation exposure would occur as aerosols. Different dosimetric approaches would apply to

the aerosol (or particle) exposures that would result from exposure to cyanide salts, compared with exposure to HCN gas.

5.2.4. Previous RfC Assessment

An RfC for HCN of 3×10^{-3} mg/m^3 was posted on the IRIS database in 1994. This RfC was based on findings of thyroid effects and neurological symptoms in workers from the study by El Ghawabi et al. (1975). The POD for this RfC was based on an adjusted LOAEL of 7.07 mg/m^3. The LOAEL was divided by a total UF of 1,000 comprised of UFs of 10 each to account for the lack of a NOAEL and intrahuman variability. UFs of 3 each were applied to account for the use of a study of less-than-chronic duration and deficiencies in the database (lack of chronic and multigenerational reproduction studies). Since the posting of the previous IRIS RfC for cyanide, no new chronic or subchronic inhalation studies for HCN with quantitative information are available in the literature. The revised RfC for cyanide was derived from the same study as the previous assessment based on the observation of altered thyroid function (as indicated by iodine uptake) in occupationally exposed male workers. In the current assessment, a database UF of 10 was applied to account for the lack of developmental and multigenerational reproductive toxicity studies.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE AND INHALATION REFERENCE CONCENTRATION

The following discussion identifies uncertainties associated with the quantification of the RfD and RfC for cyanide. Following EPA practices and guidance (U.S. EPA, 1994b, 1993), the UF approach was applied to the chosen PODs to derive an RfD and RfC (see Sections 5.1.3 and 5.2.3). Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal study to human exposure, a diverse human population of varying susceptibilities, and database deficiencies.

The database for cyanide includes limited human data from studies of occupationally exposed workers. Endpoints observed in inhalationally exposed workers include altered thyroid function and CNS symptoms (including headache, weakness, nausea, and vomiting). The database also includes oral exposure studies in laboratory animals, including limited chronic studies, subchronic dietary exposure studies, and several developmental studies, including one specifically assessing gross and microscopic brain morphology in rats (as discussed in Section 4.3). Effects seen with low-dose oral exposure to cyanide include decreased reproductive organ weight, decreased spermatid concentration, increased thyroid weight, and histologic alterations in the CNS, kidney, testis, and adrenal glands. In addition to oral and inhalation data, the database for cyanide includes studies on absorption, distribution, metabolism, and excretion.

Uncertainty exists in the selection of the most relevant animal species for human health assessment. Studies in several species, including rodents, pigs, goats, and dogs, were considered in the development of the RfD; however, limited data exist on differential species' sensitivity to cyanide, especially in the context of long-term exposure.

The RfD was derived from a BMDL of 1.8 mg/kg-day, which was based on the observation of decreased epididymis weight in male F344 rats exposed to NaCN in drinking water for 13 weeks (NTP, 1993). This study treated male and female rats with doses of CN^- ranging from 0.16 to 12.5 mg/kg-day. Other reproductive effects observed at higher doses included decreased caudal epididymis and testis weights and decreased spermatid count. After consideration of all potential PODs, the RfD of 6×10^{-4} mg/kg-day was based on the observation of decreased cauda epididymis weight in male F344 rats following subchronic dietary administration of cyanide (NTP, 1993).

The mode of action of the decrease in cauda epididymis weight in rats is uncertain, although limited information from other model systems of hypothyroid animals suggests that it may be related to thyroid disruption from the primary metabolite, thiocyanate (see Section 4.5.4). However, the critical study used as the basis for the RfD (NTP, 1993) did not assess thyroid-related parameters, such as T_4, T_3, TSH, or thyroid weight. Therefore, it is not known whether direct indicators of disruption of thyroid homeostasis accompanied the observed reproductive effects.

Additional studies exist that determined different effects at lower doses, as discussed in Section 5.1.1, including behavioral changes and decreased serum T_4 in pigs (Jackson, 1988) and kidney, adrenal, and testicular effects in dogs (Kamalu, 1993). Selection of either of these studies would result in a lower RfD as portrayed in Table 5-3 and Figure 5-1. Ultimately, these studies were deemed of lower confidence, due to issues concerning study design and reporting (see Section 5.1.1), than the 3-month dietary study in rats and mice conducted by NTP (1993) and thus, were not chosen as the principal study. To derive the RfD, UFs were applied to the POD determined through BMD modeling of the critical effect of reduced cauda epididymis weight in male rats. This study was well designed and conducted with several dose levels, sufficient numbers of animals, and a wide range of tissues and endpoints assessed; however, significant areas of uncertainty exist in the animal data relied upon for the RfD. UFs associated with the extrapolation from the POD derived from an animal study to a diverse human population of varying susceptibilities were applied.

Uncertainty exists in the selection of the BMR level utilized in the BMD modeling of the critical effect (decreased cauda epididymis weight) to determine the POD. At this response level in the cauda epididymis, no alteration in epididymal sperm count was detected, and thus, fertility in these animals would not likely be affected. However, human males have markedly lower fertility than do rats (U.S. EPA, 1996; Working et al., 1988) and thus, changes in male reproductive endpoints may therefore be expected to have greater impact in humans as compared

to rodents. In the absence of clear information to determine the level of change in cauda epididymis weight related to a biologically significant change, a decrease of 1 SD in organ weight was selected to represent a minimally adverse change.

The choice of BMD model is not expected to introduce a considerable amount of uncertainty since 1 SD in the reduction of cauda epididymis weight is within the observable range of the data. Other available continuous, constant variance models available in the BMD software (i.e., the power and linear models) also showed acceptable fits ($p > 0.1$) and had AIC values within 1 unit of the selected polynomial model. However, the polynomial model had superior visual fit to the data, especially in the low-dose range. The BMDL estimates for various models are not within a factor of 3, indicating some model dependence; therefore, in accordance with the *Benchmark Dose Technical Guidance Document* (External Review Draft, U.S. EPA, 2000b), the model with the lowest BMDL estimate was selected.

Additional BMD modeling for other data sets, including additional reproductive endpoints from the NTP (1993) study, was also conducted to provide other PODs for comparison purposes (see Appendix B). A graphical representation of these and other potential PODs and resulting reference values is shown in Figure 5-1 (see Section 5.1.4).

The default UF of 10 for the extrapolation from animals to humans accounts for toxicokinetic differences and toxicodynamic differences. Physiologically based toxicokinetic models can be useful for the evaluation of interspecies toxicokinetics; however, the cyanide database lacks an adequate model that would inform potential differences. Data from workers occupationally exposed to cyanide provide some information on the absorption, metabolism, and elimination of cyanide in humans and indicate qualitatively that the toxicokinetics of cyanide are similar between humans and animals. Additionally, some biological effects, including thyroid enlargement and neurological symptoms observed in animals and humans (such as ataxia, weakness, and behavioral changes), are similar in nature, indicating similar toxicodynamics. However, the magnitude of the similarities or differences in toxicokinetic and toxicodynamic parameters cannot be calculated due to uncertainties regarding routes of exposure and doses for the occupationally exposed workers. Therefore, a 10-fold UF to account for interspecies differences was used.

A 3-fold UF was applied to account for less-than-chronic exposure duration in the occupationally exposed workers. Uncertainty exists as to whether additional or more severe effects would be expected in these workers over longer durations.

Limited data exist on effects of cyanide in populations of occupationally exposed workers. However, since potential variability in responses to cyanide in the greater human population is unknown, the default UF of 10 for intrahuman variability was used. Human variation may be larger or smaller; however, chronic cyanide-specific data to examine the potential magnitude of human variability of response were not found.

Uncertainties associated with data gaps in the cyanide database have been identified. Effects on reproductive organ weight and sperm parameters have been identified in rats and mice subchronically exposed to cyanide in the diet. However, data more fully characterizing potential multigenerational reproductive effects are lacking. Gross developmental effects have not been observed in the few, limited developmental studies available (Imosemi et al., 2005; Malomo et al., 2004; Tewe and Maner, 1981). Due to thiocyanate's proposed mode of action of competitive inhibition of iodide uptake in the thyroid, the lack of studies evaluating subtle neurodevelopmental and behavioral outcomes adds uncertainty to this assessment. It is unclear from the available database whether perturbation of thyroid function sufficient for the induction of subclinical or clinical hypothyroidism would be expected to occur below the POD for reduced epididymis weight in rats identified in NTP (1993), since thyroid hormone levels were not measured in this study (though examination of the thyroid showed no increase in weight or histologic lesions). Therefore, a 3-fold UF for database deficiencies was applied to the POD to account for uncertainty regarding potential neurological effects during development and for the lack of data on multigenerational reproductive toxicity.

The lack of specific immune-related data on cyanide represents a data gap. Subchronic and chronic studies on cyanide and cyanide-related compounds have evaluated limited immune endpoints, such as organ weights, histopathology (NTP, 1993; Monsanto Co., 1985a, b; Lewis et al., 1984; Howard and Hanzal, 1955), and hematological parameters (NTP, 1993; Blanc et al., 1985; Monsanto Co., 1985a, b; Howard and Hanzal, 1955), and are generally negative (see Section 4.4.4). Two studies have found an elevation in percent lymphocytes in exposed workers as compared to controls (Leeser et al., 1990; El Ghawabi et al., 1975). This finding is of unclear significance, considering the nonspecific nature of this hematological parameter. Overall, although the examined immune endpoints in the cyanide database appear normal, the lack of functional immune assays precludes a confident conclusion regarding potential immune toxicity of cyanide.

The HCN inhalation database contains limited exposure-response data. Additionally, no chronic or subchronic HCN inhalation studies exist in animals. Several of the available studies may not meet current standards for study design, reporting, and peer review due to technological and experimental developments that may have occurred from the time of reference publication. The only available studies reporting exposure data and potential health effects of inhalation of HCN are occupational exposure studies. In consideration of this limited inhalation database, El Ghawabi et al. (1975) was selected as the most appropriate study for the derivation of the RfC.

The RfC was derived from a LOAEL$_{(ADJ)}$ of 2.5 mg/m^3 HCN, which was based on thyroid enlargement and altered iodide uptake in a cohort of workers in three electroplating facilities who had been exposed to HCN for 5–15 years (El Ghawabi et al., 1975). This study is the only extended duration epidemiologic study in which concurrent exposure concentrations were measured. The mean cyanide air concentrations in the breathing zone of workers at the

three plants were 7.07–11.5 mg/m^3 HCN. The lowest mean concentration recorded in the three factories, 7.07 mg/m^3 HCN, is designated as a LOAEL. Twenty male volunteers of the same age group and socioeconomic status who had no occupational exposure to cyanide were chosen as controls. Effects observed in exposed workers included thyroid enlargement and increased iodide uptake in the thyroid. The effects observed are consistent with known effects of cyanide and reported effects in other studies of cyanide-exposed workers and are also supported by similar effects observed in animals orally exposed to cyanide. However, significant areas of uncertainty exist in the human data relied upon for the RfC.

Some uncertainties exist in the exposure doses measured. The individual breathing zone concentrations of HCN were measured in the three different factories over a period of 2 months. No information was given regarding how the current cyanide environmental monitoring data may compare to conditions over the last several years. Furthermore, the authors acknowledged that workers were co-exposed to other chemicals during the electroplating process, including gasoline, alkali, and acid, but did not quantitate the magnitude of these exposures. It is unclear how these exposures would be expected to impact the effects observed in El Ghawabi et al. (1975). However, the thyroid effects, specifically the thyroid enlargement, are supported by human and animal data and are consistent with the mode of action of the cyanide metabolite, SCN$^-$ (see Section 4.6). Additionally, as with most occupational exposure scenarios, the possibility exists for exposure through the dermal route. However, Table 4-1 provides evidence indicating that the individual breathing zone measurements of HCN closely correlated with an internal measure of exposure (urinary SCN$^-$).

No NOAEL was identified in the El Ghawabi et al. (1975) study. Effects in this study were found at the lowest exposure concentrations measured; therefore, the LOAEL identified for this study does not indicate where a threshold of effects would lie and the data provided in the study are not sufficient for a dose-response analysis. To account for the uncertainty in the use of a LOAEL for the POD, a factor of 10 was applied in the derivation of the RfC.

Several assumptions were made in the conversion of the LOAEL observed in El Ghawabi et al. (1975) to an adjusted dose. The average temperatures in the factories were not included in the study; therefore, conversions from ppm to mg/m^3 exposure concentrations were based on standard temperature and pressure. Additionally, the daily and weekly cyanide exposure durations were not explicitly stated in the study; therefore, an 8-hour/day, 5-day/week exposure was assumed. Other uncertainties in the exposure assessment of the workers include potential variability in exposures among workers based on specific duties or locations in the factories; however, this uncertainty is limited since breathing zone samples from individual workers were averaged to determine mean factory exposure.

Although significant areas of uncertainty remain in the human epidemiologic data relied upon for the RfC, the use of human exposure data eliminates the substantial uncertainty inherent in the extrapolation of an animal study to humans. The study by El Ghawabi et al. (1975) was

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conducted by using a small population of male workers (n = 36) and cannot be expected to capture the human variability of response to cyanide exposure. Additionally, the workers included in the study may represent a low-sensitivity group with other more affected workers not continuing employment (i.e., the healthy worker effect). Therefore, in the absence of cyanide-specific data to account for the heterogeneity of human sensitivity, a factor of 10 was used to account for uncertainty associated with human variation in the derivation of the RfC. Human variation in response to cyanide exposure may be larger or smaller; however, chemical-specific data to assess the potential magnitude of variability are unavailable. Of the 36 workers, average exposure duration was about 7 years, with the minimum exposure duration being 5 years and the maximum being 15 years.

Uncertainty exists regarding whether progression of effects would be expected with longer exposure time. Twenty of the 36 exposed workers had thyroid enlargement rated as being mild to moderate; however, the authors found no correlation between duration of employment at the factory and either incidence or magnitude of enlargement. Additionally, it is not known whether CNS symptom incidence or severity would be expected to increase with increasing exposure duration or whether CNS effects would be detected in chronically exposed workers at lower concentrations. It is also possible that different sensitive endpoints may be detected in studies of longer duration. For instance, chronic inhalation exposure in workers at a metal tempering plant indicated some deterioration in pulmonary function (Chatgtopadhyay et al., 2000). However, no exposure monitoring from this study was available for dose-response comparison to the El Ghawabi et al. (1975) study, identified as the principal study. Therefore, to account for uncertainties regarding the exposure duration of the POD, a 3 fold UF was applied.

Uncertainties associated with data gaps in the cyanide database have been identified. The database includes limited human data from epidemiologic studies of occupationally exposed workers. No animal studies exist that employed extended duration inhalation exposure to HCN, although inhalation studies on the related compounds ACH and $(CN)_2$ exist. Studies to assess developmental or multigenerational reproductive toxicity of HCN for the inhalational route of exposure are not available. Thus, a 10-fold UF was applied to account for limitations in the inhalation database.

5.4. CANCER ASSESSMENT

The only available chronic study of cyanide that analyzed a wide variety of tissues following near lifetime exposure is an oral rat study (Howard and Hanzal, 1955); no tumors or lesions were associated with either dose group following dietary administration of cyanide at doses up to 10.8 mg/kg-day for 2 years. This study is limited by small sample sizes (10/group), histopathologic assessment of only a subset of potential target organs of carcinogenicity, and uncertainty regarding dose due to volatility. Overall, the data are inadequate for an assessment of the human carcinogenic potential of cyanide, based on EPA's *Guidelines for Carcinogenic*

Risk Assessment (U.S. EPA, 2005a). Therefore, no quantitative cancer assessment was conducted.

Uncertainty exists as to the potential of cyanide to induce thyroid tumors. Treatment of rodents with chemicals that cause decreased levels of circulating thyroid hormones may result in compensatory increased TSH levels, increased cellularity and size of the thyroid gland, and finally tumors of the thyroid (U.S. EPA, 1998b). In the case of cyanide, there is evidence of decreases of circulating thyroid hormones and compensatory increases in TSH levels followed by the formation of goiter in rodents and humans. However, the rat study by Howard and Hanzal (1955) did not identify thyroid tumors following oral exposure to HCN for 2 years. Additionally, studies examining cancer incidence in occupationally exposed cyanide workers are not available. Several case-control studies have indicated that development of goiter is a significant risk factor for the development of thyroid cancer (Truong et al., 2005; Ron et al., 1987). Smokers, a subpopulation with elevated exposure to HCN, have consistently been shown to have a decreased risk of thyroid cancer in case-control studies (Kreiger and Parkes, 2000; Galanti et al., 1996). Additionally, two case-control studies did not find an association between thyroid cancer and intake of food high in cyanogenic compounds (Bosetti et al., 2002; Kolonel et al., 1990). However, a recent case-control study in a population with a high background risk of thyroid cancer has associated high consumption of goitrogenic food and low iodine intake with increased incidence of thyroid cancer in women (Truong et al., 2010). Therefore, the potential for cyanide to influence the development of thyroid tumors is unclear, but merits further investigation.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Cyanide compounds are used in a number of industrial processes, including mining, metallurgy, manufacturing, and photography, due to their ability to form stable complexes with a range of metals. Cyanide has been employed extensively in electroplating, in which a solid metal object is immersed in a plating bath containing a solution of another metal with which it is to be coated in order to improve the durability, electrical resistance, and/or conductivity of the object. Hydrogen cyanide (HCN) has also been used in gas chamber executions and in chemical warfare. The cyanide salts, sodium cyanide (NaCN) and potassium cyanide (KCN), have also been used as rodenticides. Use in industrial processes is the main origin of cyanide in the environment, but cyanide is also released from biomass burning, volcanoes, and natural biogenic processes from higher plants, bacteria, and fungi (ATSDR, 2006). Additionally, cyanogenic compounds, which are converted to cyanide in the body, naturally occur in many plant foods, including cassava root, almonds, millet sprouts, lima beans, soy, spinach, bamboo shoots, and sorghum. Exposure to cyanide also occurs from smoking. Thiocyanate (SCN^-), the primary metabolite of cyanide, is found in plasma or blood at approximately 0.5–4 µg/L in nonsmokers and approximately 6–22 µg/L in smokers (Chandra et al., 1980).

The available data show that cyanide is rapidly and extensively absorbed via the oral, inhalation, and dermal routes, although quantitative data on the percent or extent of absorption are limited. At physiological pH, cyanide is distributed in the body as HCN, and thus, the toxicokinetics for freely dissociating cyanide compounds are the same. Cyanide distributes rapidly and fairly uniformly throughout the body following absorption. Inhaled or dermally absorbed HCN enters the systemic circulation immediately. In contrast, ingested cyanide is primarily converted to thiocyanate via first-pass metabolism in the liver. Immediately following oral exposure in humans, tissues containing cyanide included the liver, brain, spleen, blood, kidneys, and lungs (Ansell and Lewis, 1970; Gettler and Baine, 1938). Following acute inhalation exposure in humans and animals, cyanide is found in the lung, heart, blood, kidneys, and brain (Ballantyne, 1983, as cited in ATSDR, 2006; Gettler and Baine, 1938). The major metabolic pathway for cyanide is conversion to thiocyanate, primarily by rhodanese. Detoxification of cyanide by rhodanese is rapid with the concentration of sulfur-containing donor molecules as the rate-limiting factor. Rhodanese is widely distributed throughout the body, but is located at the highest concentration in the liver. Toxicokinetic studies in animals indicate rapid decreases in cyanide blood concentration within 3 hours following dosing, with the half-life of elimination for thiocyanate for all species about 10 times longer (Sousa et al., 2003).

Cyanide is primarily excreted in the urine as thiocyanate following both inhalation and oral exposures and is not thought to accumulate in the blood and tissues.

Several reports on occupationally exposed workers indicate that chronic inhalational exposure to low concentrations of cyanide can cause thyroid effects and CNS symptoms (Banerjee et al., 1997; Blanc et al., 1985; El Ghawabi et al., 1975). The results of these occupational studies suggest that chronic exposure to cyanide may be associated with alterations in thyroid gland function, including enlargement, altered iodine uptake, and decreased thyroid hormones, and subjective CNS symptoms. Another study also suggests that chronic exposure to cyanide fumes in a metal-tempering plant may reduce pulmonary function in chronically exposed workers (Chatgtopadhyay et al., 2000). Chronic or subchronic inhalation studies of HCN in experimental animals were not found.

Epidemiologic studies of populations in developing countries consuming cyanogenic compounds in food have been conducted (Madhusudanan et al., 2008; Oluwole et al., 2003; Osuntokun, 1973; Makene and Wilson, 1972). These studies are confounded by the presence of other potentially toxic dietary components associated with cyanogenic foods, such as the cyanogenic glycoside linamarin, and the high prevalence of iodine, protein, and vitamin deficiencies in the studied populations. Because of the aforementioned challenges, use of epidemiologic studies of human dietary cyanogenic exposure is limited for the purposes of this hazard assessment for cyanide.

No epidemiologic studies exist of long-term human exposure to cyanide by the oral route. Information on human oral exposure to cyanide is limited to acute effects following suicide attempts or accidental poisoning. Acute oral exposure to cyanide has been observed to result in typical signs of cyanide poisoning, including CNS depression, convulsions, coma, and death. Chronic and subchronic oral studies in experimental animals indicate that the thyroid, CNS, and male reproductive organs are sensitive targets of cyanide toxicity (Manzano et al., 2007; Soto-Blanco et al., 2002a, b; NTP, 1993; Jackson, 1988).

Histologic changes in the CNS have been observed following longer-term exposure to cyanide in some animal models. In rats exposed to cyanide in the diet for 1 year, increased vacuolation in the spinal cord white matter and exacerbation of methionine deficiency-induced spinal cord demyelination were observed (Philbrick et al., 1979). In addition, histopathologic effects, including neuronal loss, spheroids, damaged Purkinje cells, and loss of white matter in various CNS structures, were observed in rats following a 12-week oral exposure period (Soto-Blanco et al., 2002a) and in goats following 5 months of oral exposure (Soto-Blanco et al., 2002b). However, histopathology of the nervous system has not been identified in other chronic or subchronic studies (NTP, 1993; Howard and Hanzal, 1955) conducted at doses lower than that administered by Philbrick et al. (1979). In addition to histologic changes observed in some studies, subtle behavioral changes were noted in pigs orally exposed to 1.2 mg/kg-day cyanide in drinking water for 6 months.

Chronic and subchronic exposure to cyanide is known to induce thyroid effects due to the cyanide metabolite, thiocyanate. Thiocyanate adversely affects the thyroid gland via competitive inhibition of iodide uptake and perturbation of the homeostatic feedback mechanisms that regulate the synthesis and secretion of essential thyroid hormones. Philbrick et al. (1979) reported decreased serum T_4 levels and increased thyroid weights in rats but no histopathologic changes in the thyroid gland. Subchronic studies in rats and mice (NTP, 1993) conducted with a range of doses lower than the single dose tested by Philbrick et al. (1979) did not observe adverse histopathology or increased weight of the thyroid gland, although thyroid hormone levels were not evaluated. Studies in pigs have noted increased thyroid weights, altered thyroid histology, and decreased thyroid hormones (Manzano et al., 2007; Jackson, 1988) at doses in the range of the NTP (1993) study and several times lower than the Philbrick et al. (1979) study. It is apparent through comparisons of thyroid effects in animal models that sensitivity of the thyroid to the effects of cyanide appears to vary widely among species.

Reproductive effects, including decreased epididymis, cauda epididymis, and testis weights and decreased sperm parameters (epididymal sperm motility and testicular spermatid counts), have been observed in rats in a subchronic dietary study by NTP (1993). Decreases in the cauda epididymis and epididymis weights were also seen in mice (NTP, 1993). Histologic examination of reproductive organs did not reveal any lesions. Additionally, reproductive effects, specifically, alterations in testicular histology, have also been observed in a 14-week study in dogs (Kamalu, 1993). The mode of action of the reproductive effects following subchronic cyanide exposure in rodents is unclear, although some data in hypothyroid animal models suggest that these effects may be secondary to thyroid perturbation.

Cyanide exerts its acute effects, including CNS depression, convulsions, coma, and death, by binding with cytochrome c oxidase, a key enzyme in the production of ATP by way of oxidative phosphorylation. The steep dose response occurring with acute high-dose exposures is thought to be due to cyanide overload, resulting in saturation of detoxification pathways that metabolize cyanide to less acutely toxic intermediate compounds. At lower dose rates, an efficient detoxification system (primarily via rhodanese with sulfur donors as the rate-limiting factor) catalyzes the transformation of cyanide to thiocyanate, its primarily metabolite. Thiocyanate is not known to be acutely toxic, although long-term exposures can adversely affect the thyroid gland via iodide uptake inhibition and decreased thyroid hormone synthesis. The chronic effects of cyanide and thiocyanate on other organ groups are not clear.

6.2. DOSE RESPONSE
6.2.1. Noncancer—Oral

The male reproductive effects observed by NTP (1993) have been identified as the critical effects for the development of the cyanide RfD. The NTP (1993) study was well designed with five treatment groups with doses spanning two orders of magnitude. Numerous

tissues and endpoints were assessed in both rats and mice. This study identified a suite of statistically significant reproductive effects in both species, including decreased epididymis weights (cauda and whole), decreased testes weight, and altered sperm parameters. BMD analysis of the observed reproductive data from rats and mice indicated decreased cauda epididymis weight to be the most sensitive reproductive effect observed. Thus, decreased cauda epididymis weight was chosen as the critical effect. This effect is believed to be one that would likely precede substantial decrements in sperm parameters and fertility in this test species. The cyanide database contains additional, limited support for the specific endpoint of reproductive toxicity. Altered testicular histopathology, including a significantly decreased percentage of tubules in stage VIII of the spermatogenic cycle, was observed in dogs ingesting 1 mg/kg-day cyanide for 14 weeks (Kamalu, 1993).

In addition to the reproductive effects observed in NTP (1993), other sensitive effects observed in animals, including increased thyroid weight, altered thyroid hormones, and altered testicular, kidney, and adrenal histopathology, were also considered as potential critical effects (see discussion in Section 5.1.1). Though these effects were not ultimately selected for the derivation of the RfD, RfDs for these endpoints were quantified for comparison purposes.

BMD modeling was conducted to calculate potential PODs for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound confidence limit ($BMDL_x$). A BMR level was selected corresponding to a change in the mean response equal to 1 control SD from the control mean for cauda epididymis weight. In this case, a 1 SD change in cauda epididymis weight was selected under an assumption that it represents a minimal biologically significant change. Using the best fitting model for this data set, a 1 SD change was equivalent to a 7% decrease in cauda epididymis weight. Additional BMD modeling for other amenable data sets was also conducted to provide other PODs for comparison purposes (see Appendix B). PODs for these endpoints and other PODs determined through a NOAEL/LOAEL approach were considered for the derivation of the RfD. Tabular and graphical representations of these potential PODs and resulting potential reference values are shown in Table 5-2 and Figure 5-1, respectively.

The RfD of 6×10^{-4} mg/kg-day was calculated from a $BMDL_{1SD}$ of 1.9 mg/kg-day based on decreased cauda epididymis weight in rats in the subchronic oral study conducted by NTP (1993). A total UF of 3,000 was applied to the POD: 10 for the extrapolation from animals to humans (UF_A), 10 for the extrapolation from a subchronic to chronic exposure duration (UF_S); 10 for human intraspecies variability (UF_H); and 3 to account for database deficiencies (UF_D). Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans and the potential variability in human susceptibility; thus, the interspecies and intraspecies UFs of 10 were applied. To address the subchronic, 13-week study duration of the principal study, a 10-fold UF (UF_S) was also applied. Additionally, a 3-fold

database UF was considered necessary due to the lack of information regarding potential multigenerational reproductive effects and the lack of a sensitive neurodevelopmental study.

The overall confidence in the RfD is low to medium. Confidence in the principal study (NTP, 1993) is medium. This study was well conducted, involved a sufficient number of animals per group (including both sexes of two species), used several dose levels, and assessed a wide range of tissues and endpoints. However, this study did not evaluate thyroid endpoints and was only 90 days in duration. Confidence in the database is low to medium. The cyanide database includes occupational inhalation exposure studies in humans, chronic and subchronic dietary exposure studies in laboratory animals, and several developmental studies in laboratory animals. However, the database is lacking a multigenerational reproductive toxicity study, a sensitive neurodevelopmental study, and a chronic study evaluating noncancer endpoints. Therefore, reflecting low to medium confidence in the database and medium confidence in the principal study, the overall confidence in the RfD is low to medium.

6.2.2. Noncancer—Inhalation

No new chronic or subchronic inhalation studies for HCN (with quantitative information) have been published in the literature since the development of the previous HCN RfC. Therefore, the inhalation database was reevaluated, but the principal study and critical effect selected for the RfC were unchanged. The RfC is based on an occupational study reporting thyroid enlargement and altered iodide uptake. The principal study (El Ghawabi et al., 1975) identified effects on the thyroid that were consistent with the proposed mode of action of cyanide. Only a LOAEL could be identified from this study. In addition, there was potential coexposure of the workers to other substances, including gasoline, alkali, and hydrochloric acid, through the electroplating process. Nonetheless, the reported thyroid alterations are consistent with the reported effects of cyanide exposure in other occupational studies and animal studies.

The RfC of 8×10^{-4} mg/m^3 was derived from a LOAEL$_{(ADJ)}$ of 2.5 mg/m^3 HCN, which was based on thyroid enlargement and altered iodide uptake in a cohort of workers in three electroplating facilities who had been exposed to HCN for 5–15 years (El Ghawabi et al., 1975). The authors recorded multiple individual breathing zone samples and reported the mean HCN exposure level for each factory. The lowest mean HCN concentration was designated as the LOAEL. The study authors did not report the data in a manner that allowed evaluation of an exposure duration response or a concentration response. Other studies of occupationally exposed workers either did not provide exposure data or included higher exposure levels and did not control for confounding variables.

A total UF of 3,000 was applied to the POD: 10 for the extrapolation of a LOAEL to a NOAEL (UF$_L$), 3 for the extrapolation from a subchronic study (UF$_S$), 10 for human intraspecies variability (UF$_H$), and 10 to account for database deficiencies (UF$_D$). A 1-fold UF for extrapolation across species was applied because the RfC is based on thyroid enlargement and

altered iodide uptake reported in an occupational study. The occupational study by El Ghawabi et al. (1975) identified a LOAEL and a 10-fold UF was applied (UF$_L$) to extrapolate to a NOAEL. Information was unavailable to quantitatively assess the variability in susceptibility to cyanide in the human population; therefore, a 10-fold UF for intraspecies variability was applied (UF$_H$). Additionally, a subchronic-to-chronic UF of 3 was applied to extrapolate from what is assumed to be largely subchronic exposure in exposed workers to chronic exposure. A 10-fold UF was applied for uncertainties in the database, specifically the lack of a multigenerational toxicity study and a sensitive neurodevelopmental study. Several limited occupational inhalation studies are available in the cyanide database.

Reflecting medium confidence in the principal study (El Ghawabi et al., 1975) and low to medium confidence in the inhalation database, the overall confidence in the cyanide RfC is low to medium.

6.2.3. Cancer

Cyanide has not been subjected to a complete standard battery of genotoxicity assays, although, overall, the available data indicate that cyanide is not genotoxic. No adequate carcinogenicity studies of cyanide are available in animals or humans. In a 2-year chronic study in rats, no evidence of tumorigenicity was observed (Howard and Hanzal, 1955). However, the number of animals per dose group limited the power of the study and only a limited set of target tissues was evaluated histopathologically. Based on these considerations and in accordance with the U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, there is "inadequate information to assess the carcinogenic potential" of cyanide.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The Toxicological Review of Hydrogen Cyanide and Cyanide Salts (dated August, 2009) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). An external peer-review workshop was held December 14, 2009. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases, the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

EXTERNAL PEER REVIEW PANEL COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

(A) General Comments

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Comment: The peer reviewers found the document to be generally comprehensive, logical, well-written, and clear. However, two reviewers found some parts of the document to be redundant.

Response: The structure of Toxicological Reviews is established by a template, and, therefore, information may be presented in more than one place. An effort has been made to present a streamlined assessment of the chronic toxicity of hydrogen cyanide and cyanide salts. Suggestions for document improvement submitted by the panel were incorporated into the Toxicological Review wherever possible and appropriate.

Comment: One reviewer suggested a more concise summary of the primary chronic mode of action for cyanide of thyroid perturbation, including expanded discussion of chemicals operating through the same mode of action.

Response: The mode of action of thyroid perturbation following cyanide exposures is discussed in Section 4.5.3. Several other chemicals known to act via this mode of action are included in the text.

Comment: Another reviewer suggested that cyanide be expressed as hydrogen cyanide (HCN) in the document and not cyanide ion (CN^-).

Response: Though it is acknowledged in Section 2 that HCN and CN^- can interconvert based on pH and temperature, for the sake of comparability, doses in this review are given as CN^- unless stated otherwise.

Comment: One reviewer commented that Section 4.8.1, Possible Childhood Susceptibility, should unequivocally state that the developing fetus and children are more susceptible to cyanide toxicity than adults.

Response: Text will be added in this section to highlight that the developing human is expected to be the most susceptible population to chronic cyanide toxicity.

Comment: A reviewer expressed concern with the age of the database, and commented that the document should acknowledge that much of the data are old and may not meet current standards for study design, reporting, and peer review.

Response: Text will be added to Section 5.3, Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration, addressing the age of the cyanide database.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of hydrogen cyanide and cyanide salts.

Comment: Several reviewers identified the following unpublished studies and requested they be discussed in the document:

Leeser, J.E., Tomenso, J.A., and Bryson, D.D. 1990. A cross-sectional study of the health of cyanide salt production workers. Report No. OHS/R/2, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Unpublished manuscript.

Leuschner F. and B.W. Neumann. 1989. 13-Week toxicity study of potassium cyanide
administered to Sprague-Dawley rats in the drinking water. Unpublished manuscript.

One reviewer suggested additional information about perchlorate and carbon monoxide, which is a component of smoke for most non-industrial inhalation exposures (including fires and cigarette smoke).

Response: Additional studies were considered and added where relevant. Specifically, descriptions of the unpublished studies by Leeser et al. (1990) and Leuschner and Neumann (1989) were added to the document in Sections 4.1.3. and 4.2.1, respectively, and were included in Section 4.6 and discussed in Section 5. Limited information is included in the draft assessment regarding chemicals which operate by a similar mode of action, including perchlorate (see Sections 4.5.3., 4.8.2., 4.8.3., and 5.2.1.), because a greater consideration of cumulative effects of mixtures is outside the scope of this assessment. As this Toxicological Review is intended to provide scientific hazard and dose-response information pertaining to chronic exposure to cyanide, acute data of mixed gas exposures (such as HCN and carbon monoxide) was not prioritized. However, some information regarding HCN and carbon monoxide co-exposure has been added to the document in Section 4.8.3.

(B) Oral reference dose (RfD) for Hydrogen Cyanide and Cyanide Salts

1. A 13-week drinking water study (NTP, 1993) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study. Specifically, please comment on whether Jackson (1988) or Kamalu et al. (1993) (which found potentially lower points of departure) should be given greater consideration in the determination of the RfD.

Comments: Four reviewers agreed that of the studies presented in the Toxicological Review, the study by NTP (1993) was the most appropriate study for the determination of the RfD. One reviewer suggested greater consideration of the unpublished study by Leuschner and Neumann (1989).

Response: Descriptions of the unpublished studies by Leeser et al. (1990) and Leuschner and Neumann (1989) were added to the assessment in Sections 4 and 5.

Comment: One reviewer disagreed with the selection of the NTP study and recommended the study by Jackson (1988) as the principal study. The reviewer preferred the selection of a principal study that examined thyroid related endpoints, in an effort to remain consistent with the critical effect selected in the derivation of the RfC and the chronic cyanide mechanism of thyroid disruption.

Response: One of the predominant effects observed following chronic cyanide exposure is the disruption of thyroid function. However, in the case of the derivation of the RfD, the mechanism of the decreased epididymis weight and the associated reproductive changes observed in the study selected as the basis of the RfD (NTP, 1993) is not known. Though a known mechanism or mode of action could help support the relevance of these reproductive findings in humans, the lack of definitive data to inform the mode of action does not imply that this effect is not relevant to humans, and does not constitute grounds to disregard the observed effects. Thus, the study by NTP, which observed the most sensitive effects following extended cyanide oral exposure, was retained as the basis of the RfD.

2. Decreased absolute cauda epididymis weight in male rats was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comment: Three reviewers agreed with the selection of the critical effect. One reviewer agreed with the caveat that this critical effect may be overly conservative and a biomarker of exposure rather than an adverse effect as changes in sperm parameters were not noted until higher does.

Response: Human male fertility is established to be lower than that of rodent test species, suggesting that human fertility may be more susceptible to damage from toxic agents (Working, 1998; U.S. EPA, 1996). Decreased cauda epididymis weight in rats was the most sensitive of the suite of reproductive effects observed in the study by NTP (1993), including decreased testis, epididymis, and cauda epididymis weight, decreased testicular sperm concentration, and decreased sperm motility. The data from NTP (1993) suggest that cauda epididymis weight is an effect that precedes more severe decrements in sperm parameters, such as decreased testicular spermatid count, seen at the highest dose. Therefore, decreased cauda epididymis weight, the most sensitive effect observed in this study, was retained as the critical effect.

Comment: One reviewer commented that an endpoint that is secondary to thyroid perturbation does not make scientific, biological, and public health sense. The reviewer further commented that the critical effect selected should be consistent with the available mode of action data

indicating the thyroid as the likely target of toxicity following chronic exposure, not the observed reproductive effects observed in NTP (1993).

Response: See response to comment under Charge Question B1.

3. Benchmark dose (BMD) modeling methods were applied to continuous data on absolute cauda epididymis weight to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (specifically, a decrease in the control mean of one standard deviation) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comment: The reviewers generally agreed with the use of BMD modeling to determine the POD for the RfD. Additionally, the reviewers found the BMD modeling to be appropriately conducted.

Response: No response needed.

Comment: One reviewer requested that additional clarification be provided on the basis for the selection of the BMR of 1 SD. Another reviewer believed that the use of the lower confidence limit of 1 SD from the control mean will provide a minimally significant effect level.

Response: Ideally, BMR levels are determined based on the degree of change in the endpoint considered biologically significant. In the absence of this information, EPA's Draft *Benchmark Dose Technical Guidance Document* (2000b) recommends that a BMR of 1 SD change in the control mean be used as the standardized basis for comparisons with continuous data. In this case, the BMR level of one SD change in cauda epididymis weight was selected since information regarding what magnitude of change in this endpoint would be considered adverse was not available. Clarification regarding the selection of the BMR of 1 SD has been provided in Section 5.1.2.

Comment: One reviewer agreed with the use of BMD modeling, but was uneasy with the use of a BMD which is higher than the study LOAEL.

Response: There is no inherent relationship between a NOAEL or LOAEL and a BMR dose. PODs based on the NOAEL/LOAEL approach are highly dependent on study design including dose selection, dose spacing, and sample size. NOAELs are generally based on the lack of statistical significance and do not represent a biological threshold or imply that lower doses are without risk. The BMD approach mitigates some of the limitations of the NOAEL/LOAEL approach by using information from the entire dose-response curve, and thus, is preferable in cases where data are amenable to BMD modeling.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s).

Comment: Three reviewers suggested that the UF of 10 for interspecies extrapolation could be reduced to 3. One reviewer suggested that the rat was more sensitive than the mouse; therefore, a sensitive animal model was being used and thus, this UF could be reduced. However, this reviewer acknowledged that this difference between rodents does not inform the difference between rats and humans. Two reviewers commented that data may be available to inform toxicokinetic differences and similarities between rats and humans, and thus, this UF may be reduced.

Response: The interspecies UF, which takes into consideration toxicokinetic and toxicodynamic differences between animal test species and humans, is applied to account for the uncertainty in extrapolating from laboratory animal data to average healthy humans. In the case of HCN, chemical-specific data are not available which quantitatively inform the difference in susceptibility to cyanide between rats and humans; therefore, a 10-fold UF was retained.

Comment: One reviewer suggested reducing the UF of 10 for the extrapolation from the duration of the subchronic principal study (91 days) to a chronic duration, citing what is known about the thyroid disruption mode of action of cyanide and the existence of a chronic toxicity study, though limited, that did not reveal toxicity.

Response: The 91-day study by NTP (1993) falls well short of a lifetime duration. In addition, there is a lack of data on male reproductive parameters following chronic administration of cyanide, and the mode of action of the reproductive effects observed in NTP (1993) is unclear. Therefore, it is unknown whether effects would be more severe or would be observed at lower doses with a longer exposure duration. For these reasons, the UF of 10 to extrapolate from a study with a subchronic duration was retained. Additional text has been in added to Section 5.1.3 to clarify the rationale for this UF.

Comment: Two reviewers suggested a reduction of the database UF from 3 to 1. One reviewer commented that the data gaps highlighted in the discussion of the database UF need to be rethought, not necessarily reduced, given the existing reproductive and developmental toxicity data for HCN, and questioned whether a two-generation reproductive toxicity study and a neurodevelopment study would reveal effects not already observed, as these studies are not generally sensitive to thyroid disrupting chemicals. Another reviewer commented that the database UF should be reduced provided the critical effect selected for the derivation of the RfD is a sensitive toxicological endpoint observed in a sensitive species.

Response: The UF for database deficiencies is typically applied when sensitive studies such as a developmental toxicity study and a multigenerational reproductive toxicity study are not available, or when data suggest that an additional study would likely reveal a lower POD. The magnitude of the database UF reflects the database for a particular chemical and is not meant to be a reflection of the severity of the chosen critical effect. The justification for the database UF for HCN was modified in the document to reflect the lack of a neurodevelopmental study evaluating endpoints sensitive to thyroid disruption, and a multigenerational reproductive study. In consideration of these data gaps, a 3-fold UF to account for uncertainty in the database was retained. Text has been added to Section 5.1.3 to clarify the rationale for the database UF.

Comment: A reviewer expressed concern with the age of the database, and commented that the document should acknowledge that much of the data are old and may not meet current standards for study design, reporting, and peer review.

Response: Text was added to Section 5.3, Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration, acknowledging the age of the cyanide database.

(C) Inhalation Reference Concentration (RfC) for Hydrogen Cyanide

1. The occupational inhalation study by El Ghawabi et al. (1975) was selected as the basis for the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Specifically, are the study design, methods, and findings appropriate to support the derivation of an RfC? Also, please comment on whether the scientific justification and rationale for selecting the El Ghawabi et al. (1975) study as the principal study given the potential for possible co-exposure to other chemicals is adequately described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comment: In consideration of the studies available in the inhalation database for cyanide, the reviewers generally agreed with the selection of the El Ghawabi et al. (1975) as the basis for the RfC, stating that this study was either the most appropriate, was a reasonable choice, or was the best available. However, three reviewers also suggested greater consideration of the unpublished occupational study conducted by Leeser et al. (1990), with one reviewer also recommending the Leuschner and Neumann (1989) study for consideration as the principal study.

Response: The Leuschner and Neumann study was not considered as the principal study for the RfC as animals were treated orally and not via inhalation. Additional consideration of the unpublished occupational study by Leeser et al. (1990) was added to the document in Sections 4.1.3 and 5.2.1. This study was not considered to the most appropriate study for the RfC as no biologically significant effects were observed. It is unclear whether a NOAEL for thyroid effects can be established by this study as only one, relatively insensitive indicator of thyroid function was measured (serum total T_4). Other commonly administered (and more sensitive) tests for thyroid function, including TSH and iodide uptake, were not measured. Serum T_4 levels in cyanide exposed workers were decreased in controls, but according to the study authors did not reach statistical significance (85.13 ± 2.51 vs. 89.04 ± 1.81 nmol/L in controls). Additionally, serum T_4 was below the clinical reference range (60–160 nmol/L) in 3 of the cyanide-exposed workers.. The authors claimed these three workers "were otherwise normal and other thyroid functional tests showed that there was no functional problem". The authors did not state which additional tests were conducted to confirm normal thyroid function, and they specifically state that no radiolabel studies were conducted (which likely refers to the commonly used radioactive iodide uptake test). Information regarding the Leeser et al. (1990) study and associated uncertainties has been added to the Toxicological Review in Sections 4.1.3 and 5.2.1.

2. Thyroid enlargement and altered iodide uptake were selected as the critical effects for the RfC. Please comment on whether the selection of these critical effects is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comments: The panel agreed with the selection of functional thyroid effects as the endpoint for the derivation of the RfC.

Response: No response needed.

3. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to derive the POD for the RfC. Please provide comments as to whether this approach is the best

approach for determining the POD. Has the approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comment: The reviewers agreed that, considering the available data from the El Ghawabi et al. (1975) study, using the NOAEL/LOAEL approach to derive the RfC is appropriate.

Response: No response needed.

4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

Comment: Two reviewers generally agreed with the magnitude and application of the UFs.

Response: No response needed.

Comment: One reviewer stated that the UFs were well justified, but noted that intraspecies variability could exceed an order of magnitude when considering potentially susceptible populations, such as the developing fetus of an iodine- and/or protein-deficient mother; however, this may be compensated for if any of the other UFs are overestimated.

Response: Populations especially sensitive to thyroid disruption, such as the developing fetus, have been identified in the Toxicological Review; however, the magnitude of the variability between the male workers in the El Ghawabi et al. (1975) study and pregnant women in the general population is not known. Thus, in the absence of data, a 10-fold UF was applied for this area of uncertainty and variability in the human population. Additionally, a 10-fold UF was applied for database deficiencies to account for the lack of developmental and multigenerational reproductive studies.

Comment: Two reviewers recommended reducing the LOAEL-to-NOAEL UF. One reviewer commented that the LOAEL used for the POD for the thyroid disruption was the lowest concentration among all of the factories monitored in the El Ghawabi et al. (1975) study; therefore, a full UF to extrapolate from a LOAEL to a NOAEL was not needed. Another reviewer commented that thyroid effects were not observed in the occupational study by Leeser et al. (1990) and therefore, a NOAEL could be determined for this study, effectively eliminating the NOAEL to LOAEL UF.

Response: Thyroid effects were observed across the three factories in the study by El Ghawabi et al. (1975) at concentration ranges that overlapped. The average air concentrations in the three factories (7.07, 8.9, and 11.5 mg/m^3) were similar, and the lowest mean air concentration of 7.07 mg/m^3, at which thyroid disruption was observed, was selected as the POD. A NOAEL was not identified in this study. Thus, the default LOAEL to NOAEL UF was applied.

The unpublished study by Leeser et al. (1990) was added to the toxicological review (see Sections 4.1.3 and 5.2.1) but was not selected as the basis of the RfC. A LOAEL for thyroid effects was not identified for this review based on the lack of measurement of sensitive thyroid parameters (only serum total T$_4$ was measured). Other commonly administered (and more sensitive) tests for thyroid function, including TSH, free T$_4$, or iodide uptake, were not examined as part of the study design (see response to Charge Question C1). Therefore, this study was not used to reduce the LOAEL to NOAEL UF applied to the POD for the principal study.

Comment: Two reviewers disagreed with the subchronic-to-chronic UF of 3, with one reviewer commenting that the exposures for some workers were for up to 15 years and there was no correlation between duration of exposure and severity of effect (goiter). The other reviewer theorized that thyroid enlargement probably requires months of exposure to alter the thyroid gland by blocking iodide uptake and the severity may not be a function of length of exposure.

Response: The scientific support for this UF was re-evaluated and EPA has retained a 3-fold UF to account for extrapolation from a subchronic to chronic exposure duration. The workers in the principal study were exposed to cyanide for 5–15 years. Of the 36 workers, 14 had been exposed for 5 years, 14 for 5–10 years, 7 for 10–15 years, and 1 for >15 years. The mean and median exposure times for the worker population were not reported. Twenty of the 36 exposed workers had thyroid enlargement; however, the authors found no correlation between duration of exposure and either incidence or magnitude of thyroid enlargement in the workers. In addition, following continued administration of cyanide in rats, thyroid effects were less prominent at 11 months of exposure compared to 4 months of exposure (Philbrick et al., 1979), which provides some indication (although limited) that increased duration of exposure may not lead to an increase in thyroid effects. A lack of an association could be related to the low sample size and/or the failure of the authors to consider iodine status of the workers. Therefore, it is not clear whether greater alteration in thyroid function would be observed with a longer exposure duration. In the absence of information indicating that the effects of HCN would not progress in incidence or severity, a subchronic to chronic UF of 3 was applied. The text in Section 5.2.3 was revised to better characterize the support for this rationale.

Data are unavailable that indicate that thyroid enlargement may require longer periods of exposure to alter the thyroid gland (e.g., by blocking iodide uptake). While severity may not be

a function of length of exposure, evidence is lacking to support this hypothesis. Therefore, a subchronic to chronic UF of 3 was applied.

Comment: One of the reviewers (who disagreed with the application of a subchronic to chronic UF) suggested that EPA consider consulting a clinical endocrinologist who studies iodine deficient populations to inform the subchronic to chronic UF.

Response: A clinical endocrinologist was not consulted on the proposed UF for subchronic to chronic exposure duration; however, a reviewer with expertise in thyroid physiology was present on the panel.

(D) Carcinogenicity of Hydrogen Cyanide and Cyanide Salts

1. Under EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgrd.html), the Agency concluded that data are inadequate for an assessment of the human carcinogenic potential of cyanide. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

Comment: Four reviewers supported the Agency's determination that data are inadequate for an assessment of the carcinogenic potential of cyanide. One reviewer did not comment on the cancer characterization.

Response: No response required.

Comment: One reviewer suggested additional discussion focusing on whether other known thyroid toxicants with comparable mechanisms would be expected to induce thyroid tumors. Another reviewer suggested discussing EPA's guidance document on thyroid cancer mediated by TSH.

Response: Text has been added to Section 5.4 to discuss the uncertainty surrounding potential thyroid carcinogenesis of HCN.

PUBLIC COMMENTS

Comment: EPA should utilize all available studies of cyanide regardless of exposure route and form of the compound, including HCN gas, cyanide salt, cassava, cyanogenic glycosides, and acetone cyanohydrin (ACH).

Response: The unpublished inhalation studies of ACH exposure in rats (Monsanto Co., 1985a, b) are discussed in the Toxicological Review in Sections 4.2.2 and 4.3.2, but were not selected for derivation of the RfC due to the absence of any observed effects in these studies and the availability of inhalation studies in humans demonstrating sensitive effects on the thyroid.

Studies of human exposure to foods containing high amounts of cyanogenic glycosides, primarily from cassava, provide important hazard identification and susceptibility information for cyanide. These studies were included and considered in the document, but were ultimately determined not to be the most appropriate basis for the RfD due to concerns, including limited exposure documentation, confounding dietary deficiencies of the studied African populations (particularly low dietary intake of protein, vitamin B_{12}, and/or iodine; and overall malnutrition), and confounding from other chemical constituents of cassava such as linamarin, which one study found was associated with the endemic neurotoxicity (Konzo) observed in cassava-dependent, protein-deficient populations (Banea-Mayambu et al., 1997).

The toxicological effects observed following exposure to cyanide were considered based on the route of exposure (oral and inhalation) because a PBPK model, which would allow for extrapolation across routes of exposure, is not available for cyanide.

Comment: Protecting against acute toxicity of cyanides will protect against chronic effects, as acute toxicity results when the capacity for detoxification of cyanide is exceeded, whereas repeated-dose toxicity occurs within the detoxification capacity and can be tolerated over a longer period.

Response: The effects associated with cyanide vary depending on exposure. For example, populations that have slow uptake of cyanide through diet or smoking are not reported to have acute symptoms of cyanide, but demonstrate symptoms of chronic thyroid disruption through the cyanide metabolite, thiocyanate (SCN^-). The mechanisms of cyanide toxicity following acute and chronic exposures operate via different pathways. In evaluating the acute toxicity of cyanide, both the total amount of cyanide administered and the rate of cyanide absorption are important (U.S. EPA, 1992). Acute toxicity results from exceeding the body's capacity for the detoxification of cyanide (to the metabolite, thiocyanate). Acute doses of cyanide inhibit the enzyme cytochrome C oxidase of the mitochondrial electron chain, thus preventing the formation of ATP, which can lead to respiratory arrest/cardiac arrest (see Section 4.1.1). Thus, the relevant dose metric for the occurrence of acute cyanide toxicity is the peak dose of HCN in the blood. Cyanide, operating within the reductive capacity as thiocyanate, reduces iodide uptake and

affects thyroid hormone production and secretion following chronic exposure (see Section 4.5.3).
Thus, the relevant dose metric for chronic toxicity is based on the average amount of thiocyanate
in circulation, which has a much longer half-life in the body compared to cyanide. Therefore,
the toxicity associated with acute exposures is different from chronic exposures and protection
against the acute effects of cyanide does not protect against the chronic effects of cyanide
exposure.

Comment: The reference values proposed are 3–4 orders of magnitude below background
exposure levels and significantly below a well documented point of departure for deriving safe
cyanide exposures in humans. Background levels of cyanide for the general public should be
presented for perspective.

Response: Estimates of aggregate background cyanide exposure are not available. However,
information regarding exposure concentrations of cyanide in surface water, non-urban air, and
cigarette smoke is provided in Section 2. Estimates of the cyanide intake in the American diet
were not located in the available literature, though a study which estimates dietary cyanide intake
is available for a Korean population. Several studies exist which report cyanide concentrations
in food, though these studies do not report daily food intake which would be necessary to
calculate an average daily exposure of cyanide through food.

   "Safe" chronic cyanide exposure levels have not been established in the scientific
literature. Population-based studies examining exposure of cyanide at low levels and potential
health outcomes have historically been limited to African populations with relatively high intake
of cyanide containing plants (primarily cassava). Human populations with high cyanide
exposure due to diet and/or tobacco smoking have been identified as having an elevated risk of
thyroid disruption (Vestergaard, 2002; Makene and Wilson, 1972; Osuntokun et al., 1969).

Comment: The testicular and epididymis effects reported in the 1993 NTP study were not
appropriate to use in deriving the RfD, as they are more likely the result of decreased water
consumption or stress. The commentors also stated that, in the absence of histopathology or a
proposed mode of action, the epididymal weight effects are inappropriate for use in risk
assessment.

Response: Data to support the hypothesis that decreased reproductive organ weights and sperm
parameters can be caused by slight to moderate decreases in drinking water consumption were
not found in the peer reviewed literature. At the LOAEL for decreased cauda epididymis weight,
a 7%, non-statistically significant decrease in water consumption was observed. In addition,
increases in hematocrit, blood urea nitrogen, or serum albumin, which have been identified as
objective indicators of dehydration (Campbell et al., 2009), were not observed at the LOAEL

identified for decreased epididymis weight in the NTP (1993) study. EPA considered the cauda epididymis weight changes to be biologically significant. Additionally, U.S. EPA guidelines have identified statistically significant reproductive organ weight changes as endpoints useful for reproductive risk assessment (U.S. EPA, 1996). The lack of a known mechanism or histological lesion does not imply that the decrease in cauda epididymis weight is not relevant to humans and does not constitute grounds to disregard the observed effects.

Comment: EPA should not rely on the El Ghawabi et al. (1975) study to establish the RfC given its limitations, including limited exposure data and inconsistencies regarding the urinary thiocyanate excretion in this study compared to a population in France. In addition, reported symptoms in workers with exposure to cyanide, included headache, weakness, vomiting, dyspnea, and precordial pain. It is suggested that EPA give greater consideration to the subchronic ACH inhalation study (Monsanto Co., 1985a, b).

Response: The external peer review panel agreed with the selection of the El Ghawabi et al. (1975) study for the derivation of the RfC. Strengths and limitations of the El Ghawabi et al. (1975) study are discussed in Sections 4.1.3, 5.2.1, and 5.3 of the Toxicological Review. In consideration of the limited inhalation database, the El Ghawabi et al. (1975) study was selected as the principal study, with thyroid enlargement and altered iodine uptake selected as the critical effects. This study of cyanide-exposed workers and control workers included individual breathing zone measurements from the study participants and reported a strong correlation between these cyanide measurements and urinary metabolite levels. Excretion of SCN^- in workers in the El Ghawabi et al. (1975) study was similar to average urinary excretion of SCN^- measured in a French population not occupationally exposed to cyanide (Barrere et al., 2000). This similarity could be due to the very low background SCN^- exposure of workers in the El Ghawabi et al. (1975) study (only nonsmokers were included in the study and subjects were asked to refrain from consumption of food high in cyanogenic glycosides), different methods of detection of urinary SCN^- between studies, and the high background excretion of SCN^- measured in the Barrere et al. (2000) study compared to studies in other populations (Steinmaus et al., 2007; Brauer et al., 2006; Haque and Bradbury, 1999).

The unpublished inhalation studies of ACH exposure in rats (Monsanto Co., 1985a, b) were discussed in the Toxicological Review in Sections 4.2.2 and 4.3.2, but were not selected for derivation of the RfC due to the absence of any observed effects and the availability of inhalation studies in humans demonstrating sensitive effects on the thyroid.

Comment: The review of cyanide conducted by European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) should be considered by EPA. This review established an

acceptable chronic level of 15 µg SCN⁻/mL in serum, which the authors equated to a daily occupational inhalation exposure of 7.5 mg CN⁻/m³.

Response: The ECETOC review for safe chronic exposure to cyanide was based on serum SCN⁻ concentrations from two studies and a subsequent estimation of an occupational inhalation concentration based on the serum SCN⁻ concentrations and the utilization of several assumptions (regarding absorption, metabolism, and excretion rates). The ECETOC NOAEL is based on a serum SCN⁻ level of 15 µg SCN⁻/mL, which was based on the studies by Banerjee (1997) and Cliff et al. (1986), which detected serum levels of 18 and 14.5 µg SCN⁻/mL, respectively. However, at these SCN⁻ blood levels, statistically significant increased levels of TSH were observed along with decreases in serum T_4 levels, indicative of thyroid function perturbation. The observation of thyroid function perturbations at 18 and 14.5 µg SCN⁻/mL is considered by EPA to be a LOAEL. Furthermore, the back-calculated occupational exposure value of 7.5 mg CN⁻/m³ is higher than the LOAEL identified by EPA in this document for thyroid effects in the El Ghawabi et al. (1975) occupational study. Therefore, EPA maintains the recommendation of an RfC based on data reported by El Ghawabi et al. (1975).

Comment: EPA's proposed derivation of the RfD and RfC applied large UFs, whereas the application of ECETOC's approach provides a more reliable POD and requires a lower composite factor to account for uncertainty: intraspecies UF of 10 and a factor of 3 to account for sensitive subpopulations (developmental effects).

Response: The rationale for the selection of UFs for the RfC is detailed in Section 5.2.3 and incorporates uncertainties associated with intraspecies extrapolation, extrapolation from a LOAEL to a NOAEL, extrapolation from subchronic to chronic exposure, and database deficiencies. The application of the UFs in the Toxicological Review of Hydrogen Cyanide and Cyanide Salts is in concordance with the guidance outlined in the RfC Methodology (U.S. EPA, 1994b) and the Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002). See previous comment and response with regard to ECETOC's approach to deriving a chronic exposure value.

Comment: There is sufficient information to enable EPA to conclude that cyanide does not present a carcinogenic risk based on the animal and human studies and negative data for genotoxicity.

Response: EPA concluded that there was "inadequate information to assess the carcinogenic potential" of cyanide. The descriptor of "not likely to be carcinogenic to humans" is appropriate when there are robust data indicating that there is no basis for human hazard concern. As noted

in U.S. EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, this descriptor may be applied when well-designed and well-conducted animal studies, in both sexes of two species, indicate the lack of carcinogenic potential, and is applied in the absence of other animal or human data indicating potential for cancer effects. In the case of cyanide, only one chronic animal study in 10 male and female rats is available (Howard and Hanzal, 1955). This study did not identify exposure-related tumors.

Studies examining cancer incidence in occupationally exposed cyanide workers are not available in the literature. Studies of cancer in populations exposed to thiocyanate via the diet are limited to examinations of thyroid cancer and results are generally not positive (Bosetti et al., 2002; Kolonel et al., 1990), although one recent case-control study has associated high consumption of goitrogenic food and low iodine intake with increased incidence of thyroid cancer in women (Truong et al., 2010). The database consisting of one animal study in a single species does not provide robust and convincing evidence that cyanide is "not likely to be carcinogenic to humans". Text for clarification of the available evidence for carcinogenicity was added to the weight-of-evidence narrative for carcinogenicity in Section 4.7.

APPENDIX B. BENCHMARK DOSE MODELING RESULTS

Decreased Absolute Cauda Epididymis Weight in Rats Exposed to Sodium Cyanide (NaCN) in Drinking Water for 13 Weeks (NTP, 1993)

All models for continuous variables available in the EPA BMDS version 1.4.1c, except the Hill model, were fit to the data in the Table B-1. The Hill model was not fit to these data because fitting of the Hill model requires the estimation of four parameters (i.e., intercept, v, n, and k), which necessitates having a minimum of five dose groups in order to have adequate degrees of freedom for testing model fit. The NTP (1993) study has only four dose groups, and thus, the Hill model could not be fit to these data. All models fit were constant variance models. All models tested provided adequate fit to the data, based on the summary results reported by the BMDS output and visual examination of the graphs. A summary of the goodness-of-fit statistics for the tested models and resulting BMD and BMDL is presented in Table B-2.

Table B-1. Decreased cauda epididymis weight in F344 rats following administration of NaCN in drinking water for 13 weeks

Male rats	0 ppm	30 ppm	100 ppm	300 ppm
Dose (mg/kg-d CN⁻)	0	1.4	4.5	12.5
Weight (g) ± SD Cauda epididymis, absolute	0.162 ± 0.009	0.150 ± 0.013	0.148 ± 0.013	0.141 ± 0.009

Table B-2. BMD modeling results for decreased cauda epididymis weight in rats

Study	Endpoint	Model	AIC	Goodness-of-fit *p*-value	BMD	BMDL
NTP (1993); male rats	Cauda epididymis weight (absolute)	Linear (1° polynomial)	-312.75	0.08	8.4	5.6
		Polynomial (2°)	**-313.10**	**0.11**	**3.5**	**1.9**
		Power	-312.75	0.08	8.4	5.6

All models adequately described the data as demonstrated by goodness-of-fit *p*-values > 0.1. Of the available models, the polynomial (2°) was selected, based on having the lowest AIC value. Visual inspection reveals that the model describes the data well.

Polynomial Model with 0.95 Confidence Level

16:00 02/13 2008
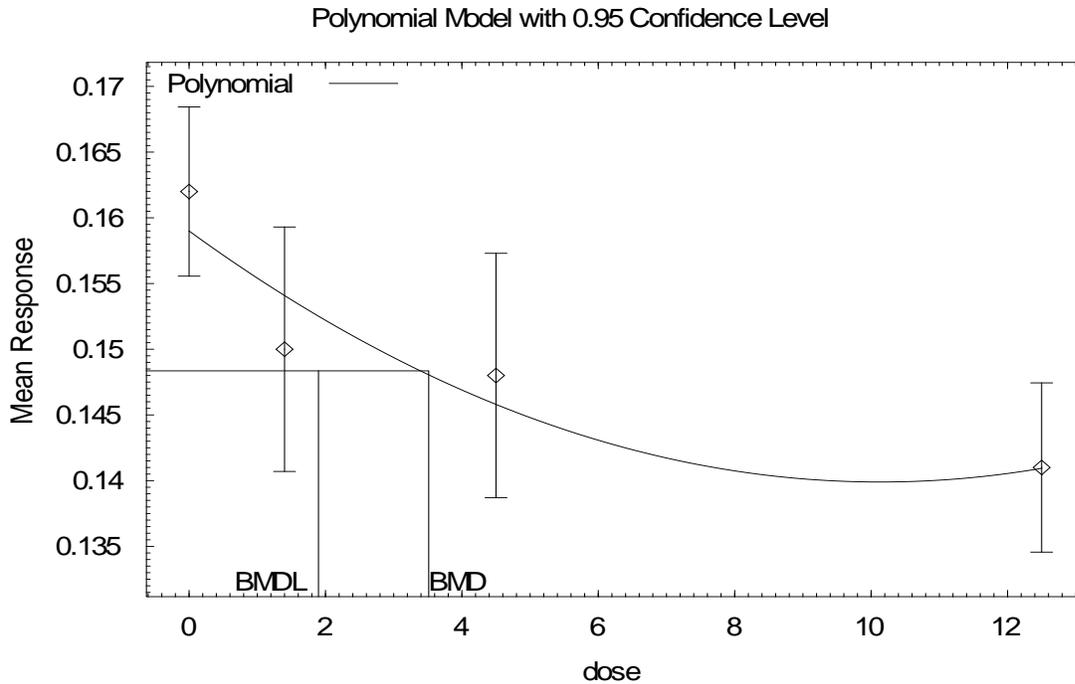
Figure B-1. Observed and predicted decrease in cauda epididymis weight in F344 rats following administration of NaCN in drinking water for 13 weeks.

The computer output for the polynomial model of decreased (absolute) cauda epididymis weight follows:

```
====================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: C:\BMDS1-4-1C\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS1-4-1C\UNSAVED1.plt
                                    Wed Feb 13 16:00:34 2008
====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = COLUMN1
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

```
                    Default Initial Parameter Values
                         alpha =      0.000125
                           rho =             0    Specified
                        beta_0 =      0.159311
                        beta_1 =   -0.00377087
                        beta_2 =      0.0001857


            Asymptotic Correlation Matrix of Parameter Estimates

  ( *** The model parameter(s)  -rho have been estimated at a boundary point,
or have been specified by the user, do not appear in the correlation matrix )


                    alpha        beta_0        beta_1        beta_2

       alpha            1     -8.4e-010     -3.3e-011      4.3e-010
      beta_0    -8.4e-010            1         -0.72          0.61
      beta_1    -3.3e-011        -0.72            1          -0.97
      beta_2     4.3e-010         0.61        -0.97             1



                           Parameter Estimates

                                                    95.0% Wald
Confidence Interval
        Variable        Estimate       Std. Err.     Lower Conf. Limit
Upper Conf. Limit
          alpha      0.000120048    2.68434e-005        6.74354e-005
0.00017266
         beta_0         0.159311     0.00302151            0.153389
0.165233
         beta_1      -0.00377087     0.00159992         -0.00690665
-0.000635079
         beta_2        0.0001857    0.000119328        -4.81776e-005
0.000419578



      Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean    Obs Std Dev   Est Std Dev   Scaled
Res.
------      ---   --------     --------    -----------   -----------   ---------
-

    0       10      0.162        0.159         0.009         0.011         0.776
  1.4       10       0.15        0.154         0.013         0.011         -1.27
  4.5       10      0.148        0.146         0.013         0.011         0.548
 12.5       10      0.141        0.141         0.009         0.011       -0.0551



  Model Descriptions for likelihoods calculated


  Model A1:          Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2

  Model A2:          Yij = Mu(i) + e(ij)
```

B-3

```
            Var{e(ij)} = Sigma(i)^2

 Model A3:          Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:          Yi = Mu + e(i)
          Var{e(i)} = Sigma^2



                    Likelihoods of Interest

          Model      Log(likelihood)   # Param's      AIC
           A1            161.851147          5     -313.702293
           A2            163.173943          8     -310.347886
           A3            161.851147          5     -313.702293
        fitted           160.552452          4     -313.104903
            R            153.631041          2     -303.262082


                    Explanation of Tests

  Test 1:  Do responses and/or variances differ among Dose levels?
           (A2 vs. R)
  Test 2:  Are Variances Homogeneous? (A1 vs A2)
  Test 3:  Are variances adequately modeled? (A2 vs. A3)
  Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
  (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              19.0858          6          0.004021
   Test 2              2.64559          3            0.4496
   Test 3              2.64559          3            0.4496
   Test 4              2.59739          1             0.107
```

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data


```
              Benchmark Dose Computation

Specified effect =               1

Risk Type        =      Estimated standard deviations from the control mean
```

```
Confidence level =          0.95

         BMD =        3.51354

        BMDL =        1.89704
```

Decreased Absolute Epididymis Weight in Rats Exposed to NaCN in Drinking Water for
13 Weeks (NTP, 1993)

      All models for continuous variables available in the EPA BMDS version 1.4.1c, except the Hill model, were fit to the data in the Table B-3. The Hill model was not fit to these data because fitting of the Hill model requires the estimation of four parameters (i.e., intercept, v, n, and k), which necessitates having a minimum of five dose groups in order to have adequate degrees of freedom for testing model fit. The NTP (1993) study has only four dose groups, and thus the Hill model could not be fit to these data. All models fit were constant variance models. All models tested provided adequate fit to the data, based on the summary results reported by the BMDS output and visual examination of the graphs. A summary of the goodness-of-fit statistics for the tested models and resulting BMD and BMDL is presented in Table B-4.

Table B-3. Decreased epididymis weight in F344 rats following administration of NaCN in drinking water for 13 weeks

Male rats	0 ppm	30 ppm	100 ppm	300 ppm
Dose (mg/kg-d CN⁻)	0	1.4	4.5	12.5
Weight (g) ± SD Epididymis, absolute	0.448 ± 0.019	0.437 ± 0.016	0.425 ± 022[a]	0.417 ± 0.016[b]

[a]Not reported as significant in NTP (1993) but significant by Dunnett's test in independent analyses conducted for this assessment, $p \leq 0.05$.
[b]Significant by Shirley's test, $p \leq 0.01$.

Source: NTP (1993).

Table B-4. BMD modeling results for decreased epididymis weight in rats

Study	Endpoint	Model	AIC	Goodness-of-fit *p*-value	BMD	BMDL
NTP (1993); male rats	Epididymis weight (absolute)	Linear (1° polynomial)	-274.73	0.22	8.2	5.6
		Polynomial (2°)	**-275.64**	**0.73**	**3.2**	**1.8**
		Power	-274.73	0.22	8.2	5.6

      All models adequately described the data as demonstrated by goodness-of-fit *p*-values > 0.1. Of the available models, the polynomial (2°) was selected, based on having the lowest AIC value. Visual inspection reveals that the model describes the data well.

Polynomial Model with 0.95 Confidence Level
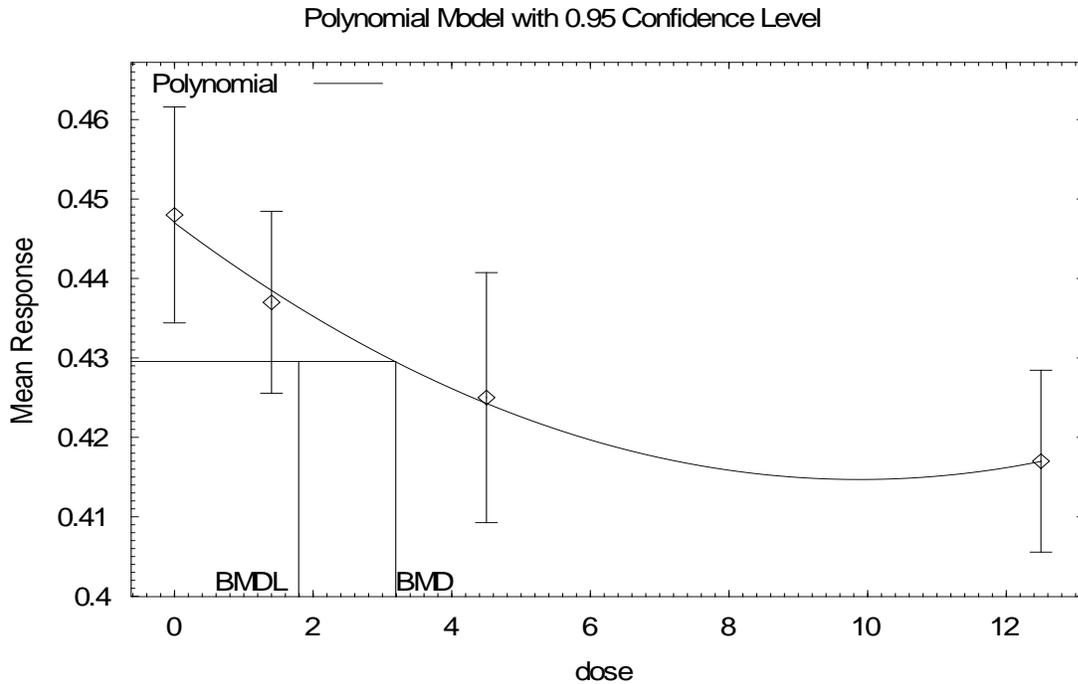
15:40 02/13 2008

**Figure B-2. Observed and predicted decrease in epididymis weight in F344
rats following administration of NaCN in drinking water for 13 weeks.**

The computer output for the polynomial model of decreased (absolute) epididymis weight
follows:

```
====================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: C:\BMDS1-4-1C\EPIDIDYMIS_WEIGHT_ABSOLUTE.(d)
        Gnuplot Plotting File:  C:\BMDS1-4-1C\EPIDIDYMIS_WEIGHT_ABSOLUTE.plt
                                        Wed Feb 13 15:40:43 2008
 ====================================================================
 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

   Dependent variable = MEAN
   Independent variable = COLUMN2
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
                 Default Initial Parameter Values
```

```
                        alpha =     0.00033925
                          rho =            0    Specified
                       beta_0 =      0.447054
                       beta_1 =      -0.00654
                       beta_2 =   0.000331286


            Asymptotic Correlation Matrix of Parameter Estimates

  ( *** The model parameter(s)  -rho have been estimated at a boundary point,
 or have been specified by the user, and do not appear in the correlation
 matrix )

                    alpha       beta_0       beta_1       beta_2

      alpha             1       1e-010     -3.9e-011     -1.8e-011

      beta_0       1e-010            1        -0.72          0.61

      beta_1    -3.9e-011        -0.72            1         -0.97

      beta_2    -1.8e-011         0.61        -0.97            1


                             Parameter Estimates

                                                        95.0% Wald
Confidence Interval
        Variable          Estimate        Std. Err.      Lower Conf. Limit
Upper Conf. Limit
           alpha        0.00030626      6.84818e-005        0.000172038
0.000440482
          beta_0          0.447054       0.00482605           0.437595
0.456513
          beta_1          -0.00654       0.00255545          -0.0115486
-0.00153141
          beta_2       0.000331286      0.000190594        -4.22712e-005
0.000704843



      Table of Data and Estimated Values of Interest

  Dose        N    Obs Mean     Est Mean    Obs Std Dev  Est Std Dev   Scaled
Res.
 ------      ---   --------     --------    -----------  -----------   --------
 -

    0        10     0.448        0.447         0.019       0.0175        0.171
  1.4        10     0.437        0.439         0.016       0.0175       -0.28
  4.5        10     0.425        0.424         0.022       0.0175        0.121
 12.5        10     0.417        0.417         0.016       0.0175      -0.0121



  Model Descriptions for likelihoods calculated


  Model A1:          Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2


                                B-8
```

```
 Model A2:          Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:          Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that were specified by the
user
 Model  R:          Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                    Likelihoods of Interest

          Model      Log(likelihood)   # Param's      AIC
           A1          141.882675          5      -273.765351
           A2          142.610833          8      -269.221665
           A3          141.882675          5      -273.765351
         fitted        141.821537          4      -275.643073
           R           134.393157          2      -264.786314


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels? (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test     -2*log(Likelihood Ratio)  Test df       p-value

   Test 1              16.4354           6           0.0116
   Test 2              1.45631           3           0.6924
   Test 3              1.45631           3           0.6924
   Test 4             0.122278           1           0.7266


The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here



The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data

            Benchmark Dose Computation

Specified effect =              1

Risk Type         =      Estimated standard deviations from the control mean

Confidence level =          0.95
```

```
BMD =          3.19201

BMDL =           1.79176
```

Table B-5. Decreased testis weight in F344 rats following administration of NaCN in drinking water for 13 weeks

Male rats	0 ppm	30 ppm	100 ppm	300 ppm
Dose (mg/kg-d CN⁻)	0	1.4	4.5	12.5
Weight (g) ± SD Testis, absolute	1.58 ± 0.094	1.56 ± 0.063	1.52 ± 0.063	1.46 ± 0.063

Table B-6. BMD modeling results for decreased testis weight in rats

Study	Endpoint	Model	AIC	Goodness-of-fit p-value	BMD	BMDL
NTP (1993); male rats	Testis weight (absolute)	**Linear (1° polynomial)**	**-167.94**	**0.82**	**7.4**	**5.1**
		Polynomial (2°)	-166.32	0.98	5.3	2.4
		Power	**-167.94**	**0.82**	**7.4**	**5.1**

Linear Model with 0.95 Confidence Level
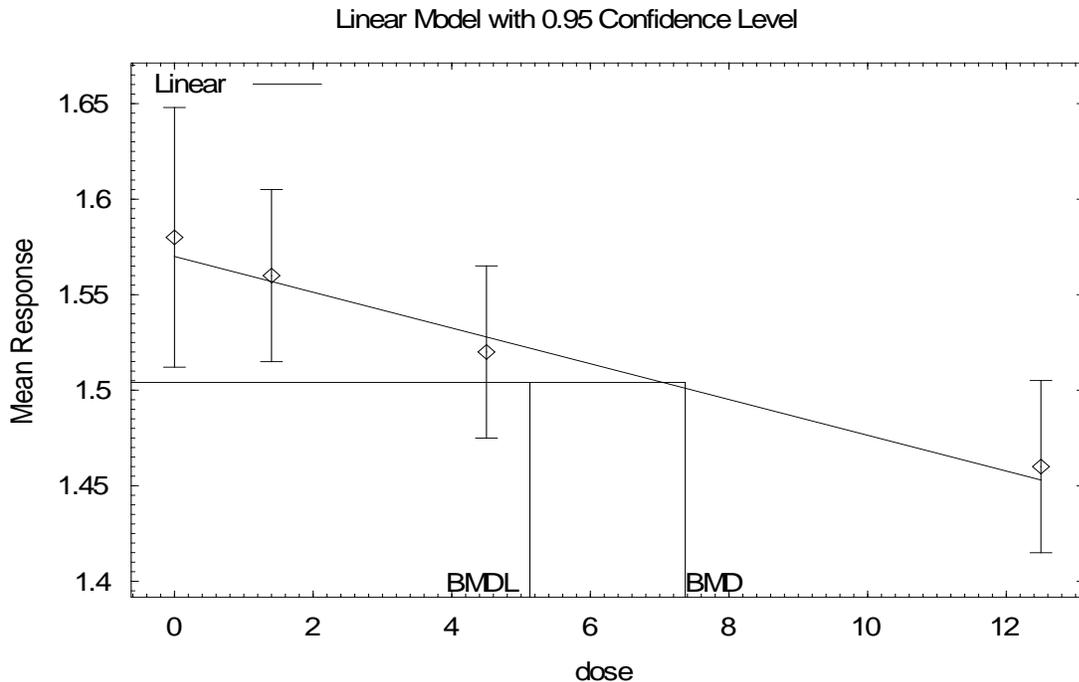
15:08 02/13 2008

Figure B-3. Observed and predicted decrease in testis weight in F344 rats following administration of NaCN in drinking water for 13 weeks.

The computer output from the linear model of decreased (absolute) testis weight follows:

```
=====================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: C:\BMDS1-4-1C\TESTIS_WEIGHT_ABSOLUTE.(d)
        Gnuplot Plotting File:  C:\BMDS1-4-1C\TESTIS_WEIGHT_ABSOLUTE.plt
                                        Wed Feb 13 15:08:00 2008
 =====================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = COLUMN2
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                        alpha =      0.005233
                          rho =             0    Specified
                       beta_0 =       1.57305
                       beta_1 =   -0.00935835


          Asymptotic Correlation Matrix of Parameter Estimates

   ( *** The model parameter(s)  -rho have been estimated at a boundary point,
or have been specified by the user, and do not appear in the correlation
matrix )

               alpha        beta_0        beta_1

     alpha          1    -4.1e-010      3.3e-011

    beta_0  -4.1e-010            1         -0.69

    beta_1   3.3e-011        -0.69            1



                          Parameter Estimates

                                             95.0% Wald
Confidence Interval
```

```
         Variable          Estimate        Std. Err.      Lower Conf. Limit
Upper Conf. Limit
         alpha         0.00475554       0.00106337          0.00267137
0.00683971
         beta_0          1.57305         0.0150381            1.54357
1.60252
         beta_1        -0.00935835       0.0022514           -0.013771
-0.00494569




     Table of Data and Estimated Values of Interest

 Dose        N     Obs Mean      Est Mean    Obs Std Dev   Est Std Dev    Scaled
Res.
------      ---   --------      --------    -----------   -----------   ---------
-

    0        10      1.58         1.57         0.095         0.069         0.319
  1.4        10      1.56         1.56         0.063         0.069       0.00244
  4.5        10      1.52         1.53         0.063         0.069        -0.501
 12.5        10      1.46         1.46         0.063         0.069          0.18



  Model Descriptions for likelihoods calculated


 Model A1:         Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:         Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:         Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:          Yi = Mu + e(i)
          Var{e(i)} = Sigma^2


                 Likelihoods of Interest

          Model      Log(likelihood)    # Param's       AIC
           A1           87.162621           5        -164.325243
           A2           88.584611           8        -161.169222
           A3           87.162621           5        -164.325243
         fitted         86.968887           3        -167.937775
            R           79.788144           2        -155.576289


                 Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
```

(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

    Test   -2*log(Likelihood Ratio) Test df   p-value

    Test 1   17.5929   6   0.007334
    Test 2   2.84398   3   0.4163
    Test 3   2.84398   3   0.4163
    Test 4   0.387468   2   0.8239

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

            Benchmark Dose Computation

Specified effect =   1

Risk Type   =   Estimated standard deviations from the control mean

Confidence level =   0.95

          BMD =   7.36887

         BMDL =   5.12669

Table B-7. Decreased testicular spermatid concentration in F344 rats following administration of NaCN in drinking water for 13 weeks

Male rats	0 ppm	30 ppm	100 ppm	300 ppm
Dose (mg/kg-d CN⁻)	0	1.4	4.5	12.5
Mean/10^{-4} mL suspension $\pm$ SD Spermatid count	89.28 $\pm$ 9.64	84.68 $\pm$ 12.74	82.90 $\pm$ 9.99	77.10 $\pm$ 6.96

Table B-8. BMD modeling results for decreased testicular spermatid concentration in rats

Study	Endpoint	Model	AIC	Goodness-of-fit *p*-value	BMD	BMDL
NTP (1993); male rats	Spermatid concentration (testis)	**Linear (1° polynomial)**	**227.04**	**0.70**	**11.2**	**6.9**
		Polynomial (2°)	228.73	0.53	8.5	2.9
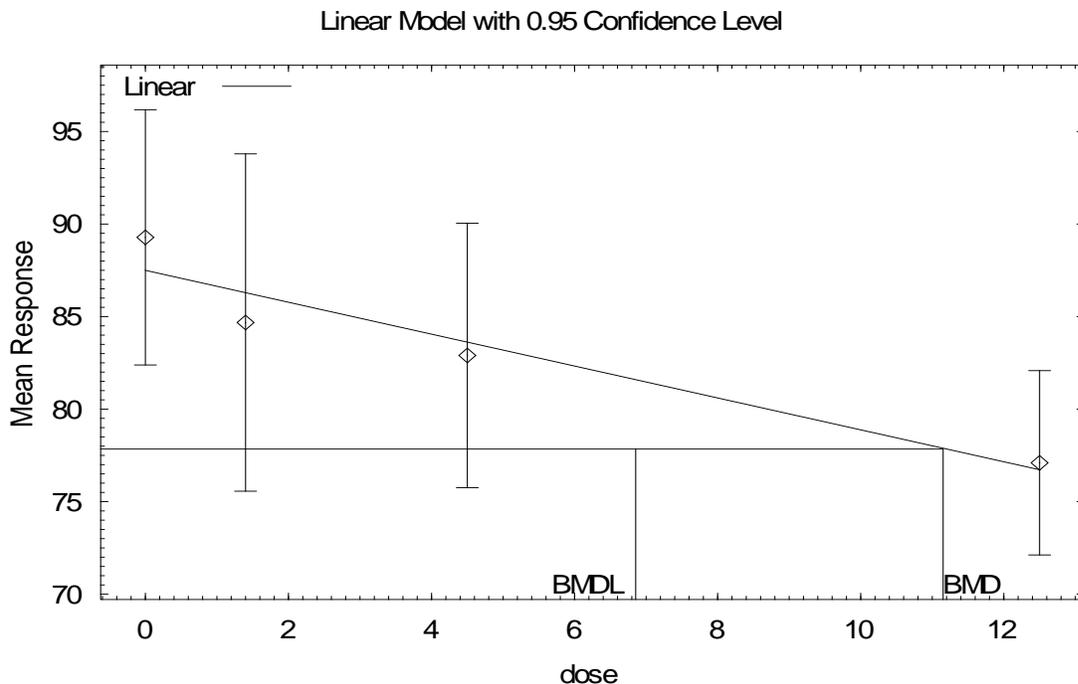
Figure B-4. Observed and predicted decrease in testicular spermatid concentration in F344 rats following administration of NaCN in drinking water for 13 weeks.

The computer output from the polynomial model of testicular spermatid concentration follows:

```
======================================================================
         Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: C:\BMDS1-4-1C\UNSAVED1.(d)
         Gnuplot Plotting File:  C:\BMDS1-4-1C\UNSAVED1.plt
                                         Thu Feb 21 16:40:27 2008
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = COLUMN1
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                   Default Initial Parameter Values
                         alpha =        100.87
                           rho =             0    Specified
                        beta_0 =       87.4548
                        beta_1 =     -0.861906


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been
specified by the user,
                 and do not appear in the correlation matrix )

                  alpha        beta_0         beta_1

      alpha           1       1.1e-010      -2.5e-013

     beta_0     1.1e-010            1          -0.69

     beta_1    -2.5e-013        -0.69             1



                            Parameter Estimates

                                             95.0% Wald
Confidence Interval
```

```
          Variable          Estimate        Std. Err.      Lower Conf. Limit
Upper Conf. Limit
          alpha            92.3886          20.6587            51.8982
132.879
          beta_0           87.4548          2.09605            83.3466
91.563
          beta_1          -0.861906         0.313806          -1.47695
-0.246857




     Table of Data and Estimated Values of Interest

 Dose        N     Obs Mean     Est Mean    Obs Std Dev   Est Std Dev   Scaled
Res.
------      ---   --------     --------     ----------    ----------    ---------
-

   0        10      89.3         87.5          9.64          9.61          0.6
  1.4       10      84.7         86.2          12.7          9.61         -0.516
  4.5       10      82.9         83.6          9.99          9.61         -0.222
 12.5       10      77.1         76.7          6.96          9.61          0.138




 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

 Model  R:        Yi = Mu + e(i)
          Var{e(i)} = Sigma^2


                  Likelihoods of Interest

        Model      Log(likelihood)    # Param's      AIC
         A1         -110.169386            5       230.338773
         A2         -108.417108            8       232.834216
         A3         -110.169386            5       230.338773
       fitted       -110.520064            3       227.040129
         R          -113.975552            2       231.951103


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
```

(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test   -2*log(Likelihood Ratio) Test df   p-value

   Test 1   11.1169   6   0.08483
   Test 2   3.50456   3   0.3202
   Test 3   3.50456   3   0.3202
   Test 4   0.701356   2   0.7042

The p-value for Test 1 is greater than .05. There may not be a
difference between responses and/or variances among the dose levels
Modeling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

             Benchmark Dose Computation

Specified effect =   1

Risk Type   =   Estimated standard deviations from the control mean

Confidence level =   0.95

          BMD =   11.1519
          BMDL =   6.85