Appendix A. Background Information for Uranium

A.1 Toxicokinetics

Studies conducted in human adults indicate that gastrointestinal absorption of ingested soluble uranium salts is <5%. On the basis of studies in animals, absorption of more water soluble uranyl compounds is greater than less soluble oxides and tetrahalide compounds. Studies in animals also provide evidence that gastrointestinal absorption of uranium may be increased by fasting and diets deficient in iron, and is higher in neonates than in adults. The rate of absorption of uranium compounds deposited in the respiratory tract varies with solubility of the uranium compound; more water soluble uranyl compounds are absorbed more readily than less soluble oxides and tetrahalide. Absorbed uranium appears to distribute initially to kidney, liver, and other soft tissues; however, under steady state conditions, the kidneys and skeleton contain most of the uranium in the body. Most of the uranium entering the kidney and soft tissue is lost over a period of days, but a small amount is retained for years. Uranium is lost from bone in multiple phases having half-lives of days, months, and years. As a result, the major depot for uranium is the bone within months or years after exposure ceases. Uranium is not known to be metabolized. The uranyl ion forms complexes with bicarbonate, citrate, and other soluble anionic species, and binds to proteins in tissue and plasma. Absorbed uranium is excreted primarily in urine (ATSDR 1999b).

Uranium radioisotopes, although they emit alpha radiation, behave in a chemically identical manner relative to stable uranium isotopes. As such, no differences in the kinetics of stable and radioactive uranium isotopes are expected, and the kinetics of distribution of the emitted radiation, which has a very short path length, can be predicted based on the pharmacokinetic parameters of stable uranium.

A.2 Health Effects

Absorbed uranium is nephrotoxic. Clinical case studies indicate that uranium can produce nephrotoxic effects in humans (ATSDR 1999b; Lussenhop et al. 1958). Epidemiological studies have found indications of possible nephrotoxicity (e.g., tubular proteinuria, aminoaciduria, glucosuria, and enzymuria) in uranium mill workers and in populations exposed to uranium in well water (ATSDR 1999b). Nephrotoxicity has been observed in a variety of animal species including dogs, rabbits, and rats exposed to uranyl salts (e.g., uranyl acetate, fluoride or nitrate) by the oral route, in rats exposed by the

inhalation route, in dogs, rabbits, and rats subjected to whole-body exposures to air-borne uranium, or after parenteral dosing (reviewed in ATSDR 1999b and Diamond 1989). The functional and morphological changes that have been observed in these studies are reasonably consistent across species and uranyl salts; these include, depending on the dosage and timing of observations, lesions of the glomerulus and renal proximal tubule and a variety of related functional impairments, including decreased glomerular filtration and renal blood flow, glucosuria and amino aciduria, proteinuria, and enzymuria. Studies of rats and rabbits have shown that ingestion of soluble uranium salts can produce histological changes in the thyroid gland and liver lesions in addition to renal lesions (ATSDR 1999b).

The studies reported in Maynard and Hodge (1949) and Maynard et al. (1953) were conducted as part of the health physics program of the *Manhattan Project*. The reporting of study outcomes focused primarily on three indicators of toxicity: growth depression, mortality, and nephrotoxicity, and, despite the above limitations, provided data on the relative toxicity of a wide variety of water soluble and relatively water insoluble uranium compounds. In general, studies of rats (the rat was the most extensively explored animal model) demonstrated that the more water soluble compounds (e.g., UO_2F_2, $UO_s(CH_3COO)_2{\cdot}2H2O$, $UO_2(NO_3)_2{\cdot}6H_2O$, UO_4) have higher toxic potencies than the relatively water insoluble uranium compounds (e.g., UO_2, UO_3, U_3O_8). Yuile (1973) summarized the relative potency for selected uranium compounds as follows, based on a comparison of the dietary concentrations (%) that produced equal body weight depressions in the chronic rat studies: UO_2F_2, 0.25% (155 mg U/kg-day); $UO_2(NO_3)_2{\cdot}6H_2O$, 1% (380 mg U/kg-day); UF_4, 20% (12,000 mg U/kg-day); and UO_2, >20% (>14,000 mg U/kg-day).

Inhaled uranium particulate can produce lung disease. A relatively large body of epidemiological literature exists on the topic of health outcomes associated with working in the uranium ore mining industry (e.g., Bruske-Hohlfeld et al. 1997; Hnizdo et al. 1997; Hornung et al. 1998; Roscoe 1997; Roscoe et al. 1995). These studies have shown excess risks of lung cancer and other respiratory tract diseases among miners. Uranium exposures in these populations is to a complex mixture of radon gas and daughter isotopes as well as to airborne dusts containing a variety of uranium compounds including various water insoluble uranium oxides. Exposures to radon daughter isotopes are thought to be major contributors to the increased risk of diseases of the respiratory tract, including cancer, in uranium miners. Health outcomes associated with working in the uranium processing industry also have been studied. Here again, exposure in these industries is primarily to dusts of relatively insoluble uranium compounds and, possibly, to aerosols of more soluble uranium compounds. Internal exposure to alpha radiation may be a major contributor to increased lung cancer risks, which have been reported in some studies. Chronic

exposures to uranium dioxide produced nephrotoxic changes and lung fibrosis in dogs and monkeys (ATSDR 1999b).

A small number of studies of the reproductive and developmental effects of uranyl salts have been reported (ATSDR 1999b; Paternain et al. 1989). The results of these studies suggest that maternal exposures to uranyl acetate during pregnancy can be maternally toxic and fetotoxic.

Only limited data exist on the toxicity of uranium radiation. From the standpoint of noncancer effects, the effects noted in studies of uranium, even radioisotopes of uranium, are believed to be solely chemical (ATSDR 1999b). Although radiation exposure has been generally assumed to be carcinogenic at all dose levels, no correlation has been established at low doses such as occur from exposure to natural radiation background levels. This is largely attributable to two factors: (1) it is difficult to construct and obtain meaningful data from epidemiological studies where exposure is near background exposure levels, and (2) the data are not statistically significant enough to substantiate a detectable health impact.

A.3 Mechanisms of Action

Mechanisms of uranium-induced nephrotoxicity have been extensively explored in animal models (reviewed in Diamond 1989). Decreased glomerular filtration rate, proteinuria, impairment of tubular function, and tubular injury are prominent features of nephrotoxicity in animals exposed to uranyl compounds. Mechanisms for decreased glomerular filtration appear to involve multiple factors including changes in renal plasma flow, glomerular capillary hydrostatic pressure, tubular hydraulic pressure, and glomerular ultrafiltration coefficient. Tubular impairments include glucosuria, amino aciduria, enzymuria, and osmotic diuresis. Prominent features of tubular injury are initial necrosis of the terminal segments of the proximal tubule with subsequent involvement of the distal tubule. Tubular impairment may represent a combination of direct effects of uranyl ion on transport proteins and the effects of tubular necrosis. Mechanisms of proteinuria have not been elucidated, and may have a glomerular and/or tubular origin.

Mechanisms of liver lesions and histological changes in the thyroid observed in rabbits and rats have not been elucidated. Mechanisms of uranium-induced lung disease are not completely understood. Involvement of inflammation related to the deposition of insoluble particulates in the lung is thought to be a contributor where the inhalation exposure is to insoluble uranium compounds. Radiogenic cancers may also arise from local irradiation of lung and lymph tissue from alpha-activity of uranium isotopes.

Ionizing radiation, including alpha particles such as are emitted from uranium isotopes, is believed to result in ionization events leading to a number of harmful cellular processes, including free radical formation, lipid peroxidation, and deoxyribonucleic acid (DNA) damage. A thorough review of the mechanisms of ionizing radiation is found in the ATSDR Toxicological Profile for Ionizing Radiation (ATSDR 1999a).

A.4 Health Guidelines

ATSDR (1999b) has derived an MRL of 8.0×10^{-3} mg U/m^3 for intermediate-duration inhalation exposure to insoluble compounds of uranium based on a no-observed-adverse-effect level (NOAEL) of 1.1 mg U/m^3 for renal effects in dogs (Rothstein 1949b).

ATSDR (1999b) has derived an MRL of 4.0×10^{-4} mg U/m^3 for intermediate-duration inhalation exposure to soluble compounds of uranium based on a lowest-observed-adverse-effect level (LOAEL) of 0.15 mg U/m^3 for renal effects in dogs (Rothstein 1949a).

ATSDR (1999b) has derived an MRL of 3.0×10^{-4} mg U/m^3 for chronic-duration inhalation exposure (365 days or more) to soluble compounds of uranium based on a NOAEL of 0.05 mg U/m^3 for renal effects in dogs (Stokinger et al. 1953).

ATSDR (1999b) has derived an MRL of 2.0×10^{-3} mg/kg/day for intermediate-duration oral exposure to soluble compounds of uranium based on a LOAEL of 0.05 mg U/kg/day for renal effects in rabbits (Gilman et al. 1998). This MRL was considered to be protective for chronic exposures as well.

EPA established a chronic oral RfD of 3×10^{-3} mg U/kg-day for soluble uranium salts (IRIS 2002). The RfD is based on weight loss and nephrotoxicity in rabbits observed in a 30-day feeding study (Maynard and Hodge 1949). EPA (IRIS 2002) has not established a chronic inhalation reference concentration (RfC) or cancer risk assessment for uranium compounds.

EPA (1995) has established slope factors for carcinogenicity from ingestion or inhalation of radioisotopes of uranium (EPA 1995). EPA (1999) also has established risk coefficients for radiogenic cancer morbidity and mortality from inhalation, tap water, and dietary intakes of radioisotopes of uranium.

A.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to uranium were derived for endpoints affected by one or more of the other chemicals in the uranium, fluoride, cyanide, and nitrate mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include renal, neurological, and reproductive (testicular) effects. Where data are available, chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001c, Section 2.3.2). Of the endpoints of concern for the mixture, data are available only for renal effects of uranium. The derivations are based on data presented in ATSDR (1999b). Due to inadequate data, TTDs were not derived for uranium radiation.

Renal Effects

ATSDR (1999b) has derived an MRL of $2.0x10^{-3}$ mg/kg/day for intermediate-duration oral exposure, and stated that this MRL is likely to be protective for chronic-duration oral exposure, to soluble compounds of uranium based on a LOAEL of 0.05 mg U/kg/day for renal effects in rabbits (Gilman et al. 1998). The MRL was derived by applying an uncertainty factor of 30 (3 for use of a minimal LOAEL and 10 for intrahuman variability) to the LOAEL.

Summary (TTDs for Uranium)

$MRL_{RENAL} = 2x10^{-3}$ mg/kg/day

A.6 References

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Appendix B. Background Information for Fluoride

B.1 Toxicokinetics

Studies on the rate and extent of fluorine absorption are not available, but evidence suggests that fluorine is too reactive to be absorbed unchanged, and instead is absorbed as fluoride. A study in rats suggests that hydrogen fluoride is absorbed primarily by the upper respiratory tract, and that removal of hydrogen fluoride from inhaled air by the upper respiratory tract approaches 100% for exposures that range from 30 to 176 mg fluoride/m^3 (Morris and Smith 1982). The studies of Collings et al. (1951) and Rye (1961) have demonstrated the absorption of fluoride from fluoride-containing dusts, but did not quantify the rate or extent of the absorption.

Ingested dietary fluoride is readily absorbed from the gastrointestinal tract as the undissociated hydrogen fluoride molecule by passive absorption (Whitford and Pashley 1984). Since the neutral undissociated molecule can penetrate cell membranes and be absorbed much better than the fluoride ion, decreasing the stomach pH increases absorption. The absorption of soluble fluoride in humans is rapid and extensive (~97%) (ATSDR 2001d) with maximum plasma fluoride concentrations attained as early as within 30 minutes following exposure (Ekstrand et al. 1977). However, additional factors can affect absorption. For example, the absorption of fluoride as calcium fluoride is increased when the material is given with meals (Trautner and Einwag 1987).

Following absorption, distribution of fluoride to the blood is rapid. Immediately following 40 minutes of intermittent inhalation exposure, plasma fluoride concentrations correlated closely (correlation coefficient=0.98; $p<0.01$) with the concentration of hydrogen fluoride in the air passed through the surgically isolated upper respiratory tract. Plasma levels were not measured at time points <40 minutes. Reports of skeletal fluorosis and elevated bone fluoride levels after occupational exposure to hydrogen fluoride and fluoride dusts indicate that fluoride is distributed to bone, mainly in regions undergoing active ossification or calcification, and accumulates there after inhalation exposure (ATSDR 2001d).

Long-term retention and accumulation of fluoride are primarily confined to calcified tissue in humans, though soft tissue concentrations of fluoride do rise transiently following ingestion of fluoride (ATSDR 2001d). Teeth and bone readily take up fluoride following oral exposure (ATSDR 2001d). While the rate of fluoride uptake in human teeth may decrease with age, it is apparent that the total fluoride content

of teeth and bone increases throughout life, and that the amount deposited is dependent on the exposure concentration. With the exception of the aorta and kidney, there is no evidence of accumulation or retention of fluoride in soft tissues in humans (ATSDR 2001d). Upon cessation of exposure, fluoride levels in bone are expected to decrease slowly; however, the time period over which this would occur in humans is not known.

Fluoride is believed to replace the hydroxyl ion and possibly the bicarbonate ion associated with hydroxyapatite—a mineral phase during formation of bone (ATSDR 2001d). The resultant material is hydroxyfluorapatite. Once absorbed, a portion of the fluoride is deposited in the skeleton, and the remainder is excreted in the urine, feces, sweat, and saliva within 24 hours (ATSDR 2001d). Thus, skeletal sequestration and renal excretion are the two major means by which the body prevents circulation of toxic amounts of fluoride ion. Fluoride in the skeleton is removed approximately at the rate of bone remodeling. Urinary excretion is markedly decreased in the presence of decreased renal function (ATSDR 2001d).

The fluoride ion carried in human blood serum exists in two forms, namely as an inorganic ion F^- and in combination with an organic molecule (Halton et al. 1984). The toxicological significance, if any, of the latter form is unknown. A portion of the circulating inorganic fluoride acts as an enzyme inhibitor because it forms metal-fluoride-phosphate complexes that interfere with the activity of those enzymes requiring a metal ion cofactor. In addition, fluoride may interact directly with the enzyme or the substrate. It is a general inhibitor of the energy production system of the cell, and of glycolysis in particular (ATSDR 2001d). Although much is known about enzyme inhibition by fluoride, the human health significance remains to be determined. The studies on enzymatic inhibition by fluoride were *in vitro* studies and used fluoride concentrations that were significantly higher than concentrations that would be normally found in human tissues.

No data were located regarding excretion of fluoride following human inhalation exposure to fluorine. Urinary fluoride levels were increased in dogs and rabbits exposed to levels as low as 0.8 mg/m^3 for 5–6 hours/day, 6 days/week for 35 days (Stokinger 1949). No quantitative data were reported at this level, but urinary fluoride levels in rabbits exposed to 3 mg/m^3 were 1.5 times normal. Studies in humans indicate that fluoride absorbed from inhaled hydrogen fluoride over an 8-hour work shift is excreted even during exposure, with urinary excretion peaking approximately 2–4 hours after cessation of exposure (about 10 hours following beginning of exposure) (Collings et al. 1951; Rye 1961).

The principal route of elimination of ingested fluoride is via the urine as demonstrated in a variety of species. In general, urine accounts for about 50–70% of the fluoride intake and feces accounts for 5–10%. Estimates of total elimination range from about 50% (Spencer et al. 1970) to about 100% (McClure et al. 1945). These varying estimates lead to widely varying estimates of the amount of fluoride that is stored in the body. There is a striking linear relationship between the concentration of fluoride in drinking water and in the urine of humans exposed continuously to fluoride. However, plasma fluoride levels are reflected better by the urinary fluoride excretion rate than by the concentration of fluoride in the urine (Ekstrand and Ehrnebo 1983). Large amounts of fluoride were excreted for prolonged periods by persons who lived for many years in areas with high fluoride water levels and who subsequently moved to areas with low fluoride levels, which indicated the excretion of fluoride that was mobilized from bone (Likins et al. 1962).

B.2 Health Effects

The primary effects of fluorides following acute inhalation exposure consist of irritation of the respiratory tract, with hematologic, renal, and hepatic effects seen in animal studies. Humans exposed to fluorine, which is thought to be rapidly converted to fluoride upon contact with tissues, have reported nasal irritation at exposures as low as 50 parts per million (ppm) for 3 minutes (Keplinger and Suissa 1968). Animal studies have established 60-minute 50% lethal concentration (LC_{50}) values ranging from 150 to 185 ppm for fluorine and from 325 to 1,610 ppm for hydrogen fluoride (ATSDR 2001d). Effects following subchronic inhalation to fluorine and hydrogen fluoride are similar to the acute effects, with nasal irritation being the most sensitive effect reported in humans, and respiratory tract irritation, hemorrhage, and edema being the most sensitive effects seen in animal studies (ATSDR 2001d). No studies of the health effects of chronic inhalation exposure to fluoride in humans or animals were identified.

Eichler et al. (1982) reported that a 3-year-old boy who had consumed a single dose of 16 mg fluoride/kg died 7 hours following ingestion. Upon autopsy, hemorrhagic edema of the lungs, hemorrhagic gastritis, and massive cerebral edema were observed. The hemorrhagic edema observed in the lungs was probably due to aspiration of the gastric contents. Cloudy swelling was observed in the cells of the liver, heart, and kidney. In rats, LD_{50} values for sodium fluoride administered by oral gavage range from 31 to 101 mg fluoride/kg (ATSDR 2001d). These LD_{50} values for rats may vary with strain, weight, and gender. An LD_{50} of 44.3 mg fluoride/kg was reported for mice (Lim et al. 1978). No reproductive effects were seen in mice exposed for 5 days to 32 mg fluoride/kg/day, nor were developmental effects reported in rats

exposed to 12.26 mg fluoride/kg/day on gestational days 6–15 or in mice exposed to 13.21 mg fluoride/kg/day (ATSDR 2001d).

Data are not available on the effects of intermediate-duration exposure to fluoride in humans. Intermediate-duration exposure of animals to fluoride has resulted in effects on a number of organ systems, including bone, testes, kidney, neurobehavioral effects, and developmental effects. Effects on the bone are commonly reported, including decreased bone growth, alterations in tooth enamel, delayed bone healing, and increased bone formation rate. A number of studies in rats, mice, and guinea pigs have reported testicular effects, including reduced fertility, decreased sperm counts, and histologic alterations of the seminiferous tubules and Leydig cells. Two studies in rats have demonstrated alterations in spontaneous behavior and decreased spontaneous activity in rats exposed for 6 weeks or 60 days, while two studies in mice have demonstrated renal effects of ingested fluoride. A study in rats reported that exposure of dams to 11.4 mg fluoride/kg/day resulted in an increased number of fetuses per litter with skeletal variations—no other developmental effects of fluoride were identified (ATSDR 2001d).

An extensive database on the effects of oral exposure to fluoride in humans exists, identifying effects on bone as the most sensitive effect of chronic exposure (ATSDR 2001d). Numerous studies have examined the possible relationship between chronic exposure to fluoride in drinking water and the risk of bone fractures. Many of these studies examined communities with high level of fluoride in the water or fluoridated water (ATSDR 2001d); a few prospective or retrospective studies have also examined this possible association. These studies have found conflicting results, with studies finding a lower or higher incidence of hip fractures or no differences in hip fracture between humans exposed to fluoride in drinking water. The chronic oral MRL is based on a LOAEL of 0.56 mg fluoride/kg/day for increased fracture rates in osteoporotic postmenopausal women.

Fluoride results in thickened bones and exostoses (skeletal fluorosis) when ingested in large doses for an extended period of time (ATSDR 2001d). Signs of skeletal fluorosis range from increased bone density to severe deformity, known as crippling skeletal fluorosis. Crippling fluorosis is characterized by complete rigidity of the spine, often accompanied by kyphosis (humpback) or lordosis (arched back). Reported cases are found almost exclusively in developing countries, particularly India, and are associated with malnutrition. The incidence of early skeletal fluorosis in the United States is unknown, since it appears that the early signs can only be identified radiologically. Fluoride may also have effects on the kidney, with a case report demonstrating that exposure to high levels of fluoride resulted in renal insufficiency and interstitial nephritis (ATSDR 2001d).

While animal studies, particularly in minks, have confirmed the toxicity of fluoride in bone, chronic oral studies in animals have also identified effects of fluoride in organs other than bone (ATSDR 2001d). Rabbits exposed to 5 mg fluoride/kg/day as sodium fluoride showed a roughened duodenal mucosa, while exposure of rabbits to 4.5 mg fluoride/kg/day as sodium fluoride resulted in serious testicular effects, with structural damage to the developing spermatids and a complete cessation of spermatogenesis, as well as decreased levels of total primary and secondary antibody titers, suggesting an impaired immune response.

Numerous epidemiological studies have examined the issue of a connection between fluoridated drinking water and cancer. The weight of evidence indicates that no such connection exists. However, all of the investigations were ecologic studies, and the sensitivity limit of even the most sensitive analysis in these studies appears to be a 10–20% increase. Since any carcinogenic effect of fluoride at the levels found in water supplies would probably be below this level of sensitivity, a National Toxicology Program (NTP) cancer bioassay was conducted to assess the effect of fluoride in the drinking water on cancer incidence in animals (Bucher et al. 1991; NTP 1990). The NTP study found equivocal evidence of a fluoride-related increase in osteosarcomas in male rats, and no evidence of any fluoride-related neoplasm in female rats or male or female mice. A lifetime oral study sponsored by Proctor and Gamble (Maurer et al. 1990) found no evidence of fluoride carcinogenicity in either male or female rats exposed in the feed. Both studies contain limitations that preclude strong conclusions. The NTP is presently carrying out additional experiments on the relationship, if any, between fluoride and cancer.

B.3 Mechanisms of Action

A number of mechanisms are involved in the toxicity of fluoride to bone. Fluoride ions are incorporated into bone by substituting for hydroxyl groups in the carbonate-apatite structure to produce hydroxy-fluorapatite, thus altering the mineral structure of the bone (Chachra et al. 1999). Unlike hydroxyl ions, fluoride ions reside in the plane of the calcium ions, resulting in a structure that is electrostatically more stable and structurally more compact (Grynpas and Rey 1992). Following administration of fluoride, there is a shift in the mineralization profile towards higher densities and increased hardness (Chachra et al. 1999). Although fluoride administration is associated with an increase in bone mass, *in vivo* and *in vitro* animal studies have found a negative association between fluoride-induced new bone mass and bone strength, suggesting that the quality of the new bone was impaired by the fluoride (ATSDR 2001d). Because bone strength is thought to derive mainly from the interface between the collagen and the mineral (Catanese and Keavney 1996), alteration in mineralization probably affects strength. The wider crystals, which are formed after fluoride exposure, are presumably not associated with collagen fibrils and

thus, do not contribute to mechanical strength. Turner et al. (1997) found that the crystal width was inversely correlated with bending strength of the femur. Thus, although there is an increase in hardness and bone mass and unaltered structure, the mechanical strength of bone is decreased (Chachra et al. 1999).

In addition to the physicochemical effect of fluoride on the bone, at high doses, fluoride can be mitogenic to osteoblasts (ATSDR 2001d) and inhibitory to osteoclasts. The osteoblasts are still active, although there are fewer plump, cuboidal, highly secretory osteoblasts; whereas fluoride is mitogenic to osteoblastic precursors (Bonjour et al. 1993), it is toxic to individual osteoblasts at the same concentration (Chachra et al. 1999). The effect of fluoride on osteoclasts is not well understood; it appears that fluoride decreases the amount of bone resorbed by osteoclasts (Chachra et al. 1999).

Studies in humans and animals suggest that the effect of fluoride on bone strength is biphasic. In rats administered 1–128 ppm fluoride as sodium fluoride in drinking water for 16 weeks, both increases and decreases in bone strength were found; the maximum femoral bone strength occurred at 16 ppm (Turner et al. 1992). A biphasic relationship between femoral bone strength and bone fluoride content was found. The biphasic nature of bone effects is supported by data from clinical trials in women with postmenopausal osteoporosis (Haguenauer et al. 2000). The meta-analyses of 12 studies found a significant increase in the relative risk of nonvertebral fractures in subjects ingesting high doses of fluoride; in subjects administered low fluoride doses or slow-release formulations, there was no effect on nonvertebral fractures. Similarly, there was no effect on vertebral fracture risk in high fluoride dose subjects, but a decrease in this risk in subjects administered low fluoride doses or slow-release formulations was found.

Fluoride has been shown to interfere with glycolysis. Because the central nervous system relies heavily on this energy source, hypotheses have been advanced as to a mechanism for fluoride effects on the central nervous system. Although effects on glycolytic enzymes could explain the neuromuscular symptoms seen frequently in cases of fluoride poisoning (e.g., tetany, paresthesia, paresis, convulsions), other studies tend to indicate that hypocalcemia caused by fluoride binding of calcium causes these symptoms.

B.4 Health Guidelines

ATSDR (2001d) derived an acute inhalation MRL of 0.01 ppm for fluorine, based on a NOAEL of 10 ppm for irritation of the eyes and skin during a 15-minute exposure of volunteers. The NOAEL was adjusted for a 24-hour continuous exposure, and an uncertainty factor of 10 for intrahuman variability was applied to yield the MRL of 0.01 ppm.

ATSDR (2001d) also derived an acute inhalation MRL of 0.03 ppm fluoride for hydrogen fluoride, based on a NOAEL of 98 ppm fluoride for nasal irritation in rats exposed for 60 minutes. The NOAEL was converted to a human equivalent concentration, duration-adjusted, and an uncertainty factor of 30 (3 for animal to human extrapolation using dosimetric adjustment, and 10 for intrahuman variability) to give the MRL of 0.03 ppm fluoride.

An intermediate-duration inhalation MRL of 0.02 ppm fluoride (ATSDR 2001d) was derived for hydrogen fluoride based on a duration-adjusted LOAEL of 0.75 ppm fluoride for slight irritation of the respiratory tract in volunteers exposed to hydrogen fluoride for 15–50 days. An uncertainty factor of 30 (3 for use of a minimal LOAEL and 10 for intrahuman variability) was applied to the LOAEL to derive the MRL of 0.02 ppm fluoride.

No inhalation MRLs were derived for fluorides other than hydrogen fluoride. No chronic inhalation MRLs were derived for fluorine or hydrogen fluoride (ATSDR 2001d).

No oral MRLs were derived for fluorine or hydrogen fluoride (ATSDR 2001d).

No acute or intermediate MRLs were derived for fluoride (ATSDR 2001d).

A chronic-duration oral MRL of 0.06 mg fluoride/kg/day was derived for fluoride (ATSDR 2001d), based on a LOAEL of 0.56 mg fluoride/kg/day for increased fracture rate in osteoporotic postmenopausal women. The MRL was derived by applying an uncertainty factor of 10 for use of a LOAEL in a sensitive human subpopulation.

EPA has derived an oral RfD of 0.06 mg/kg/day for fluoride (ATSDR 2001d), based on a NOAEL of 0.06 mg/kg/day for dental fluorosis in chronically-exposed children (Hodge 1950). An uncertainty factor of 1 was applied to the NOAEL since the study was a chronic study in a sensitive population of humans.

No RfC for fluoride has been derived, and fluoride has not undergone an evaluation of carcinogenic potential by EPA.

B.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to fluoride were derived for endpoints affected by one or more of the other chemicals in the uranium, fluoride, cyanide, and nitrate mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include renal, neurological, and reproductive (testicular) effects. Where data are available, chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001c, Section 2.3.2). The derivations are based on data presented in ATSDR (2001d).

Musculoskeletal Effects

A number of human studies have investigated the toxicity, particularly potential skeletal toxicity, of fluoride (for review of these studies, see ATSDR 2001d). The vast majority of these studies were ecological studies examining the possible relationship between fluoride in drinking water and the occurrence of hip fractures. These studies, as well as retrospective cohort studies, have found decreases, increases, and no effect on hip fracture occurrence in communities consuming fluoridated water. Limitations in the study designs of many of these studies preclude using these data to establish a causal relationship between fluoride and risk of hip fractures. In addition to these epidemiology studies, several human experimental studies have examined the effect of fluoride administration for the treatment of osteoporosis. One study found significant increases in lumbar spine and femoral head and trochanter bone mineral density, decreases in radius bone mineral density, no effect on vertebral fracture rate, and increases in nonvertebral fracture rate among postmenopausal women with osteoporosis ingesting a capsule containing 34 mg fluoride/day as sodium fluoride for 4–6 years. Another study did not find any effect on bone mineral density or vertebral or nonvertebral fracture rates among postmenopausal women with spinal osteoporosis ingesting 34 mg fluoride/day as sodium fluoride. A meta-analysis of these data, as well as other clinical studies, found a significant correlation between exposure to high levels of fluoride and an increased relative risk of nonvertebral fractures. The LOAEL of 34 mg fluoride/day (0.56 mg fluoride/kg/day) was selected as the basis of a chronic-duration oral MRL for fluoride. The MRL of 0.06 mg fluoride/kg/day was derived by dividing the LOAEL by an uncertainty factor of 10 to account for the use of a LOAEL in a sensitive subpopulation.

Renal Effects

Greenberg (1982) reported a LOAEL of 1.9 mg fluoride/kg/day for degeneration of the nephron in mice exposed to sodium fluoride in the drinking water for 280 days. To this LOAEL, an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) was applied to yield a provisional TTD_{RENAL} of $2x10^{-2}$ mg/kg/day. However, as this value, derived from animal data, is lower than the chronic MRL of 0.06 mg/kg/day, which is based on chronic human data examining the most sensitive known endpoint of fluoride toxicity, the MRL value of 0.06 mg/kg/day was adopted as the TTD_{RENAL} for fluoride.

Reproductive Effects

In CD rats exposed to sodium fluoride for 60 days in the diet, Araibi et al. (1989) reported a LOAEL of 2.3 mg fluoride/kg/day for alterations in the seminiferous tubule diameter. To this LOAEL, an uncertainty factor of 1,000 (10 for a LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) to yield a provisional TTD_{REPRO} of $2x10^{-3}$ mg/kg/day. However, as this value, derived from animal data, is lower than the chronic MRL of 0.06 mg/kg/day, which is based on chronic human data examining the most sensitive known endpoint of fluoride toxicity, the MRL value of 0.06 mg/kg/day was adopted as the TTD_{REPRO} for fluoride.

Neurological Effects

Mullenix et al. (1995) identified a NOAEL of 5.5 mg fluoride/kg/day and a LOAEL of 7.5 mg fluoride/kg/day for alterations in spontaneous behavior in Sprague-Dawley rats exposed to sodium fluoride in the drinking water for 6 weeks. To this LOAEL, an uncertainty factor of 1,000 (10 for a LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) to yield a provisional TTD_{NEURO} of $5x10^{-3}$ mg/kg/day. However, as this value, derived from animal data, is lower than the chronic MRL of 0.06 mg/kg/day, which is based on chronic human data examining the most sensitive known endpoint of fluoride toxicity, the MRL value of 0.06 mg/kg/day was adopted as the TTD_{NEURO} for fluoride.

Summary (TTDs for Fluoride)

TTD_{RENAL} = 0.06 mg/kg/day

TTD_{REPRO} = 0.06 mg/kg/day

TTD_{NEURO} = 0.06 mg/kg/day

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Appendix C. Background Information for Cyanide

C.1 Toxicokinetics

Cyanide is rapidly absorbed (within seconds) following inhalation exposure. Humans retained 58% of hydrogen cyanide in the lungs after inhaling the gas through normal breathing (Landahl and Herrmann 1950). During inhalation exposure of dogs to an unknown concentration of hydrogen cyanide (Gettler and Baine 1938), one dog reportedly absorbed 16.0 mg (1.55 mg/kg); the other dog absorbed 10.1 mg (1.11 mg/kg). These doses were fatal to the dogs in 15 and 10 minutes, respectively. More recent quantitative data were not available. Following oral exposure, cyanide is rapidly absorbed, as evidenced by the death of an exposed dog as early as 8 minutes following exposure (Gettler and Baine 1938); the absorption of cyanide varied from 17 to 74% in the three exposed dogs. A more recent study in rats (Farooqui and Ahmed 1982) indicated that at least 53% of a single oral dose of cyanide in rats was absorbed within 24 hours of exposure. Evidence for dermal absorption of cyanide comes from studies in animals (ATSDR 1997) wherein systemic toxicity was observed following dermal contact with cyanide compounds; however, quantitative data on the dermal absorption of cyanide are not available.

Following absorption, cyanide is rapidly distributed by the blood throughout the body. After inhalation exposure in humans, tissue cyanide levels, expressed per gram of wet tissue, were highest in the lung, followed by the heart, blood, kidney, brain, and liver (ATSDR 1997). Similar distribution patterns were seen in animals after cyanide inhalation. In humans who had died of oral cyanide overdose, cyanide levels were generally greatest in the stomach contents, with significant levels reported in the spleen, lungs, blood, liver, brain, and kidney (ATSDR 1997). Following oral exposure in animals, a similar pattern was seen, with the greatest levels in the stomach contents, with significant levels in the liver, lung, blood, and kidney (ATSDR 1997). Cyanide in the blood was found mainly (95%) in the hemolysate, with 70% of the total cyanide found in the heme-containing fraction (Farooqui and Ahmed 1982). Cyanide has not been reported to accumulate in the body.

Reports of ingestion of cyanides by humans and reports of occupational exposure indicate that cyanide is transformed into thiocyanate. A plasma half-life of 20 minutes to 1 hour has been estimated for cyanides in humans after nonlethal exposures (Hartung 1982). Animal data indicate that the primary pathway of cyanide metabolism involves transformation to thiocyanate by either rhodanese or 3-mercaptopyruvate sulfur transferase, accounting for 60–80% of the administered dose (ATSDR 1997). Species and tissue

distribution of rhodanese is highly variable, with dogs possessing the lowest levels of all species examined (Himwich and Saunders 1948). Minor pathways of cyanide metabolism include (1) conversion to 2-aminothiazoline-4-carboxylic acid; (2) incorporation into a 1-carbon metabolic pool; or (3) combining with hydroxocobalamin to form cyanocobalamin (vitamin B_{12}).

Cyanide metabolites are normally excreted in urine with small amounts eliminated through the lungs. Urinary excretion of thiocyanate was monitored in a man after ingestion of ~3–5 grams of potassium cyanide (15–25 mg CN^-/kg) (ATSDR 1997). The results indicated that the patient excreted 237 mg of thiocyanate over a 72-hour period. This quantity was substantially more than the normal average amount of thiocyanate in urine, which varies between 0.85 and 14 mg/24 hours. Thirty-one children who had consumed flour made from insufficiently processed cassava, which therefore had significant concentrations of cyanide, had mean urinary thiocyanate levels of 757 µmol/L, compared with 50 µmol/L in those children who had consumed sufficiently processed cassava. When rats were given 2 mg CN^-/kg of radiolabeled potassium cyanide, urinary excretion of radioactivity reached 47% of the dose within 24 hours following administration. When [^{14}C] sodium cyanide was injected subcutaneously into rats at a level of 8.3 µmol, no difference in radioactivity eliminated was observed between the group pretreated for 6 weeks with a diet containing 0.7 mg CN^-/kg as potassium cyanide and their matching controls. Most of the radioactivity was detected in the urine (89% by 24 hours). Thiocyanate was the major metabolite. About 4% of the radioactivity was expired, mostly as carbon dioxide.

C.2 Health Effects

Studies of the acute effects of cyanide inhalation have generally been limited to the examination of serious and lethal effects. Acute inhalation exposure to high levels of cyanide, regardless of the form, leads quickly to death that is preceded by dyspnea, convulsions, and central nervous system depression (ATSDR 1997). A human 10-minute LC_{50} of 524 ppm for cyanide inhalation has been estimated (ATSDR 1997), while Singh et al. (1989) reported that a man exposed to 192 ppm died within 3 days of exposure. Rat LC_{50} values of 483 ppm cyanide for a 5-minute exposure and 137 ppm cyanide for a 60-minute exposure have been reported (ATSDR 1997). Mouse LC_{50} levels are similar to those in rats, with LC_{50} values of 310 ppm cyanide for a 5-minute exposure and 159 ppm cyanide for a 30-minute exposure (ATSDR 1997). Other acute effects of cyanide inhalation include peripheral vision loss in a male human exposed for 13 minutes to 434 ppm cyanide as hydrogen cyanide and dyspnea, bradycardia, arrhythmia, and EEG alterations in monkeys exposed for 30 minutes to 96 ppm cyanide as hydrogen cyanide.

Data on the effects of subchronic or chronic inhalation exposure to cyanide in humans and animals are limited. In an early study, four dogs were exposed to 43 ppm cyanide as hydrogen cyanide for 30 minutes every other day for 28 days. One dog of four died, while other affected endpoints included the respiratory (dyspnea), gastrointestinal (vomiting, tenesmus, and diarrhea), and neurological (tremors, ataxia, stiffness, cellular atrophy) effects. A later study reported increased creatine phosphokinase activity in rats following five 12.5-minute exposures, at 4-day intervals, to 192 ppm cyanide as hydrogen cyanide (ATSDR 1997).

Two chronic studies of humans occupationally exposed to cyanide are reported in ATSDR (1997). El Ghawabi et al. (1975) described a cohort of workers chronically exposed (5–15 years) to 6.4–10.4 ppm of an unspecified cyanide form evolved from sodium cyanide and copper cyanide during electroplating. Reported symptoms included dyspnea, lacrimation, precordial pain, increased hemoglobin and lymphocytes, and vomiting, as well as significant neurological effects, including confusion, hallucination, headache, weakness, and dizziness. A later study (Blanc et al. 1985) described health effects in workers exposed to 15 ppm hydrogen cyanide (14 ppm cyanide) in a silver-reclaiming facility. Symptoms were similar to those reported by El Ghawabi et al. (1975), and included dyspnea, palpitations, chest pain, nausea, altered thyroid hormone levels, rash, decreased body weight, and neurologic effects, including persistent headache, dizziness, and paresthesia. Both of these studies, however, are limited by their inability to control for co-exposure to other compounds.

Case reports of acute oral exposures to cyanide in humans have identified a number of health effects. Stertorous, deep, and rapid breathing was reported in a man who ingested ~15 mg CN^-/kg as potassium cyanide in a suicide attempt (Liebowitz and Schwartz 1948), while shortness of breath and dyspnea were observed in two reports of suicide attempts; one man ingested 7.6 mg CN^-/kg (Goodhart 1994) and the other man ingested 0.57 mg CN^-/kg (Saincher et al. 1994), both as potassium cyanide. Acute neurologic effects vary with the amount of cyanide consumed, ranging from headache at low doses, to tremor and coma at higher doses (ATSDR 1997). There is evidence that acute oral exposures to cyanide can lead to the development of Parkinsonism (ATSDR 1997). Other reported effects of acute oral cyanide exposure in humans include shallow pulse, albuminuria, increased serum creatinine and serum creatinine kinase, and metabolic acidosis (ATSDR 1997). Hamsters exposed from gestational days 3–14 showed no effects on the number of implantations or resorptions at concentrations up to 10.4 mg CN/kg/day as cassava, but 1 mg CN/kg/day as cassava resulted in significantly decreased fetal weight and delayed bone ossification (Frakes et al. 1986).

Studies of humans orally exposed to cyanide for intermediate duration are lacking. The intermediate-duration oral MRL for cyanide is based on a 13-week drinking water study performed by NTP (1993) which defined a NOAEL of 4.5 mg/kg/day and a LOAEL of 12.5 mg/kg/day for decreased weights of male reproductive organs and altered spermatogenesis in male F344 rats. The study did not report effects on other organ systems, including neurological effects, in rats at doses up to 12.5 mg/kg/day or in mice at doses up to 24.3 mg/kg/day in males and 28.8 mg/kg/day in females. In contrast, Gerhart (1986, 1987a, 1987b) reported altered posture and hypoactivity in Sprague-Dawley rats exposed by gavage for 90 days to 0.8 mg CN/kg/day as $KAg(CN)_2$ or 0.14 mg CN/kg/day as CuCN, with labored respiration seen at 0.8 mg CN/kg/day as $KAg(CN)_2$ or 4.35 mg CH/kg/day as CuCN. Gerhart (1986, 1987a, 1987b) also reported increased testicular weight at 2.6 mg CN/kg/day as $KAg(CN)_2$ or 14.5 mg CN/kg/day as CuCN. A 14-week study in male dogs identified LOAELs of 1.04 mg CN/kg/day, regardless of whether the food contained NaCN or cassava, for cardiac swelling and hemorrhage, proximal tubule damage, kidney congestion and vacuolation, adrenal cortex swelling and fibrosis, and destruction of germ cells in the seminiferous tubules (Kamalu 1993). However, as dogs have very low levels of rhodanese relative to humans, and thus a higher sensitivity to the effects of cyanide, the implication of these studies relative to risks in humans is uncertain.

Data on the effects of chronic oral exposure to cyanide, including studies of carcinogenicity, in humans and animals are lacking.

C.3 Mechanisms of Action

Cyanide (as hydrogen cyanide), originating *in vivo* by dissociation of potassium cyanide, sodium cyanide, and other cyanogenic compounds or arising from catabolism of cyanogenic glycosides, exerts its acute toxic effects by complexing with the ferric iron atom in metalloenzymes, resulting in histotoxic anoxia through inhibition of cytochrome c oxidase (ATSDR 1997), metalloenzymes that function as the terminal oxidase of the inner mitochondrial membrane respiratory chain. A two-step process has been proposed: cyanide as hydrogen cyanide first penetrates a protein crevice of cytochrome c oxidase and binds to the protein. Hydrogen cyanide then binds to the trivalent iron ion of the enzyme, forming a relatively stable (but reversible) coordination complex. One mole of hydrogen cyanide is bound to one mole of cytochrome c oxidase. As a result, the enzyme becomes unable to catalyze the reactions in which electrons would be transferred from reduced cytochrome to oxygen. Cellular oxygen utilization is thus impaired, with resultant reduction in or cessation of aerobic metabolism (ATSDR 1997). Glucose catabolism then shifts from the aerobic pathway to anaerobic metabolism including the pentose phosphate

pathway, resulting in increased blood glucose, pyruvic acid, lactic acid, and nicotinamide adenine dinucleotide phosphate (NADPH) levels, and a decrease in the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio.

The inhibition of oxygen use by cells (termed histoxic hypoxia) causes oxygen tensions to rise in peripheral tissues. This results in a decrease in the unloading gradient for oxyhemoglobin; thus, oxyhemoglobin is carried in the venous blood. Inhibition of oxygen utilization is thought to occur rapidly after cyanide exposure (ATSDR 1997). Tadic (1992) determined that inhibition of cytochrome c oxidase activity in rat brains was most pronounced between 15 and 20 minutes after administration of sodium cyanide (12 mg/kg or 1.3xLD_{50}). In addition to binding to cytochrome c oxidase, cyanide also binds to catalase, peroxidase, methemoglobin, hydroxocobalamin, phosphatase, tyrosinase, ascorbic acid oxidase, xanthine oxidase, and succinic dehydrogenase.

The central nervous system is the primary target for acute cyanide toxicity in humans and animals. Acute inhalation of high concentrations of cyanide provokes a brief central nervous system stimulation followed by depression, convulsions, coma, and death in humans and in animals (ATSDR 1997). The effects are probably due to rapid biochemical changes in the brain, such as changes in ion flux, neurotransmitter release, and possibly peroxide formation (ATSDR 1997).

Cyanide poisoning likely involves mechanisms in addition to inhibition of cytochrome c oxidase activity. Cyanide is a strong nucleophile with multiple effects including release of secondary neurotransmitters, release of catecholamines from adrenal glands and adrenergic nerves, and inhibition of antioxidant enzymes in the brain. However, the extremely low concentration of cyanide required to inhibit the oxidase, the rapid interaction of hydrogen cyanide with the enzyme and the key role of cytochrome c oxidase in aerobic metabolism all combine to make cyanide inhibition of the terminal step of electron transport (ATSDR 1997) the key molecular target in cyanide poisoning.

C.4 Health Guidelines

ATSDR (1997) did not derive inhalation MRLs for cyanide for any exposure duration, because available studies were not adequate. Many of the animal and human studies used lethality, or serious effects, as the endpoint. Two available epidemiology studies were not used because of inadequate exposure characterization or co-exposure to other chemicals.

ATSDR (1997) did not derive an acute oral MRLs for cyanide because most of the available studies reported lethality as the endpoint, and because of a general lack of information as to acute systemic effects of cyanide.

ATSDR (1997) derived an intermediate oral MRL of 0.05 mg/kg/day for cyanide based on a NOAEL of 4.5 mg/kg/day for reproductive effects, such as decreased epididymal weight, decreased testis weight, and alterations in spermatozoa in male rats (NTP 1993). The MRL was derived by applying an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for intrahuman variability) to the NOAEL. The rats were exposed for 13 weeks to sodium cyanide in the drinking water.

ATSDR (1997) has not derived a chronic oral MRL for cyanide, due to limitations of exposure analysis in the available human data and a lack of reported effects in the one reported chronic animal study.

EPA oral RfDs have been established for cyanide and its compounds. These RfDs range from $2x10^{-1}$ mg/kg/day for potassium cyanide to $5x10^{-3}$ mg/kg/day for copper cyanide (IRIS 2002). The RfDs for potassium silver cyanide and potassium cyanide were based on weight loss and thyroid effects in several rat studies (Howard and Hanzel 1955; Philbrick et al. 1979), while the RfD for copper cyanide was based on decreased body and organ weights and liver and kidney effects in a intermediate-duration rat study (Gerhart 1986). An EPA RfC exists only for hydrogen cyanide; this RfC is $3x10^{-3}$ mg/m^3. The RfC was based on central nervous system and thyroid effects in a human occupational study (El Ghawabi et al. 1975).

The EPA has determined that cyanide is not classifiable as to its human carcinogenicity (Group D) (IRIS 2002). No cancer classifications exist for the NTP, Integrated Risk Information System (IRIS), or the International Agency for Research on Cancer (IARC) (no available data).

C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to cyanide were derived for endpoints affected by one or more of the other chemicals in the uranium, fluoride, cyanide, and nitrate mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include renal, neurological, and reproductive (testicular) effects. Where data are available, chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001c, Section 2.3.2). The derivations are based on data presented in ATSDR (1997).

Reproductive Effects

ATSDR (1997) derived an intermediate oral MRL of 0.05 mg/kg/day for cyanide based on a NOAEL of 4.5 mg/kg/day for reproductive effects, such as decreased epididymal weight, decreased testis weight, and alterations in spermatozoa in male rats (NTP 1993). The MRL was derived by applying an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for intrahuman variability) to the NOAEL. The rats were exposed for 13 weeks to sodium cyanide in the drinking water.

Neurological Effects

While a number of human and animal studies have reported neurologic effects from the ingestion of cyanide-containing compounds, none has done so in such a way as to allow for a definitive dose-response analysis of the effect of cyanide on neurological endpoints. For example, Gerhart (1987a, 1987b) examined the effects of copper cyanide and potassium silver cyanide in rats, but was not able to clearly delineate the effects of cyanide from the effects of the metals. Similarly, a number of studies in humans who consumed cassava, which contains considerable levels of cyanide, are complicated by the presence of scopoletin, which may have contributed to the neurological effects seen. As such, none of the available studies are suitable for derivation of a TTD for neurologic effects of cyanide. The MRL of 0.05 mg/kg/day will be adopted as the TTD_{NEURO} for cyanide.

Renal Effects

Available studies in humans and animals have suggested that renal effects may result from prolonged exposure to cyanide, though reliable quantitative data are limited. The study of Gerhart (1987b), while having the limitation of co-exposure to silver, identified a NOAEL of 2.6 mg/kg/day and a LOAEL of 7.8 mg/kg/day for increased blood urea nitrogen. Studies in dogs have identified lower LOAEL values, but dogs are a poor model for cyanide toxicity in humans due to a lack of rhodanese enzyme levels. Application of an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) would yield a TTD_{RENAL} of $3x10^{-2}$ mg/kg/day. However, as this would fall below the MRL, the MRL of 0.05 mg/kg/day will be adopted as the TTD_{RENAL} for cyanide.

Summary (TTDs for Cyanide)

MRL_{REPRO} = 0.05 mg/kg/day

TTD_{NEURO} = 0.05 mg/kg/day

TTD_{RENAL} = 0.05 mg/kg/day

C.6 References

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Appendix D. Background Information for Nitrate

D.1 Toxicokinetics

Available studies indicate that oral absorption of nitrate is nearly 100% (for reviews, see EPA 1990 and WHO 1978). Witter (1979, cited in EPA 1990) administered oral radioactive nitrate ion to two male volunteers; one received the nitrate 1 hour after a large meal, the other about 10 hours after eating. In the subject who had recently eaten, the radioactivity had a disappearance half-life from the stomach of about 30 minutes, but the radioactivity in the pylorus remained constant, suggesting that the nitrate had moved to the small intestine rather than being absorbed through the stomach. In the second subject, the disappearance half-life was 10 minutes. Studies in animals have also demonstrated that the bulk of an orally-administered nitrate exposure is absorbed through the small intestine, likely through the upper portion of that organ. Absorbed nitrate is distributed throughout the body, but does not appear to accumulate in any organ (EPA 1990).

The major metabolic pathway for nitrate is conversion to nitrite, and then to ammonia. Small amounts of nitrate, perhaps 5–10% of the total exposure, are converted to nitrite by bacteria in the saliva, stomach, and small intestine. This reaction is pH dependent, with no nitrate reduction occurring below pH 4 and above pH 9, and the presence of oxygen inhibits the reduction of nitrite to ammonia. Absorbed nitrite rapidly reacts with hemoglobin in the blood to form methemoglobin, which in adults, is rapidly converted to oxyhemoglobin, then back to hemoglobin. In infants, particularly those under 3 months old, these reducing systems are not fully developed, which may result in a buildup of methemoglobin in the blood. Due to the higher stomach pH typically found in infants, it is believed that they also convert more nitrate to nitrite in the stomach than adults. There are large species differences in the rate of reaction of nitrite with hemoglobin, paralleled by similar differences in the rates of reduction of methemoglobin, making extrapolation of results from animal data to humans problematic. Another potential metabolic pathway, though less prevalent than the reaction with hemoglobin, is the reaction of nitrite with endogenous molecules to form N-nitroso compounds, many of which have toxic effects, including carcinogenicity.

Available data in humans have demonstrated that elimination of ingested nitrate is rapid, with elimination almost exclusively in the urine (EPA 1990; WHO 1978). Animal data support this observation. In both humans and animals, considerably more nitrate is eliminated in the urine than is ingested in a normal diet, implying that there is significant endogenous nitrate formation.

Parks et al. (1981, cited in EPA 1990) reported that following intratracheal instillation of trace amounts of nitrate to BALB/C mice, absorption from the lungs was complete within a 10-minute period. Additional studies of the toxicokinetics of inhaled nitrate are not available; however, the behavior of absorbed nitrate following inhalation exposure is not expected to differ from nitrate absorbed following oral exposure.

D.2 Health Effects

The most sensitive known effects of exposure to nitrate result from increased levels of methemoglobin arising from the nitrite-hemoglobin reaction. In healthy adults, methemoglobin formation and reduction is continuous, with steady-state methemoglobin levels in healthy adults being 2.5% of the total hemoglobin content or lower (EPA 1990). Due to the large excess capacity of the blood to carry oxygen, levels of methemoglobin up to 10% typically do not cause significant clinical signs. Levels above 10% may result in cyanosis, weakness, rapid pulse, and, at levels exceeding 50%, death. Other reported effects of nitrate in animals include altered thyroid function, amyloidosis of the liver, kidney, spleen, and adrenal glands, and altered lung and liver weights.

Because of greater numbers of nitrate-reducing bacteria in the gastrointestinal tract and diminished methemoglobin-reducing capacity, infants, particularly those 3 months and younger, are particularly susceptible to nitrate/nitrite-induced methemoglobinemia. A study by Bosch et al. (1950) examined 139 cases of methemoglobinemia in young children (90% occurred in children <2 months of age). Examination of the wells used to supply water to the children revealed that none of the wells supplied <10 mg/L nitrate-nitrogen, with all but two of the wells containing >25 mg/L. Walton (1951) presented the results of a survey on morbidity and mortality among infants due to methemoglobinemia. The results of the survey revealed 239 cases of infant methemoglobinemia, 39 of them fatal. Of the 214 cases where quantitative data were available on nitrate levels in water, none occurred in infants consuming water with <10 mg/L nitrate-nitrogen, 5 cases occurred in infants exposed to 11–20 mg/L nitrate-nitrogen, 36 cases in infants exposed to 21–50 mg/L nitrate-nitrogen, and 173 cases in infants exposed to >50 mg/L nitrate-nitrogen. Many other studies have examined the effects of high (>20 mg/L) levels of nitrate in the drinking water of infants, and have found increased methemoglobin levels and signs of clinical methemoglobinemia in exposed infants (for reviews, see EPA 1990 and WHO 1978).

D.3 Mechanisms of Action

The known toxic effects of nitrate exposure result from the conversion of nitrate to nitrite. The conversion is mainly the result of bacterial oxidation reactions within the gastrointestinal tract. Exposure of hemoglobin to nitrite results in the oxidation of the Fe^{2+} ion in the heme of hemoglobin to Fe^{3+}, resulting in the formation of methemoglobin. Methemoglobinemia results in the majority of the symptoms seen following high-dose acute nitrate exposure in humans. Under normal conditions, healthy adults will have <2.5% methemoglobin in the blood. Methemoglobin can be reduced back to hemoglobin by both spontaneous (nicotinamide adenine dinucleotide phosphate [NADH]-dependent) and dormant (NADPH-dependent) methemoglobin reductase enzymes.

Infants are particularly susceptible to methemoglobinemia due to their high gut content of nitrate-reducing bacteria, their lower enzymatic capacity to reduce methemoglobin to hemoglobin, and to the presence of hemoglobin F, which is more susceptible to oxidation by nitrite. The high pH of the infant gastrointestinal system favors the growth of nitrate-reducing bacteria, particularly in the stomach and especially after ingestion of contaminated waters, since the ingested bacteria are likely to flourish in the stomach. The stomach of adults is typically too acidic to allow for significant bacterial growth and the resulting conversion of nitrate to nitrite. Additionally, the enzymes involved in the conversion of methemoglobin to hemoglobin do not fully develop in humans until between 3 and 6 months after birth, resulting in an increased susceptibility to methemoglobinemia.

As mentioned in Section D.1, the reaction rates for the nitrite-hemoglobin reaction vary considerably across species (many animal species lack nitrate-reducing bacteria), as do the rates of the reactions reducing methemoglobin back to functional hemoglobin. Also, since the rates of conversion of nitrate to nitrite by bacteria can vary within individuals, the extent of nitrate toxicity can also vary greatly depending on age and other factors within both humans and animals.

D.4 Health Guidelines

ATSDR has not published a toxicological profile for nitrates. No MRL values are available.

EPA (IRIS 2002) has derived an oral RfD of 1.6 mg/kg/day for nitrate, based on a NOAEL of 1.6 mg/kg/day for methemoglobinemia in exposed infants (Bosch et al. 1950; Walton 1951). An uncertainty factor of 1 was applied to the NOAEL since the study was performed in a sensitive population

of humans (infants age 0–3 months). No RfC for nitrate has been derived, and nitrate has not undergone an evaluation of carcinogenic potential by EPA.

D.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

ATSDR has not published a toxicological profile for nitrates; no MRLs exist for exposure to nitrate by any route of exposure. As no shared targets of toxicity for nitrate and any of the other components of the mixture exist, no TTDs for nitrate were derived.

D.6 References

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