INTERACTION PROFILE FOR:
CYANIDE, FLUORIDE, NITRATE, AND URANIUM

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program (NTP), initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found. The Food Quality Protection Act (FQPA) of 1996 requires that factors to be considered in establishing, modifying, or revoking tolerances for pesticide chemical residues shall include the available information concerning the cumulative effects of substances that have a common mechanism of toxicity, and combined exposure levels to the substance and other related substances. The FQPA requires that the Administrator of the Environmental Protection Agency (EPA) consult with the Secretary of the Department of Health and Human Services (which includes ATSDR) in implementing some of the provisions of the act.

To carry out these legislative mandates, ATSDR's Division of Toxicology (DT) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists in collaboration with mixtures risk assessors and laboratory scientists have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence (WOE) approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

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All reviewers were selected in conformity with the conditions for peer review specified in CERCLA Section 104(I)(13).

Scientists from ATSDR have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

SUMMARY

Uranium and fluoride are used in conjunction with nitrate when separating isotopes of uranium via the gaseous diffusion process. This process has been used at several U.S. Department of Energy (DOE) facilities, and continues to be used today. In addition, cyanide has been reported with great frequency as a contaminant at NPL sites. Review of ATSDR's documents with site-specific information showed that uranium, fluoride, cyanide, and nitrate were reported at one site (Pantax Plant site), while three-component submixtures were reported at eight additional sites: Eastern Michaud Flats, Monticello Mill, Alcoa/Lavaca Bay, Depue/New Jersey Zinc/Mobil Chemical, Hipps Road Landfill, Riverbank Army Ammunition Plant, Savanna Army Depot, and Santa Susana Field Laboratory. The purposes of this profile are to: (1) evaluate data (if available) on health hazards, and their dose-response relationships, from oral exposure to this four-component mixture; (2) evaluate data on the joint toxic actions of components of this mixture; and (3) make recommendations for exposure-based assessments of the potential impact of joint toxic action of the mixture on public health.

Evaluation of the available environmental fate data for the components of the mixture suggests that in the event of exposure, the primary route of exposure of nearby populations to mixtures of these chemicals in soil is likely to be oral, resulting from contamination of soil and/or groundwater. ATSDR toxicological profiles are available for cyanide, uranium, and fluoride (ATSDR 1997, 1999b, 2001d, respectively); these documents are the primary sources of information presented in the Appendices concerning the toxicokinetics, health effects, mechanisms of action, and health guidelines for these chemicals. Neither a toxicological profile nor Minimal Risk Levels (MRLs) are available for nitrate; however, U.S. EPA (IRIS 2002) has derived an oral reference dose (RfD) for nitrate.

No studies were located that examined health effects in humans or animals exposed to mixtures exclusively containing uranium, fluoride, cyanide, and nitrate, and no physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for this mixture have been developed. A component-based approach (ATSDR 2001b, 2001c) was applied, wherein the potential influence of individual components on the toxicity of other components in the mixture is evaluated. For the purposes of component analysis, the toxicity from uranium radiation was considered as a separate element from the chemical toxicity of uranium. As joint action data are lacking for the majority of the component pairs, the mechanisms of action for each component pair were also analyzed for evidence of potential joint toxic actions. The weight-of-evidence analysis suggests greater-than-additive joint actions for one component pair (fluoride and cyanide, in both directions), and less-than-additive joint actions for two of the

component pairs (cyanide's effect on the toxicity of uranium radiation and nitrate's effect on cyanide toxicity).

Component-based approaches that assume endpoint-specific additive joint toxic action are recommended for exposure-based assessments of possible noncancer or cancer health hazards from oral exposure to uranium, fluoride, cyanide, and nitrate, because there are no direct data available to characterize health hazards (and dose-response relationships) from the four-component mixture. The weight-of-evidence analysis indicated that data are inadequate to characterize the modes of joint action of the majority of the components, but the additivity assumption appears to be suitable in the interest of protecting public health.

A target-organ toxicity dose (TTD) modification of the hazard index approach is recommended for conducting exposure-based assessments of noncancer health hazards. Where data are available, TTDs for several toxicity targets have been recommended for each of the components, including TTDs for renal, reproductive, and neurological effects.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ATSDR	Agency for Toxic Substances and Disease Registry
ATP/ADP	adenosine triphosphate/adenosine diphosphate
BINWOE	binary weight-of-evidence
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
DNA	deoxyribonucleic acid
DOE	Department of Energy
DT	Division of Toxicology
EPA	Environmental Protection Agency
Fe	iron
FQPA	Food Quality Protection Act of 1996
IARC	International Agency for Research on Cancer
ICRP	International Commission on Radiological Protection
IRIS	Integrated Risk Information System
kg	kilogram
L	liter
LC_{50}	50% lethal concentration
LD_{50}	50% lethal dose
LOAEL	lowest-observed-adverse-effect level
mg	milligram
MRL	Minimal Risk Level
NADH	nicotinamide adenine dinucleotide phosphate (reduced form)
NADPH	nicotinamide adenine dinucleotide phosphate (oxidized form)
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
PBPK	physiologically based pharmacokinetic
PBPK/PD	physiologically-based pharmacokinetic/pharmacodynamic
ppm	parts per million
RfC	reference concentration
RfD	reference dose
TTD	target-organ toxicity dose
µmol	micromole
UO_2F_2	uranyl fluoride
U.S.	United States
WOE	weight-of-evidence
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to

1. Introduction

The primary purpose of this Interaction Profile for uranium, fluoride, and cyanide is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture Minimal Risk Level (MRL), and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence (WOE) approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Division of Toxicology's (DT) recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

Uranium and fluoride are used in conjunction with nitrate when separating isotopes of uranium via the gaseous diffusion process. This process has been used at several U.S. Department of Energy (DOE) facilities, and continues to be used today. In addition, cyanide has been reported with great frequency as a contaminant at National Priorities List (NPL) sites. Review of ATSDR's documents with site-specific information showed that uranium, fluoride, cyanide, and nitrate were reported at one site (Pantax Plant site), while three-component submixtures were reported at eight additional sites: Eastern Michaud Flats, Monticello Mill, Alcoa/ Lavaca Bay, Depue/New Jersey Zinc/Mobil Chemical, Hipps Road Landfill, Riverbank Army Ammunition Plant, Savanna Army Depot, and Santa Susana Field Laboratory.

Table 1. Data from 2001 CERCLA Priority List of Hazardous Substances

Compound	NPL Ranking
Uranium	94
Hydrogen Fluoride	248
Fluoride	261
Cyanide	28
Hydrogen Cyanide	139
Nitrate	216

Data on the rankings of the individual compounds in the mixture, based on the 2001 CERCLA Priority List of Hazardous Substances, are presented in Table 1. Evaluation of the available environmental fate data for the components of the mixture suggests that in the event of exposure, the primary route of exposure of nearby populations to mixtures of these chemicals in soil is likely to be oral, resulting from contamination of soil and/or groundwater.

ATSDR toxicological profiles are available for cyanide, uranium, and fluoride (ATSDR 1997, 1999b, 2001d); these documents are the primary sources of information presented in the Appendices concerning the toxicokinetics, health effects, mechanisms of action, and health guidelines for these chemicals. No toxicological profile or MRLs are available for nitrate; however, U.S. EPA (IRIS 2002) has derived an oral reference dose (RfD) for nitrate. The bases for available MRLs as well as other pertinent health effects are presented in Table 2 and in Appendices A, B, C, and D.

Table 2. Potential Health Effects of Concern for Intermediate and Chronic Oral Exposure to the Mixture Uranium, Fluoride, Cyanide, and Nitrate (see Appendices A, B, C, and D)[a]

Uranium	Uranium Radiation	Fluoride	Cyanide	Nitrate
Renal **Hepatic** Endocrine (Thyroid)	Cancer	***Musculoskeletal*** Reproductive (Testicular) Neurological Renal	***Reproductive (Testicular)*** **Developmental** **Neurological** Renal	**Hematological**

[a]The basis for the MRL is bolded and italicized; other sensitive effects are bolded; and less sensitive effects in common across two or more compounds are listed without bold or italics.

2. Joint Toxic Action Data for the Mixture of Concern and Component Mixtures

This chapter provides a review and evaluation of the literature pertinent to joint toxic action of the mixture and its components. The text is generally organized so that human data are presented first, and studies are grouped by route, and by endpoint where that is feasible.

2.1 Mixture of Concern

No studies were located that examined health effects in humans or animals exposed to mixtures containing uranium, fluoride, cyanide, and nitrate. No physiologically-based pharmacokinetic (PBPK) models were found for mixtures of these three components.

2.2 Component Mixtures

The following subsections present evaluations of health effects data and discussions of mechanistic information pertinent to the joint toxic action of each pair of components. For clarity, the radiologic effects of uranium are discussed separately from the chemical effects.

2.2.1 Uranium and Fluoride

Uranium and fluoride are often found in combination in the nuclear power industry, where uranium hexafluoride is used to enrich uranium mixtures to increase the activity. Upon contact with moisture, including moisture in the air, uranium hexafluoride rapidly hydrolyzes to uranyl fluoride (UO_2F_2) and hydrogen fluoride. As these uranium compounds see heavy industrial use, many of the studies of the toxicity of uranium have examined one or both of them. It is not possible to determine the effect of the fluoride ions in these studies on the measured toxicity of uranium; however, similar toxic effects have been noted in animal studies when uranium compounds not containing fluoride were examined, providing strong evidence that the major effects of the studies is from the uranium ion. None of the available studies of uranium and fluoride have specifically examined for the effects of fluoride; thus, the potential modulation of fluoride-induced toxic effects by uranium cannot be determined.

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between uranium and fluoride in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to uranium and fluoride were located. The primary sensitive shared target of toxicity following oral

exposure to uranium and fluoride compounds is renal effects. No other shared targets of uranium and fluoride were identified from the available literature.

The most sensitive effects of chronic oral fluoride exposure are on skeletal endpoints, where fluoride alters the structure of the carbonate-apatite crystals, resulting in an increased bone mass, but decreased bone strength. Data are not available as to whether uranium could affect this incorporation and therefore modify the primary toxic action of fluoride. Uranium has an affiliation for bone tissue, which is a concern with, e.g., veterans with depleted-uranium shrapnel. Thus, the potential for interaction bears investigation. Uranium's most sensitive effects are on the kidney. It is possible that renal damage might reduce the elimination of fluoride and thus either enhance or prolong its toxicity. Studies have shown that urinary excretion of fluoride is markedly decreased in the presence of decreased renal function (ATSDR 2001d). Fluoride may also have effects on the kidney, with a case report noting that exposure to high levels of fluoride resulted in renal insufficiency and interstitial nephritis (ATSDR 2001d). The potential mechanisms involved in this effect are not understood. Fluoride has also been shown to have toxic effects on the testes and to elicit neurological effects. As these endpoints are not sensitive endpoints for uranium toxicity, and no mechanistic or joint action data are available examining the effect of uranium on the toxicity of fluoride for these effects, a reliable projection as to the potential joint toxic action of uranium and fluoride cannot be made.

The most sensitive effects of exposure to uranium compounds are renal effects, resulting in both functional (e.g., proteinuria, enzymuria, glucosuria) and morphologic (e.g., proximal tubule necrosis) changes. Uranium-induced renal changes occur primarily in the glomerulus and proximal tubule. While fluoride has also been shown to elicit effects on the kidney, the mechanisms behind these effects are not understood. Neither is it known whether co-exposure to fluoride will alter the mechanisms of uranium nephrotoxicity. Exposure to higher levels of uranium can also result in effects on other organs, including the liver and the thyroid. However, the mechanisms of these effects have not been elucidated, so reliable projections as to the effect of co-exposure to fluoride on the mechanisms of uranium-induced hepatic and thyroid effects cannot be made.

2.2.2 Uranium Radiation and Fluoride

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between uranium radiation and fluoride in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to uranium radiation and fluoride were located. While it is generally accepted that exposure to ionizing radiation may result in increased incidence of tumors, data on the carcinogenic effects of

uranium radiation are not available, and available studies of the carcinogenicity of fluoride have presented equivocal evidence at best, with the majority of examinations demonstrating no carcinogenic effects. No additional shared targets of toxicity for uranium radiation and fluoride were located.

The most sensitive effects of chronic oral fluoride exposure are on skeletal endpoints, where fluoride alters the structure of the carbonate-apatite crystals, resulting in an increased bone mass, but decreased bone strength. Under steady state conditions, the majority of the retained uranium in the body is in the kidneys and skeleton. As such, the skeleton may also be a target of uranium radiation. However, as alpha particles do not penetrate deeply into tissues, particularly hard tissues, it is not known if uranium radiation will influence the skeletal toxicity of fluoride. It is feasible that damage from exposure to uranium radiation to cells involved in bone restructuring might result in an enhanced skeletal toxicity of fluoride, but no human or animal data are available to confirm this hypothesis. Based on the pharmacokinetic behavior of uranium, the kidney would be expected to be exposed to the greatest share of uranium radiation. However, as the mechanism of renal effects of fluoride are not well understood, reliable projections as to the effect of uranium radiation on fluoride-induced renal toxicity cannot be made. Similarly, data are not available examining the potential effect of uranium radiation on other endpoints of fluoride toxicity, such as testicular and neurological effects, precluding estimation of the potential effects of uranium radiation on fluoride toxicity. While the most sensitive target of uranium radiation is believed to be carcinogenesis, available studies of fluoride toxicity have demonstrated only equivocal evidence of carcinogenic effects at best, with other studies showing no carcinogenic effects of oral fluoride exposure.

Exposure to ionizing radiation is known to have carcinogenic effects; as such, cancer is a potentially sensitive endpoint for exposure to uranium radiation. The mechanism of this effect would be similar to other radionuclides, in that ionization events result in damage to cellular macromolecules and eventual transformation. However, studies demonstrating carcinogenic effects from chronic exposure to uranium radiation are lacking, likely due to the fact that the chemical effects of uranium are generally overriding, even for enriched uranium. Data are not available examining the potential effect of fluoride on the possible carcinogenic effects of uranium alpha radiation, and understanding of the mechanisms of fluoride toxicity and possible carcinogenicity are not sufficient to make reliable predictions as to the effect that co-exposure to fluoride may have on the toxicity of uranium radiation.

2.2.3 Uranium and Cyanide

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between uranium and cyanide in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to uranium and cyanide were located. The primary shared targets of toxicity following oral exposure to uranium and cyanide compounds are endocrine (thyroid) and renal effects.

The most sensitive effects of prolonged exposure to cyanide are effects on the testes, with developmental and neurological effects also being sensitive effects, and renal and thyroid effects reported at higher exposure levels. Cyanide is believed to cause its toxic effects by binding to iron-containing proteins, with a particular affinity for cytochrome c oxidase, a mitochondrial enzyme involved in oxidative metabolism. Binding of cyanide to this enzyme inhibits mitochondrial electron transport and results in histoxic hypoxia, leading to severe decrements in cellular energy availability. No data are available examining potential effects of uranium on the mechanisms of cyanide toxicity.

The most sensitive effects of exposure to uranium compounds are renal effects, resulting in both functional (e.g., proteinuria, enzymuria, glucosuria) and morphologic (e.g., proximal tubule necrosis) changes. Uranium-induced renal changes occur primarily in the glomerulus and proximal tubule. Oral exposure to cyanide has also been demonstrated to result in renal toxicity, though renal endpoints are less sensitive targets than other cyanide-induced changes. Data are not available that examine the potential effect of cyanide, and its resulting inhibition of metalloenzymes including cytochrome c oxidase, on the nephrotoxic effects of uranium. Exposure to higher levels of uranium can also result in effects on the thyroid. However, the mechanisms of these effects have not been elucidated, so reliable projections as to the effect of co-exposure to fluoride on the mechanisms of uranium-induced thyroid effects cannot be made.

2.2.4 Uranium Radiation and Cyanide

Treatment with cyanide immediately before irradiation has been shown to provide protection against the effects of ionizing radiation. Schubert (1991) reported that 100% of mice exposed to KCN 2 minutes prior to a lethal dose of gamma radiation survived, as opposed to 0% in controls. Treatment with thiosulfate, which counteracts the effects of cyanide, 5 minutes prior to KCN injection resulted in 0% survival, while treatment 3 minutes postirradiation had no effect (100% survival). A similar protective effect was described in Schubert et al. (1992), who reported that mice injected with KCN 2 minutes prior to gamma irradiation showed fewer chromosomal aberrations than irradiated control mice.

Similarly, Biaglow and Durand (1978) and Mikaelsen (1954) each reported that *in vitro* pretreatment with cyanide results in a decreased incidence of chromosomal damage in cells exposed to gamma radiation.

The mechanism involved in cyanide-induced radioprotection has not been fully elucidated, but it is believed that it involves either a decreased susceptibility owing to the hypoxic state of the cells or an increased availability of cellular oxygen resulting from the inhibition of oxidative phosphorylation (Biaglow and Durand 1978; Mikaelsen 1954; Schubert 1991; Schubert et al. 1992). However, available studies have demonstrated this effect only for X and gamma radiations, whereas uranium isotopes emit alpha particles. While the fundamental mechanisms of the various types of ionizing radiation are similar (ionization events leading to cellular effects), data are not available that specifically examine the effects of cyanide on the toxicity of alpha radiation. Furthermore, the available interaction studies are of acute duration. It is unknown whether cyanide would offer protection on a more chronic basis.

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between uranium radiation and cyanide in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to uranium radiation and cyanide were located. While radiation is believed to result in increased incidence of tumors, chronic studies of the effects of cyanide are inadequate to assess the potential carcinogenicity of cyanide. No additional shared targets of toxicity for uranium radiation and cyanide were identified by the available literature.

2.2.5 Uranium and Nitrate

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between uranium and nitrate in affecting health-related endpoints in humans or animals. While the literature contains reports of studies examining the effects of uranium nitrate, they have focused exclusively on the effects of the uranium ion. No PBPK models for co-exposure to uranium and nitrate were located. From the available data, uranium and nitrate do not appear to have any sensitive shared targets of toxicity. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of uranium and nitrate.

2.2.6 Uranium Radiation and Nitrate

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between uranium radiation and nitrate in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to uranium radiation and nitrate were located. From the available data, uranium radiation and

nitrate do not appear to have any sensitive shared targets of toxicity. Similarly, our understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of uranium radiation and nitrate.

2.2.7 Fluoride and Cyanide

Both fluoride and cyanide ions have been demonstrated to affect cellular energy metabolism, with fluoride primarily resulting in decreased glycosolation reactions, while cyanide is an inhibitor of oxidative phosphorylation. Szabo et al. (1973) demonstrated that fluoride and cyanide have opposite effects on cellular glucose metabolism, with fluoride treatment resulting in a decrease in cellular glucose uptake in cultured cells, while cyanide treatment resulted in increased uptake; the increased glucose uptake resulting from cyanide exposure is thought to represent an increased use of the glycolytic pathways subsequent to inhibition of oxidative metabolism. Co-exposure of the cells to the same concentrations of both fluoride and cyanide resulted in decreased glucose uptake, but not to the same extent as fluoride alone. However, no *in vivo* studies in humans or animals that examined possible joint toxic actions of fluoride and cyanide in affecting health-related endpoints in humans or animals were located. No PBPK models for co-exposure to fluoride and cyanide were located.

Shared targets of toxicity of fluoride and cyanide include reproductive (testicular), neurological, and renal effects. The intermediate oral MRL for cyanide is based on decreased testicular weight and altered spermatogenesis in F344 rats exposed for 13 weeks in drinking water (NTP 1993). Fluoride has also been shown to elicit effects on the testes, though only at doses approximately 10-fold greater than the most sensitive effects for fluoride. Similarly, high-dose exposure to either fluoride or cyanide can result in neurological and renal changes. Cyanide does not appear to have an effect on skeletal endpoints, which are the critical effect for the chronic MRL for fluoride. As cyanide has a negative effect on oxidative metabolism and fluoride has been shown to reduce glycolysis (Szabo et al. 1973), it is possible that joint effects on cellular energy status may result in nonadditive effects of fluoride and cyanide. However, the available data are sufficient neither to predict the direction or extent of this potential interaction nor the organ or organs in which it may potentially occur.

2.2.8 Fluoride and Nitrate

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between fluoride and nitrate in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to fluoride and nitrate were located. From the available data, fluoride and nitrate do not appear to have any

sensitive shared targets of toxicity. Similarly, understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of fluoride and nitrate. A study by Whitford and Pashley (1991) demonstrated that renal acidification resulting from an injection of sodium nitrite would increase the renal reabsorption of fluoride ions in dogs; however, the potential influence of this on the toxicity of fluoride has not been established.

2.2.9 Cyanide and Nitrate

No studies directly examining the toxic effects of simultaneous oral exposure to cyanide and nitrate were located in the literature. However, as described in Appendix D, approximately 5–10% of an oral dose of nitrate will be converted to nitrite by gastrointestinal bacteria. Nitrate has long been administered, alone or in combination, as an antidote against the acute lethal effects of cyanide; in recent years, a combination of nitrite and sodium thiosulfide has been used. Increased levels of methemoglobin, resulting from nitrite exposure, compete with cytochrome c oxidase for cyanide ions, forming cyanmethemoglobin and thereby reducing the lethality of cyanide, usually on the order of a 3- to 5-fold reduction. For example, Burrows and Way (1979) reported that injection of 22 mg/kg sodium nitrite 5 minutes after a single oral dose of sodium cyanide in sheep resulted in an increase in the 50% lethal dose (LD_{50}) from 3.7 to 14.1 mg/kg, but no change in the slope of the dose-response curve. Similarly, Cannon et al. (1994) reported that in mice, the LD_{50} values for a subcutaneous injection of potassium cyanide with and without pretreatment with an injection of 100 mg/kg sodium nitrite were 10.1 and 28.6 mg/kg, respectively, while Chen and Rose (1952) reported a 5-fold increase in the LD_{50} of subcutaneous sodium cyanide in dogs given intravenous sodium nitrite.

The traditionally proposed mechanism of nitrite-induced protection against cyanide toxicity has been well-established. For example, Tadic (1992) reported that injection of sodium nitrite 30 minutes after subcutaneous injection of 20 mg/kg sodium cyanide resulted in a restoration of brain cytochrome oxidase activity, while Isom and Way (1974) reported that subcutaneous injection of 100 mg/kg sodium nitrite reduced the increase in glycolysis normally seen with cyanide intoxication. More recent examinations have discovered other potential mechanisms that are believed to come into play with combination treatments of sodium nitrite and other compounds (Paitian et al. 1985; Way et al. 1988). However, these mechanisms may also be the result of co-administration of nitrite and the other compounds, rather than from nitrite itself. The potential contribution of these mechanisms to nitrite's reduction of cyanide toxicity is not known, though they are believed to result in more efficient detoxification. Additional characterizations of non-methemoglobin mechanisms of nitrite-induced protection against cyanide

toxicity will be required in order to adequately assess the potential role they may play in the joint toxic actions of nitrate and cyanide.

Data are not available examining the potential effects of cyanide on the toxicity of nitrate, and mechanistic understanding is not sufficient to predict the direction and/or magnitude of any potential effect of cyanide exposure on nitrate toxicity. No PBPK models for co-exposure to cyanide and nitrate were located. Cyanide and nitrate do not appear to have any sensitive shared targets of toxicity.

2.3 Relevance of the Joint Toxic Action Data and Approaches to Public Health

Mixtures containing uranium, fluoride, cyanide, and nitrate may be found together at hazardous waste sites, most notably those located at present or former DOE facilities. No studies examining a complete mixture of these compounds were located in the literature. No PBPK models are available for the complete mixture, or for any of the two- or three-component submixtures.

In the absence of studies that examine relevant endpoints and describe dose-response relationships following oral exposures to mixtures that contain these chemicals (e.g., in food or in soil), component-based approaches to assessing their joint action that assume dose additivity for noncancer effects appear to be reasonable for practical public health concerns. Carcinogenic effects are believed to be a sensitive health effect only for uranium radiation, and even in that case, data demonstrating carcinogenic effects of uranium radiation following oral exposure are not available. Therefore, an approach focusing on the carcinogenic risks of that component as being of greatest concern for the mixture seems to be reasonable.

In the introduction to this document, Table 2 presented an overview of the potential health effects of concern from oral exposure to uranium, uranium radiation, fluoride, cyanide, and nitrate. Each of the four compounds affects a variety of target organs and endpoints. There are few target organs in common across two or more of the components of the mixture. In the cases where an endpoint is shared, it is generally a sensitive target of one compound, but a high-dose effect of the other. The exception to this is the shared target of testicular effects of fluoride and cyanide. As shown in Table 3, however, the oral MRLs for uranium, fluoride, and cyanide are based on different endpoints, and oral MRLs have not been derived for uranium radiation and nitrates. Available data on possible binary interactions among these four chemicals are limited for most of the pairs. PBPK models that predict the disposition of these chemicals are not available for the complete mixture, for ternary submixtures, or for any of the binary component pairs of the mixture. Tables 4 through 7 describe binary weight-of-evidence (BINWOE) evaluations for the pairs of the chemicals of concern using the classification scheme summarized in

Table 3. Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern (see Appendices A, B, C, and D)

Duration of Exposure	Uranium	Uranium Radiation	Fluoride	Cyanide	Nitrate
Acute	None derived, inadequate data	None derived, inadequate data	None derived, inadequate data	None derived, inadequate data	None derived, no Toxicological Profile
Intermediate	Renal effects in rabbits	None derived, inadequate data	None derived, inadequate data	Reproductive effects in rats	None derived, no Toxicological Profile
Chronic	None derived, inadequate data	None derived, inadequate data	Increased risk of bone fractures in humans	None derived, inadequate data	None derived, no Toxicological Profile

Figure 1 and in ATSDR (2001b). The selection of target organs or endpoints for BINWOE development takes into account the critical effects of the individual components. In addition, and particularly if the components do not have the same critical effect, the selection also takes into account other relatively sensitive effects in common across two or more components of the mixture. The conclusions in these tables were based on the evaluations of the pertinent literature presented in Section 2.2. The BINWOEs focus on repeated simultaneous oral exposure, since this is the exposure scenario of most interest for public health concerns for the subject chemicals and their mixture. A summary discussion of the BINWOEs follows this paragraph and precedes the descriptive tables.

There are no pertinent interaction data, and understanding of mechanisms of action is too incomplete to make projections of joint toxic actions between the following pairs of chemicals:

- Uranium and fluoride;
- Uranium and cyanide;
- Uranium and nitrate;
- Uranium radiation and fluoride;
- Uranium radiation and nitrate; and
- Fluoride and nitrate;

Evidence of varying quality and quantity is available supporting projections of joint toxic action for the following pairs of chemicals:

- Uranium radiation and cyanide (Tables 4 and 5);
- Fluoride and cyanide (Tables 6 and 7); and
- Cyanide and nitrate (Tables 8 and 9)

For uranium radiation and cyanide and cyanide and nitrate, data are available in only one direction. For example, data are available on the effect of cyanide on the toxicity of uranium radiation, but not for the effect of uranium radiation on the toxicity of cyanide. While data suggesting joint actions of fluoride and cyanide are limited, they suggest that the effects will occur in both directions.

In summary, there are no data that suggest that non-additive interactions occur for the majority of the component pairs, though it should be emphasized that studies designed to identify and characterize mode of joint toxic action of the components are, for the most part, unavailable. In two cases, the effect of cyanide on the toxicity of uranium radiation and the effect of nitrite on the toxicity of cyanide, the available data suggest less-than-additive joint action of the component pairs, and in one case, the joint action of fluoride and cyanide, the available data suggest a greater-than-additive joint action for the component pair.

Figure 1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

Classification		Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	-1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanism(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from –1 through 0 to +1

*Source: ATSDR 2001b, 2001c

Table 4. Effect of **Uranium Radiation** on **Cyanide**

BINWOE: ? (0)

Direction of Interaction - The direction of the interaction cannot be predicted in the absence of (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with uranium radiation will influence the toxicity of cyanide; or (3) mechanistic understanding leading to an unambiguous projection of interactions between uranium radiation and cyanide.

Mechanistic Understanding - The most sensitive effects of cyanide are effects on the testes, with developmental and neurological effects also being sensitive effects, and renal and thyroid effects reported at higher exposure levels. Cyanide is believed to cause its toxic effects by binding to iron-containing proteins, with a particular affinity for cytochrome c oxidase, a mitochondrial enzyme involved in oxidative metabolism. Binding of cyanide to this enzyme inhibits mitochondrial electron transport and results in histoxic hypoxia, leading to severe decrements in cellular energy availability. No data are available examining potential effects of uranium radiation on the mechanisms of cyanide toxicity.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. No studies were located in which treatment with uranium radiation before cyanide exposure was examined.

Additional Uncertainties - Uncertainties have been addressed in the above discussion.

Table 5. Effect of **Cyanide** on **Uranium Radiation**

BINWOE: <IIB2ii (-1 x 0.71 x 0.71 x 0.79 x 0.79 = -0.31)
for carcinogenic effects

Direction of Interaction - Several studies have established that treatment with cyanide immediately prior to an exposure to ionizing radiation results in decreased toxicity (lethality, chromosomal damage) of the radiation exposure (Biaglow and Durand 1978; Mikaelsen 1954; Schubert 1991; Schubert et al. 1992). The proposed direction of interaction is therefore less than additive.

Mechanistic Understanding - The mechanism by which cyanide reduces the susceptibility to ionizing radiation is not fully understood. Cyanide must be actively inhibiting cytochrome c oxidase to be radioprotective, as demonstrated by Schubert (1991), who reported that injection of mice with KCN 2 minutes prior to irradiation with an otherwise 100% lethal dose of gamma radiation resulted in complete protection (100% survival), unless thiosulfate, a cyanide antagonist, was given 5 minutes prior to irradiation, in which case survival was 0%. While these studies were performed using isotopes that were not alpha emitters, the mechanism of action for alpha and gamma radiations (ionization events leading to cellular damage) is expected to be similar. Therefore, the mechanisms resulting in a protective effect of cyanide against gamma and X-irradiation are expected to function against ionizations caused by alpha radiation, such as is emitted by uranium isotopes. However, because of the different average path lengths of the radiations, the distribution of the emissions from uranium will likely be quite different from a whole-body gamma radiation exposure. A confidence rating of "II" was therefore assigned for mechanistic understanding, reflecting mechanistic data from related compounds.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. The studies of Schubert (1991) and Schubert et al. (1992) examined toxicologically relevant endpoints, specifically lethality and chromosomal aberrations, following pretreatment with cyanide prior to gamma irradiation, but no studies examining pretreatment with cyanide prior to exposure to uranium radiation were located. Both Biaglow and Durand (1978) and Mikaelsen (1954) reported that *in vitro* pretreatment with cyanide results in a decreased incidence of chromosomal damage in cells exposed to gamma radiation; no *in vitro* studies examining the effect of pretreatment with cyanide on the effects of uranium radiation were located. All interaction studies were of acute duration; it is unknown whether cyanide would offer protection on a more chronic basis. A confidence rating of "B" was assigned for toxicological significance.

Modifying Factors - Available studies of the radioprotective effects of cyanide have been conducted using acute injection exposures. Therefore, modifying factors of 0.79 for different exposure duration/ sequence (2) and 0.79 for different exposure route (ii) were applied to the BINWOE.

Additional Uncertainties - The rating of II (and the corresponding 0.71 weighting factor) for mechanistic understanding and the modifying factor of 0.79 for different exposure route do not fully express the uncertainties associated with the applicability of extrapolation of data on the effects of gamma radiation to the effects of alpha radiation.

Table 6. Effect of **Fluoride** on **Cyanide**

BINWOE: >IIIC2b (1 x 0.32 x 0.32 x 0.79 x 0.79 = 0.06)

Direction of Interaction - The direction of action for the effects of fluoride on the toxicity of cyanide is expected to be greater than additive based on mechanistic studies of fluoride and cyanide.

Mechanistic Understanding - Both fluoride and cyanide ions have been demonstrated to affect cellular energy metabolism, with fluoride primarily resulting in decreased glycosolation reactions, while cyanide is an inhibitor of oxidative phosphorylation. These two appear to act independently on different sites of energy metabolism. Szabo et al. (1973) demonstrated that fluoride and cyanide have opposite effects on cellular glucose uptake, with fluoride treatment resulting in a decrease in cellular glucose uptake in cultured cells, while cyanide treatment resulted in increased uptake; the increased glucose uptake resulting from cyanide exposure is thought to represent an increased use of the glycolytic pathways subsequent to inhibition of oxidative metabolism. Co-exposure of the cells to the same concentrations of both fluoride and cyanide resulted in decreased glucose uptake, but not to the same extent as fluoride alone. However, no studies directly examining the effect of fluoride on the toxicity of cyanide were located. A rating of "III" was therefore applied for mechanistic understanding.

Toxicological Significance - No *in vivo* studies of the joint action of fluoride and cyanide were located in the literature. The only study that examined the joint action of fluoride and cyanide examined a metabolic endpoint, rather than a known toxic effect of cyanide. A rating of "C" was therefore selected for toxicological significance.

Modifying Factors - Available data on joint action are limited to single-exposure *in vitro* studies. As such, modifying factors for different exposure duration (2) and *in vitro* data (b) were applied.

Additional Uncertainties - Uncertainties have been addressed in the above discussion.

Table 7. Effect of **Cyanide** on **Fluoride**

BINWOE: >IIIC2b (1 x 0.32 x 0.32 x 0.79 x 0.79 = 0.06)

Direction of Interaction - The direction of action for the effects of cyanide on the toxicity of fluoride is expected to be greater than additive based on mechanistic studies of cyanide and fluoride.

Mechanistic Understanding - Both cyanide and fluoride ions have been demonstrated to affect cellular energy metabolism, with fluoride primarily resulting in decreased glycosolation reactions, while cyanide is an inhibitor of oxidative phosphorylation. These two appear to act independently on different sites of energy metabolism. Szabo et al. (1973) demonstrated that fluoride and cyanide have opposite effects on cellular glucose metabolism, with fluoride treatment resulting in a decrease in cellular glucose uptake in cultured cells, while cyanide treatment resulted in increased uptake; the increased glucose uptake resulting from cyanide exposure is thought to represent an increased use of the glycolytic pathways subsequent to inhibition of oxidative metabolism. Co-exposure of the cells to the same concentrations of both fluoride and cyanide resulted in decreased glucose uptake, but not to the same extent as fluoride alone. However, no studies directly examining the effect of cyanide on the toxicity of fluoride were located. A rating of "III" was therefore applied for mechanistic understanding.

Toxicological Significance - No *in vivo* studies of the joint toxic action of cyanide and fluoride were located in the literature. The only study that examined the joint action of cyanide and fluoride examined a metabolic endpoint, which was a known toxic effect of fluoride. A rating of "C" was therefore selected for toxicological significance.

Modifying Factors - Available data on joint action of cyanide and fluoride are limited to single-exposure *in vitro* studies. As such, modifying factors for different exposure duration (2) and *in vitro* data (b) were applied.

Additional Uncertainties - Uncertainties have been addressed in the above discussion.

Table 8. Effect of **Cyanide** on **Nitrate**

BINWOE: ? (0)

Direction of Interaction - The direction of the interaction cannot be predicted in the absence of (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with cyanide will influence the toxicity of nitrate; or (3) mechanistic understanding leading to an unambiguous projection of interactions between cyanide and nitrate.

Mechanistic Understanding - Both cyanide and nitrate exert their main effects through fairly well-characterized mechanisms of action. It is believed that the bulk of the known effects of cyanide result from its interaction with the iron atom of cytochrome c oxidase. The only known effects of nitrate result from its metabolism to nitrite and resulting methemoglobinemia. While it is believed that the actions of nitrate may decrease the toxicity of cyanide (see Table 9 below), our understanding of the mechanisms and effects of nitrate-induced methemoglobinemia does not allow for accurate predictions as to whether or not co-exposure to cyanide will influence the toxicity of nitrate.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. No studies were located in which treatment with cyanide before nitrate exposure was examined.

Additional Uncertainties - Uncertainties have been addressed in the above discussion.

Table 9. Effect of **Nitrate** on **Cyanide**

BINWOE: <IA2ii (-1 x 1 x 1 x 0.79 x 0.79 = -0.62**)**
for neurological effects
BINWOE: <IIB2ii (-1 x 0.71 x 0.71 x 0.79 x 0.79 = -0.31**)**
for all other effects of cyanide

Direction of Interaction - The direction of action for the effects of nitrate on the toxicity of cyanide is expected to be less than additive, based on mechanistic studies of nitrate metabolism and numerous studies of the use of nitrite-containing compounds as antidotes to acute cyanide toxicity.

Mechanistic Understanding - As discussed in Appendix D, a small percentage, perhaps 5–10%, of an oral nitrate dose is converted to nitrite by bacteria of the gastrointestinal tract. Nitrite-induced formation of methemoglobin, either alone or in combination with other agents, has been used for many years as an antidote to acute cyanide toxicity in humans. Methemoglobin competes with cytochrome c oxidase for cyanide, resulting in diminished inhibition of respiratory function as a result of cyanide exposure. Re-establishment of brain levels of cytochrome oxidase enzyme levels has been demonstrated in acute *in vivo* studies (Isom and Way 1974; Tadic 1992); the mechanistic understanding for neurologic effects of cyanide was therefore given a rating of "I". The effect of nitrate (or nitrite) on other potential effects of cyanide has not been directly examined. However, the mechanism discussed above (methemoglobin competition for binding of cyanide ions) is likely to be applicable to these other endpoints as well. They were therefore assigned a confidence rating of "II" for mechanistic understanding. While more recent studies have suggested that nitrite may protect against cyanide toxicity by other mechanisms as well, these mechanisms are not presently well-elucidated.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. No studies were located in which treatment with nitrate before or after cyanide exposure was examined. As discussed above, a metabolite of nitrate, nitrite, has been shown to be protective against the neurologic effects of acute cyanide toxicity. A rating of "A" was therefore assigned for toxicological significance for neurological effects, and a rating of "B" was assigned for toxicological significance for other effects of cyanide.

Modifying Factors - Available studies of nitrite-induced protection against cyanide toxicity have been mainly of acute duration and intravenous exposure. Thus, modifiers of 0.79 for different exposure duration/sequence (2) and 0.79 for different exposure route (ii) were applied.

Additional Uncertainties - Uncertainties have been addressed in the above discussion.

2.4 Recommendations for Data Needs

Neither *in vivo* data from human or animal studies nor *in vitro* data examining the toxicity of the four-component mixture, or for three-component submixtures, are available. Similarly, PBPK models describing the behavior of the four-component mixture or the three-component submixtures are not available. In the absence of data for the complete mixture, a component-based approach was utilized. However, data on the joint toxic action of the component pairs of the mixture are lacking, with no adequate joint action data available for any of the nine component pairs of the mixture. Data on the potential mechanistic interactions between the component pairs are also lacking for the majority of the component pairs, with only the effect of nitrate on cyanide having a solid mechanistic basis for determination of potential joint action. Data are also available on the effect of cyanide on uranium radiation, but the data are limited to studies of related compounds, specifically other types of radiation. Fluoride and cyanide affect separate targets involved in energy metabolism, but studies of their joint toxicity are not available.

For the individual components, chronic oral MRLs are available for uranium (the intermediate MRL is believed to be protective for chronic exposure) and fluoride. An intermediate oral MRL is available for cyanide, and MRLs are not available for exposure to uranium radiation or to nitrate for any duration. The U.S. Environmental Protection Agency (EPA) (IRIS 2002) has derived a RfD for nitrate, based on methemoglobinemia; in practice, health assessments may use this RfD until such time as a chronic oral MRL for nitrate is derived. Additional data are needed if MRLs are to be derived for uranium radiation. Studies furthering our understanding of the mechanisms of the toxicity of the individual components, and component pairs, may also aid in understanding of the potential modes of joint action of the component pairs of the mixture.

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

Examination of the available joint toxic action data, presented in Section 2.2, reveals that no health effects data are available for the complete mixture, or for ternary submixtures. Because suitable toxicity data, joint action models, and PBPK models are lacking for the complete mixture, the recommended approach for the exposure-based assessment of joint toxic action of this mixture is to use a hazard index approach with a target-organ toxicity dose (TTD) modification and a qualitative WOE method. The hazard index, together with the WOE approach, assesses the potential consequences of additive and interactive joint action of the components of the mixture on noncarcinogenic endpoints of concern (ATSDR 2001b). Table 10 presents a matrix of the BINWOE values, where available, for each of the component pairs of the chemicals of concern as discussed in Chapter 2. Where appropriate, TTDs for oral exposure scenarios have been derived as described in the Appendices, using the methods recommended by ATSDR (2001b). However, in some cases, particularly for fluoride and cyanide, the effects on less-sensitive endpoints are not well studied, resulting in the application of very high uncertainty factors in calculation of TTD values. When the uncertainty associated with TTD calculation would result in numerical values lower than those of the MRL, which is based on the most sensitive observed effect identified and which is, in most cases, much better studied, the MRL value was recommended instead. Table 11 lists numerical values of these TTDs, and of the MRLs where available, for the shared endpoints of concern for chronic oral exposure to this mixture: renal, reproductive, and neurological effects. Additionally, for two components, fluoride and nitrate, the most sensitive endpoint is not a shared target of toxicity for the mixture. Therefore, the chronic MRL for fluoride is also presented. Since no toxicological profile or MRLs exist for nitrate, the RfD derived by U.S. EPA (IRIS 2002), is presented. Hazard indices for these unique sensitive endpoints can be calculated based on the MRL or RfD, respectively.

Table 10. Matrix of BINWOE Determinations for Neurological, Developmental, Reproductive, Renal, and Carcinogenic Effects of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF				
		Uranium	Uranium Radiation	Fluoride	Cyanide	Nitrate
EFFECT OF	Uranium			? (0)	? (0)	? (0)
	Uranium Radiation			? (0)	? (0)	? (0)
	Fluoride	? (0)	? (0)		>IIIC2b (0.06)	? (0)
	Cyanide	? (0)	<IIB2ii (-0.31) c	>IIIC2b (0.06)		? (0)
	Nitrate	? (0)	? (0)	? (0)	<IA2ii n (-0.62) <IIB2ii r, d, k (-0.31)	

c = cancer; d = developmental; k = renal (kidney); n = neurological; r = reproductive

The BINWOE determinations were explained in Section 2.3. No pertinent interactions data were available for the pairs of metals classified as indeterminate (?), and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings were assigned to these pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001b, 2001c).

Direction: = additive (0); > greater than additive (+1): < less than additive (–1); ? indeterminate (0)

Mechanistic Understanding

I. Direct and unambiguous mechanistic data to support direction of interaction (1.0).
II. Mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71).
III. Inadequate and ambiguous mechanistic data do not clearly indicate direction of interaction (0.32).

Toxicological Significance

A. The toxicological significance of the interaction has been directly demonstrated (1.0).
B. The toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71).
C. The toxicologic significance of interaction is unclear (0.32).

Modifiers

1. Anticipated exposure duration and sequence (1.0).
2. Different exposure duration or sequence (0.79).
a. *In vivo* data (1.0)
b. *In vitro* data (0.79)
i. Anticipated route of exposure (1.0)
ii. Different route of exposure (0.79)

Table 11. Target Organ Toxicity Doses (TTDs) and MRLs for Chronic Oral Exposure to Chemicals of Concern (see Appendices A, B, C, and D for Details of Derivations)

	Chemical				
Endpoint	Uranium	Uranium Radiation	Fluoride	Cyanide	Nitrate
Renal	2.0×10^{-3} mg/kg/day (intermediate oral MRL)	ID	0.06 mg/kg/day	0.05 mg/kg/day	NA
Reproductive (testicular)	NA	ID	0.06 mg/kg/day	0.05 mg/kg/day (intermediate oral MRL)	NA
Neurological	NA	ID	0.06 mg/kg/day	0.05 mg/kg/day	NA
Musculoskeletal	NA	ID	0.06 mg/kg/day (chronic oral MRL)	NA	NA
Hematological	NA	ID	NA	NA	1.6 mg/kg/day (EPA RfD)

ID = Inadequate data to derive a TTD for the selected endpoint; NA = Selected endpoint does not appear to be a sensitive target, or data are not available

Proceeding with the TTD modification of the hazard index approach involves calculating endpoint-specific hazard indices for each endpoint of concern, as described in ATSDR (2001c, Section 2.3.2 and Figure 2 with accompanying text). For example, a hazard index for renal effects of this mixture is calculated as follows:

$$HI_{RENAL} = \frac{E_U}{MRL_{U\ RENAL}} + \frac{E_F}{TTD_{F\ RENAL}} + \frac{E_{CN}}{TTD_{CN\ RENAL}}$$

where HI_{RENAL} is the hazard index for renal toxicity, E_U is the exposure to uranium (as the oral intake in the same units as the corresponding MRL, in this case mg/kg/day, calculated as described above), E_F is the exposure to fluoride (as the oral intake in the same units as the corresponding TDD, mg/kg/day), $MRL_{U\ RENAL}$ is the MRL for the renal toxicity of uranium, and so forth. Components for which data are not available, and therefore no TTD can be derived, are not included in the endpoint-specific hazard index calculation.

Because the available evidence supports the existence of one or more non-additive joint toxic actions, consideration must be given as to the effect of these actions on evaluations of the toxicity of the entire mixture. A less-than-additive effect of nitrate on the toxicity of cyanide was reported in the BINWOEs, based on high-dose acute data on nitrite injection in humans in combination with the observation that small amounts of oral nitrate are metabolized to nitrite. However, a recommendation of the assumption of additive joint action for the nitrate and cyanide is still considered appropriate, due to (1) the uncertainty associated with application of data from acute injection studies to chronic, low-level oral exposures; (2) a lack of data supporting the assumption that significant amounts of nitrate will be converted to nitrate at very low exposure levels; and (3) a lack of data supporting the assumption that the mechanism of the acute toxicity of cyanide, which is ameliorated by nitrite-induced methemoglobinemia, is an important mechanism in determining the intermediate and chronic effects of cyanide. Given these uncertainties, and the relatively small proportion of nitrate that is converted to nitrite, a protective effect of nitrate on cyanide toxicity is expected only at very high nitrate exposure levels, where significant nitrite formation would be seen. Therefore, for chronic, low-level exposure, the assumption of additivity is recommended.

Examination of the weight of evidence also indicates the possibility of greater-than-additive action for the toxicity of fluoride and cyanide, based on their potential joint effects on energy metabolism. However, data examining this potential for joint action are extremely limited, with only a single acute *in vitro* study examining glucose uptake to support the mechanism. As such, while the available data are suggestive of a greater-than-additive joint action of fluoride and cyanide, additional joint action data will be necessary before the effect of this potential mechanism on the toxicity of the mixture can be adequately evaluated. In the absence of an adequate evaluation, the default assumption of additivity is recommended.

These methods are to be applied only under circumstances involving significant exposure to the mixture, i.e., only if hazard quotients for two or more of the compounds equal or exceed 0.1 (Figure 2 of ATSDR 2001c). Hazard quotients are the ratios of exposure estimates to noncancer health guideline values, such as MRLs. If only one or if none of the compounds have a hazard quotient that equals or exceeds 0.1, then no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. If one or more of the endpoint-specific hazard indices exceed 1, they provide preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of the components on that endpoint. As discussed by ATSDR (1992, 2001c), the exposure-based assessment of potential health hazard is used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

The default approach for a multi-component mixture for which no data on the carcinogenicity of the mixture are available and no PBPK models have been validated would involve calculating the carcinogenic risk for each component by multiplying lifetime oral exposure estimates for each component by the appropriate EPA cancer oral slope factor (an estimate of cancer risk per unit of exposure). If only one or if none of the component risks equals or exceeds $1x10^{-6}$, then no further assessment of joint toxic action would be needed due to the low likelihood that additivity and/or interactions would result in a significant health hazard. However, in the case of the present mixture, only exposure to radiation from uranium is presently believed to result in carcinogenic effects. As such, when assessing the carcinogenic risks of the mixture uranium, fluoride, cyanide, and nitrate, focus should be placed primarily on the risk of carcinogenic effects of uranium radiation. While there is a suggestion that co-exposure to cyanide may result in a reduction of the carcinogenic effects of uranium radiation, as evidenced by a less-than-additive BINWOE, neither the data on uranium radiation-induced carcinogenesis nor the data supporting a less-than-additive effect of cyanide upon those carcinogenic effects are presently sufficient to warrant an alteration of the default approach. A health-protective approach that does not assume a reduction of carcinogenesis is therefore recommended.

4. Conclusions

There are no direct data available to characterize health hazards (and dose-response relationships) from mixtures containing all four of the components. Similarly, PBPK/PD models have not yet been developed that would predict pertinent target tissue doses of the components under scenarios involving exposure to mixtures of all four components. Finally, available information on toxic actions of the individual components indicates that joint actions of uranium, fluoride, cyanide, and nitrate on three toxicity targets are plausible, specifically: reproductive effects, neurologic alterations, and renal effects. For two of the components, fluoride and nitrate, the most sensitive health effect does not appear to be a shared target with other components, but should be considered when evaluating sites containing the potential health hazards resulting from exposure to the mixture. With data on the individual components suggesting possible sites of joint toxic action, but no data available on the toxicity or behavior of the complete mixture or the relevant submixtures, a default component-based approach that assumes additive joint toxic action in exposure-based assessments of possible noncancer health hazards from oral exposure to mixtures of uranium, fluoride, cyanide, and nitrate was recommended.

Weight-of-evidence analyses of available data on the joint toxic action of mixtures of these components indicate that scientific evidence for greater-than-additive or less-than-additive interactions among these components is limited and generally inadequate to characterize the possible modes of joint action on most of the pertinent toxicity targets. For two component pairs, cyanide's effect on the toxicity of uranium radiation and nitrate's effect on cyanide toxicity, less-than-additive effects of the two compounds are predicted. For one component pair, fluoride and cyanide, the available data suggest greater-than-additive joint action in both directions. No other greater-than-additive or less-than-additive interactions were indicated from the available data. Therefore, it is recommended that additivity be assumed as a public health protective measure in exposure-based assessments of health hazards from exposure to mixtures of these components.

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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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