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.