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.