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. Few relevant data were located for the mixture of jet fuels, hydrazines, trichloroethylene, arsenic, and strontium-90.

2.1 Mixture of Concern

No studies were located that examined health effects or pharmacokinetic endpoints in humans or research animals exposed to mixtures containing jet fuels, hydrazines, trichloroethylene, arsenic, and strontium-90. No physiologically-based pharmacokinetic (PBPK) models were found for mixtures of these five components.

2.2 Component Mixtures

No studies were located that examined health effects or pharmacokinetic endpoints in humans or research animals exposed to three- or four-membered mixtures of the five components of concern. No PBPK models were found for three- or four-membered mixtures of these chemicals.

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.

2.2.1 Jet Fuels and Hydrazines

No studies were located regarding possible joint toxic actions between jet fuels and hydrazines in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to jet fuels and hydrazines were found. Jet fuels and hydrazines both produce effects in the liver and central nervous system. In the liver, both substances produce inflammation, fatty degeneration, and necrosis. For both substances, a proposed mechanism of hepatic effects involves metabolism and generation of reactive oxygen species; however, mechanistic understanding of the hepatic effects of jet fuels and hydrazines is not sufficient to make reliable predictions as to the hepatic effects of joint exposure. Both jet fuels and hydrazines have been shown to cause neurological effects. However, the mechanisms believed to be responsible for these effects differ for the two classes of compounds, with jet fuels believed

to disrupt function of nerve cell membrane proteins by physical presence of the solvent in the membrane, whereas hydrazines are believed to form hydrazones with vitamin B6 derivatives, thereby inhibiting reactions that require vitamin B6 as a cofactor and inducing a functional deficiency of vitamin B6 (see Appendices A and B). Understanding of these mechanisms is inadequate to make reliable predictions as to the neurological effects of joint exposure. Hydrazines have been demonstrated to cause multiple tumor types in animal studies. No mechanistic information was located as to potential effects of jet fuels, which have not been demonstrated to be carcinogenic, on the carcinogenic effects of hydrazines.

2.2.2 Jet Fuels and Trichloroethylene

In a cohort mortality study of 3,814 white male employees at a uranium processing plant, Ritz (1999) reported that the main exposures (classified into "light," "moderate," and "heavy;" actual exposure concentrations not reported) were to kerosene, trichloroethylene, and cutting fluids (complex mixtures of variable composition classified as straight oils, soluble, or synthetic fluids; no information was available regarding the specific cutting oils used at the plant being studied over the 30-year exposure period). Considerable overlap in exposures occurred between these three substances, though primarily only at the "light" exposure level. Moderate exposure to trichloroethylene for 5 or more years was associated with increased incidence of liver (relative risk [RR] 12.1, 95% confidence interval [CI] 1.03–144) and brain (RR 14.4, 95% CI 1.24–167) cancer, though these increases were each the result of a single case. Both light (RR 3.46, 95% CI 1.22–9.80) and moderate (RR 7.71, 95% CI 2.04–29.1) exposures to kerosene (>2 years duration) were associated with increases in cancers of the esophagus and stomach. However, no inferences as to potential joint toxic actions can be made for trichloroethylene and kerosene from this study due to co-exposure to other chemicals (i.e., cutting fluids).

Spirtas et al. (1991) reported on the mortality of a cohort of 14,457 workers at an aircraft maintenance facility. The primary exposures were to trichloroethylene, though co-exposure to a number of chemicals, including JP-4, also occurred, as reported in a subsequent exposure assessment (Stewart et al. 1991). A significant trend toward increased incidence of emphysema with increasing trichloroethylene exposure was noted in male workers. While increases in cohort cancer mortality were observed, neither trichloroethylene nor JP-4 exposure was associated with significant increases in mortality from any type of cancer examined.

No other studies were located regarding possible joint toxic actions between jet fuels and trichloroethylene in affecting health-related endpoints in humans or research animals. No PBPK models for co-

exposure to jet fuels and trichloroethylene were found. Jet fuels and trichloroethylene both produce neurological, hepatic, and immunological effects. Both jet fuels and trichloroethylene are believed to inhibit neuronal function by their physical presence in neuronal membranes, and as such, are expected to produce additive effects on the central nervous system. However, data directly corroborating this are not available. Both jet fuels and trichloroethylene are believed to elicit hepatic and immunological effects as a result of metabolism to reactive products, possibly involving reactive oxidative species. However, understanding of these mechanisms is insufficient to reliably predict the result, which might involve competitive inhibition and induction of various cytochrome P-450 isozymes, of joint exposure. Trichloroethylene is a probable human carcinogen (see Appendix C). No mechanistic information as to potential effects of jet fuels, which have not been demonstrated to be carcinogenic, on the carcinogenic effects of trichloroethylene was located.

2.2.3 Hydrazines and Trichloroethylene

No studies were located regarding possible joint toxic actions between hydrazines and trichloroethylene in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to hydrazines and trichloroethylene were found. Important targets of toxicity common to hydrazines and trichloroethylene are the liver and central nervous system. Both hydrazines and trichloroethylene have been shown to cause hepatic effects, including inflammation, fatty degeneration, and necrosis (see Appendices B and C). Both are believed to do so as a result of metabolism resulting in a reactive intermediate, possibly resulting in oxygen radical formation, although hydrazines can also act by direct binding of the parent compound to cellular macromolecules. Mechanistic understanding of the hepatic effects of hydrazines and trichloroethylene is not sufficient to make reliable predictions as to the hepatic effects of joint exposure. Both hydrazines and trichloroethylene have been shown to cause neurological effects. However, the mechanisms for these effects appear to differ for the two classes of compounds, with hydrazines believed to interact with alpha-keto acids, such as vitamin B6, whereas trichloroethylene is thought to interact directly with neuronal membranes (see Appendices B and C). Understanding of these mechanisms is inadequate to make reliable predictions as to the neurological effects of joint exposure. The carcinogenic effects of hydrazines and trichloroethylene in laboratory animals are well documented (see Appendices B and C). Limited understanding of the mechanisms of hydrazine carcinogenesis, as well as limited knowledge of the mechanisms of action of trichloroethylene, precludes a reliable prediction of the carcinogenic effects of joint exposure.

2.2.4 Jet Fuels and Arsenic

No studies were located regarding possible joint toxic actions between jet fuels and arsenic in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to jet fuels and arsenic were found. Studies examining both jet fuels and arsenic have reported neurological effects. However, the mechanisms behind arsenic-induced neurological effects are not well understood. Thus, no reliable predictions of the neurological effects of joint exposure can be made. Similarly, understanding of the mechanisms of arsenic and jet fuel-induced effects on the immune system is inadequate to assess the potential effects of joint exposure on immunotoxicity. Other sensitive endpoints of arsenic toxicity (e.g., dermal, cardiovascular, hematological, and renal effects) are not believed to be sensitive endpoints of jet fuel exposure, and mechanistic understanding is insufficient to allow for reliable predictions of the effect of co-exposure on these endpoints. Arsenic is a confirmed human carcinogen (see Appendix D). However, the mechanisms of arsenic carcinogenesis are not sufficiently understood to allow for reliable predictions of the effect of exposure to jet fuels on arsenic-induced carcinogenesis.

2.2.5 Hydrazines and Arsenic

Yamamoto et al. (1995) treated groups of male F344 rats to multiple initiators, followed by 26 weeks of exposure to dimethylarsinic acid. Animals received a single intraperitoneal injection of 100 mg/kg of diethylnitrosamine on day 0 of the experiment, then intraperitoneal injections of 20 mg/kg of N-methyl-N-nitrosourea on days 5, 8, 11, and 14, followed by subcutaneous injections of 40 mg/kg of 1,2-dimethyl-hydrazine on days 18, 22, 26, and 30. Two groups received no initiating treatments. Beginning at week 6, initiated animals then received 0, 50, 100, 200, or 400 ppm of dimethylarsinic acid in the drinking water (0, 27.1, 54.3, 108.6, or 217.1 mg As/kg/day); animals with no initiation treatments received 100 or 400 ppm (108.6 or 217 mg As/kg/day). Animals were sacrificed at 30 weeks and examined for histologic changes, including examination of glutathione S-transferase-placental (GST-P)-positive foci in the liver. Dimethylarsinic acid treatment resulted in significantly decreased body weights at concentrations of 100 ppm or greater. In initiated groups, dimethylarsinic acid treatment resulted in dose-dependent increases in the incidence of tumors of the liver, bladder, kidneys, and thyroid gland; preneoplastic lesions in the liver (GST-P-positive foci) and kidney (atypical tubules) were also increased. No tumors or preneoplastic lesions were observed in uninitiated animals. This study suggests that dimethylarsinic acid, which is a major metabolite of arsenic in mammals, can promote tumors initiated by a combination of several chemicals, one of which was 1,2-dimethylhydrazine (a liver carcinogen in laboratory animals and, although not used as a rocket fuel, is structurally similar to the hydrazines that

have been used for that purpose). However, data from this study were inadequate to assess whether any joint toxic action specifically between dimethylarsinic acid and 1,2-dimethylhydrazine was additive or greater than additive.

No other studies were located regarding possible joint toxic actions between hydrazines and arsenic in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to hydrazines and arsenic were found. Shared targets of toxicity of hydrazines and arsenic include the hematopoietic and neurological systems (see Appendices B and D). However, understanding of the mechanisms of arsenic-induced toxic effects is insufficient to allow for reliable predictions of the effects of joint exposure. The carcinogenic effects of both hydrazines and arsenic are well documented (see Appendices B and D). Arsenic is a known human carcinogen. As with toxicity endpoints, the mechanisms of action are not sufficiently understood to allow for reliable predictions of the carcinogenic effect of joint exposure.

2.2.6 Trichloroethylene and Arsenic

Constan et al. (1995, 1996) exposed groups (5/time interval) of rats to a mixture of 31 ppm arsenic (as arsenic trioxide), 50 ppm benzene, 15 ppm chloroform, 7 ppm chromium (as chromium chloride hexa-hydrate), 37 ppm lead (as lead acetate trihydrate), 34 ppm phenol, and 38 ppm trichloroethylene in drinking water for up to 6 months; control animals received untreated drinking water. No changes in weight gain, body weight, liver weight, or liver-associated plasma enzymes were reported. The authors noted an increase in hepatocellular proliferation, as measured by increased bromo-deoxyuridine (BrdU) staining, that was seen around the large hepatic veins on days 3 and 10, and at 1 month of exposure. Similarly, at day 10 and 1 month of exposure, apoptosis of hepatocytes, assessed by TdT-mediated dUTP digoxigenin nick end labeling (TUNEL) stain, was elevated in large hepatic veins. Neither proliferation nor apoptosis were significantly different from controls at 3 and 6 months of treatment.

In a follow-up study, Benjamin et al. (1999) pretreated groups of rats with an intraperitoneal injection of 20 mg/kg of diethylnitrosamine on day 0, then exposed them to the same mixture (referred to as 10x), or the mixture at 1/10th the concentration (3.1 ppm arsenic [as arsenic trioxide], 5.0 ppm benzene, 1.5 ppm chloroform, 0.7 ppm chromium [as chromium chloride hexahydrate], 3.7 ppm lead [as lead acetate trihydrate], 3.4 ppm phenol, and 3.8 ppm trichloroethylene; referred to as 1x) in drinking water for 21 or 56 days. Treatment at the 1x concentration resulted in a significant increase in the area, but not total number, of GST-P-positive (i.e., preneoplastic) foci in the liver relative to the deionized water controls.

Treatment with the 10x concentration did not significantly affect either the number or area of the foci. The researchers concluded that there was no evidence of tumor promotion in this study.

Pott et al. (1998a) reported that oral administration of a mixture of arsenic, trichloroethylene, vinyl chloride, and 1,2-dichloroethane, after 2 weeks of initiation with diethylnitrosamine, in male F344 rats resulted in a dose-related decrease in the area of hepatocellular foci, as well as a decrease in the number of large foci per animal. No pulmonary adenomas were seen in any of the treated groups, while animals initiated with diethylnitrosamine averaged 0.25 adenomas per animal; the difference was statistically significant. The incidence of pulmonary hyperplasia was also significantly lower in treated groups compared to the initiation-only control group.

In a series of studies, Vodela et al. (1997a, 1997b) exposed male and female broiler chickens to drinking water containing mixtures of either 0.8 ppm arsenic, 1.3 ppm benzene, 5.0 ppm cadmium, 6.7 ppm lead, and 0.65 ppm trichloroethylene (low) or the same components at 10-fold higher concentrations (high). In the first experiment (Vodela et al. 1997a), male broiler chickens were exposed to the low or high concentrations of the mixture in the drinking water for 49 days. Exposed animals showed decreased water intake, food intake, and body weight gain, as well as statistically significant, dose-related decreases in cell-mediated and humoral immune response in both dose groups, relative to pair-watered controls. In the second experiment (Vodela et al. 1997b), female chickens were exposed to the low- or high-dose levels of the mixture from week 29 to 39 of age (10 weeks). Water consumption was significantly decreased in the high-dose animals, but not the low-dose animals; pair-watered controls were therefore used. Body weights were linearly ($p \leq 0.01$) decreased in exposed hens. Increasing concentration of the exposure mixture resulted in decreasing egg production and decreased egg weights, neither of which were due to reduced water consumption.

All of these studies were performed with mixtures that included other chemicals in addition to arsenic and trichloroethylene. It is uncertain which, if any, effects were influenced by these two chemicals, and what any joint toxic actions may have been. No other studies were located regarding possible joint toxic actions between arsenic and trichloroethylene in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to arsenic and trichloroethylene were found. The most sensitive effects of trichloroethylene exposure are neurological effects, believed to result from an interaction between trichloroethylene and the neuronal membrane (see Appendix C). Although arsenic also produces neurological effects, the available data are not sufficient to reliably predict the neurological effect of joint exposure. Similarly, while both arsenic and trichloroethylene have been shown to affect

the immune system and kidneys, data are inadequate to reliably predict the effect of joint exposure. Other sensitive endpoints of arsenic toxicity (e.g., dermal, cardiovascular, and hematological effects) are not believed to be sensitive endpoints of trichloroethylene exposure, and mechanistic understanding is insufficient to allow for reliable predictions of the effect of co-exposure to trichloroethylene on these endpoints. Trichloroethylene is a probable human carcinogen (see Appendix C) and arsenic is an established human carcinogen (see Appendix D), but due to limited understanding of the mechanism of action of either chemical, it is unknown what the carcinogenic effect of joint exposure might be.

2.2.7 Jet Fuels and Strontium-90

No studies were located regarding possible joint toxic actions between jet fuels and strontium-90 in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to jet fuels and strontium-90 were found. Exposures to either jet fuels or strontium-90 have been shown to result in a decreased immune response in animal studies, but the effects for jet fuels are not well studied (see A and E). Understanding of the mechanism(s) of jet fuel-induced immunotoxic effects is not sufficient to allow for reliable mechanistic inferences as to possible joint action of jet fuels and strontium-90. Other effects of strontium-90 (musculoskeletal and hematological effects and cancer) have not been demonstrated as endpoints of jet fuel toxicity, and plausible modes of joint action on these strontium-90 targets are not obvious (see Appendices A and E). Other effects of jet fuels (neurological and hepatic effects, see Appendix A) are not believed to be sensitive targets of strontium-90 radiation (see Appendix E). No data were located to indicate how exposure to radiation from strontium-90 might influence neurological effects from jet fuels itself or hepatic effects involving metabolites of jet fuels.

2.2.8 Hydrazines and Strontium-90

No studies were located regarding possible joint toxic actions of hydrazines and strontium-90 in humans or research animals. No PBPK models for co-exposure to hydrazines and strontium-90 were found. Strontium-90 is believed to cause hematological and immunological effects by localizing in bone and/or lymphatic tissues and subsequently irradiating the progenitor cells (see Appendix E). The mechanisms of hydrazine-induced hematological effects are not known with certainty, but are believed to involve either direct binding to cellular molecules, particularly alpha-keto acids, or the generation of reactive metabolites (see Appendix B). Available data are insufficient to allow for reliable predictions of hematological or immunological changes following joint exposure. The mechanism of carcinogenesis for strontium-90 (ionization events leading to damage to cellular constituents, including deoxyribonucleic

acid [DNA]) is well characterized. Hydrazines have also been shown to be genotoxic; however, the mechanisms of action of hydrazines are not sufficiently understood to allow for a reliable prediction of the carcinogenic effect of joint exposure.

2.2.9 Trichloroethylene and Strontium-90

Kilburn (1999) reported on a cohort of 154 jet engine repair workers who were exposed to a variety of metals (strontium chromate, manganese, nickel, beryllium, and others) and solvents (trichloroethylene, 1,1,1-trichloroethane, trichlorofluoroethane, and methanol) and 112 controls. Reported exposure levels, measured in six workers on a single day, were 0.006–0.29 mg/m^3 for strontium chromate and 4,800 mg/m^3 for trichloroethylene. Exposed workers were found to have significant differences in a number of respiratory parameters, including shortness of breath, wheezing, phlegm, and abnormal radiographs, relative to controls. The researchers noted that such effects are consistent with industrial bronchitis due to inhalation of welding fumes and of particulates from grinding stainless steel. Exposed workers also showed significant impairment of a number of neurological indices, including simple and choice reaction times, sway speeds (eyes open and closed), and color discrimination. The researchers tentatively attributed these effects to chlorinated solvent exposure, although it was noted that some of the metals present (e.g., manganese) may also have contributed. Due to co-exposures to other chemicals and the fact that strontium was in the form of strontium chromate, with chemical toxicity generally believed to be due to the chromate group, potential joint toxic actions between strontium-90 and trichloroethylene cannot be assessed from this study.

No other studies were located regarding possible joint toxic actions between strontium-90 and trichloroethylene in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to trichloroethylene and strontium-90 were found. Exposures to either strontium-90 or trichloroethylene have been shown to result in a decreased immune response in animal studies, but the effects for trichloroethylene are not well studied (see Appendices C and E). Understanding of the mechanism(s) of trichloroethylene-induced immunotoxic effects is not sufficient to allow for reliable mechanistic inferences as to possible joint action of trichloroethylene and strontium-90. Other effects of strontium-90 (musculoskeletal and hematological effects) have not been demonstrated as sensitive targets of trichloroethylene, and plausible modes of joint action on these strontium-90 targets are not obvious (see Appendices C and E). Other effects of trichloroethylene (neurological, hepatic, and renal effects, see Appendix C) are not believed to be sensitive targets of strontium-90 radiation (see Appendix E). No data were located to indicate how exposure to radiation from strontium-90 might influence neurological effects

from trichloroethylene itself or hepatic and/or renal effects involving metabolites of trichloroethylene. The mechanism of carcinogenesis for strontium-90 (ionization events leading to cellular damage, including DNA) is well characterized. Trichloroethylene is also carcinogenic in some species; however, understanding of the mechanisms of action of trichloroethylene is not sufficient to allow for reliable prediction of the effect of trichloroethylene on strontium-90-induced carcinogenic effects.

2.2.10 Arsenic and Strontium-90

De Kimpe et al. (1999) examined the effect of a number of compounds, including stable strontium (as strontium nitrate), on the methylation of arsenic in freshly-isolated liver cytosol from adult male Flemish Giant rabbits. This species was chosen because previous *in vivo* studies by these researchers demonstrated inorganic arsenic metabolism very similar to humans in these rabbits. Over the tested range of 0.34–8.5 μM, strontium exposure resulted in a dose-dependent decrease in both the mono- and dimethylation of arsenic. Similar results were found for many other species of trace elements and anions, as well as some, but not all, chelating agents, organic methyltransferase inhibitors, and uremic toxins. In contrast, some trace elements acted as stimulating agents for methylation, most notably Zn^{2+}. The researchers suggested that inhibition of methylation by strontium and other divalent cations may result from competitive inhibition with the stimulatory divalent cation, zinc. The researchers suggested that the inhibitory effects of the chelating agents ethylenediaminetetraacetic acid (EDTA) and oxime indicate that zinc may be an essential co-factor for As(III) methylation. This study shows that strontium inhibits methylation of arsenic *in vitro*. Because methylation of inorganic arsenic is generally considered to be a detoxification reaction, it is plausible that strontium-90 will increase the toxic effects of arsenic. However, it is not clear that strontium would be present in the liver cell in sufficient quantities to have any effect in a complete organism (approximately 99% of the total body burden is contained in the skeleton, see Appendix E) and it must be noted that the methylation products of arsenic are not without toxic effects themselves (studies have shown effects on the respiratory tissues, gastrointestinal tract, liver, kidney, reproduction, development, and genetic material [ATSDR 2000] and there is some evidence that dimethylarsinic acid is a cancer promoter [Yamamoto et al. 1995]).

Liu et al. (1999) reported that addition of 75 mg/L of arsenic trioxide (As_2O_3) to the water of Wistar rats for 6 months resulted in significantly decreased levels of naturally-occurring strontium in the kidney, but not in the liver, compared with control rats. The effect in the kidney disappeared when the animals were co-treated with sodium fluoride along with the arsenic trioxide.

No other studies were located regarding possible joint toxic actions between arsenic and strontium-90 in affecting health-related endpoints in humans or research animals. No PBPK models for co-exposure to arsenic and strontium-90 were found. Strontium-90 is believed to cause hematological and immunological effects by localizing in bone and/or lymphatic tissues and subsequently irradiating the progenitor cells (see Appendix E). While arsenic is also capable of eliciting hematological and immunological effects (see Appendix D), the mechanisms by which it does so are not well understood. Therefore, no reliable predictions as to the immunological and hematological effects of joint exposure to arsenic and strontium-90 can be made. The mechanism of carcinogenesis for strontium-90 (ionization events leading to damage to cellular constituents, including DNA) is well characterized. However, understanding of the mechanisms of action of arsenic is not sufficient to allow for reliable predictions of carcinogenic effects following joint exposure. Arsenic induces the metal-binding protein metallothionein in the liver, but binds to it with low affinity (see Appendix D). The extent to which strontium, which is sequestered in bone, might interact with metallothionein in the liver is unclear, as are any potential consequences for strontium-90 toxicity, which is focused on the bone and surrounding tissues (see Appendix E).

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

Due to the lack of data regarding toxicity of the mixture of jet fuels, hydrazines, trichloroethylene, arsenic, and strontium-90, a component-based approach is recommended to assess potential public health effects associated with exposure to this mixture. PBPK/PD models to predict dispositional and toxicological outcomes of joint action of these five components are not available, but the WOE approach can be used to evaluate the joint toxic action of the component pairs (ATSDR 2001a, 2001b).

The weight-of-evidence approach produces a qualitative binary weight-of-evidence (BINWOE) classification and associated score for the effect of each substance in the mixture on each other substance in the mixture. BINWOEs are based primarily on pairwise data regarding joint toxic action, but can also include inferences based on mechanistic understanding of the disposition and toxicity of the individual substances. Figure 1 shows the factors that contribute to a BINWOE classification and the associated scoring.

BINWOEs for the mixture of jet fuels, hydrazines, trichloroethylene, arsenic, and strontium-90 are shown in Tables 4–7. 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. See Section 1 and Appendices A–E for information on the critical and other sensitive endpoints of the individual mixture components. The BINWOEs focus on repeated simultaneous exposure, since this is the exposure scenario most relevant to evaluation of public health risk associated with exposure to these substances at a waste site.

Due to the scarcity of data available regarding joint toxic action of the component pairs for the jet fuels, hydrazines, trichloroethylene, arsenic, and strontium-90 mixture, and insufficient understanding of toxic and pharmacokinetic mechanisms of the individual substances, the type of joint toxic action could not be predicted for 17 of the 20 BINWOEs for this mixture. The only joint action that could be projected was for additive depression of the central nervous system from exposure to jet fuels and trichloroethylene (see Tables 4 and 5) and a greater-than-additive effect of strontium-90 on the general toxicity of arsenic by inhibition of methylation of the arsenic (see Table 6).

Although the BINWOEs were indeterminate for all of the remaining pairs due to insufficient data (see Table 7), the extensive overlap of toxic endpoints for the five mixture components suggests that there is a potential for joint toxic action among these substances. For example, similar effects on the liver are produced by jet fuels, hydrazines, and trichloroethylene. The possibility of joint toxic action on the central nervous system by jet fuels and trichloroethylene was recognized in the BINWOEs; in addition, the central nervous system is also affected by hydrazines and arsenic (although the peripheral nerves are a more sensitive target for this chemical). Immunosuppression is characteristic of four of the five mixture components (all but hydrazines, for which there is also some evidence of immune sensitivity), and similar hematological effects are produced by arsenic, strontium-90, and hydrazines. Renal effects, which are well-known for trichloroethylene, are also produced by arsenic. Strontium-90 and arsenic are known to be human carcinogens, while conclusive evidence has not yet established hydrazines and trichloroethylene as such. The genotoxicity of strontium-90 and hydrazines have also been demonstrated in laboratory studies, while the precise mechanisms of carcinogenicity in trichloroethylene and arsenic have not been fully elucidated.

Given this amount of overlap in toxic endpoints, it is reasonable to be cautious when evaluating public health concerns for this mixture by assuming additivity (dose additivity for noncancer effects and response additivity for cancer, as per ATSDR 2001a, 2001b).

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 mechanisms(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 2001a, 2001b

Table 4. Effect of **Jet Fuels** on **Trichloroethylene**

BINWOE: =IIIC (0)
neurological effects
BINWOE: ? (0)
hepatic effects
BINWOE: ? (0)
immunological effects

Direction of Interaction - Jet fuels and trichloroethylene are expected to produce additive effects on neurological endpoints. The direction of the interaction for hepatic and immunological effects cannot be predicted in the absence of (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with jet fuels will influence the toxicity of trichloroethylene; or (3) mechanistic understanding leading to an unambiguous projection of interactions between jet fuels and trichloroethylene.

Mechanistic Understanding - Jet fuels and trichloroethylene both produce neurological, hepatic, and immunological effects. Both jet fuels and trichloroethylene are believed to inhibit neuronal function by their physical presence in neuronal membranes, and as such, are expected to produce additive effects on the central nervous system. However, data directly corroborating this are not available; a rating of "III" was therefore assigned. Both jet fuels and trichloroethylene are believed to elicit hepatic and immunological effects as a result of metabolism to reactive products, possibly involving reactive oxidative species. However, understanding of these mechanisms is insufficient to reliably predict the influence, which might involve competitive inhibition and induction of various cytochrome P-450 isozymes, of exposure to jet fuels on the hepatic or immunological effects of trichloroethylene. Trichloroethylene is a probable human carcinogen (see Appendix C). No mechanistic information as to potential effects of jet fuels, which have not been demonstrated to be carcinogenic, on the carcinogenic effects of trichloroethylene was located.

Toxicologic Significance - The BINWOE for neurological effects contains a rating of "C" for toxicological significance because neither the joint toxic action nor any potentially related mechanistic changes have been demonstrated. Two cohort mortality studies (Ritz 1999; Spirtas et al. 1991) involving co-exposure to jet fuels and trichloroethylene have been reported. However, in both cases, high levels of co-exposure to other chemicals prevents a reliable determination of the potential joint toxic action of jet fuels and trichloroethylene. No other relevant interaction data on health effects following simultaneous exposure were located. No studies were located in which pretreatment with jet fuels prior to trichloroethylene exposure was examined.

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

Table 5. Effect of **Trichloroethylene** on **Jet Fuels**

BINWOE: =IIIC (0)
neurological effects
BINWOE: ? (0)
hepatic effects
BINWOE: ? (0)
immunological effects

Direction of Interaction - Jet fuels and trichloroethylene are expected to produce additive effects on neurological endpoints. The direction of the interaction for hepatic and immunological effects cannot be predicted in the absence of (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with trichloroethylene will influence the toxicity of jet fuels; or (3) mechanistic understanding leading to an unambiguous projection of interactions between trichloroethylene and jet fuels.

Mechanistic Understanding - Jet fuels and trichloroethylene both produce neurological, hepatic, and immunological effects. Both jet fuels and trichloroethylene are believed to inhibit neuronal function by their physical presence in neuronal membranes, and as such, are expected to produce additive effects on the central nervous system. However, data directly corroborating this are not available; a rating of "III" was therefore assigned. Both trichloroethylene and jet fuels are believed to elicit hepatic and immunological effects as a result of metabolism to reactive products, possibly involving reactive oxidative species. However, understanding of these mechanisms is insufficient to reliably predict the influence, which might involve competitive inhibition and induction of various cytochrome P-450 isozymes, of exposure to trichloroethylene on the hepatic or immunological effects of jet fuels.

Toxicologic Significance - The BINWOE for neurological effects contains a rating of "C" for toxicological significance because neither the joint toxic action nor any potentially related mechanistic changes have been demonstrated. Two cohort mortality studies (Ritz 1999; Spirtas et al. 1991) involving co-exposure to trichloroethylene and jet fuels have been reported. However, in both cases, high levels of co-exposure to other chemicals prevents a reliable determination of the potential joint toxic action of trichloroethylene and jet fuels. No other relevant interaction data on health effects following simultaneous exposure were located. No studies were located in which pretreatment with trichloroethylene prior to jet fuel exposure was examined.

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

Table 6. Effect of **Strontium-90** on **Arsenic**

BINWOE: >IIICb (+1 x 0.32 x 0.32 x 0.79 = +0.08)

Direction of Interaction - It is plausible that strontium-90 will increase the toxic effects of arsenic (>). There is evidence that strontium inhibits methylation of arsenic *in vitro* (De Kimpe et al. 1999). Because methylation of inorganic arsenic is generally considered to be a detoxification reaction (see caveat below), inhibition of methylation may reasonably be expected to produce a general increase in arsenic toxicity at targets throughout the body.

Mechanistic Understanding - Mechanistic understanding of the effect of strontium on arsenic is limited (III). Relevant data were from a single *in vitro* study (De Kimpe et al. 1999). The study was conducted in freshly-isolated liver cytosol from adult male Flemish Giant rabbits. This species has been shown to be an appropriate model for metabolism of arsenic in humans, and the liver is the primary site of arsenic methylation. While strontium was found to inhibit methylation of arsenic in this test system, so were many other inorganic ions and organic compounds. It was found that certain inorganic cations (most notably Zn^{2+}) stimulated methylation. The researchers presented some evidence to suggest that zinc may be an essential co-factor for As(III) methylation, and hypothesized that competitive inhibition between strontium (or other divalent cations) and zinc could be responsible for the observed inhibition of methylation in this test system. However, it is not clear that strontium would be present in the liver cell in sufficient quantities to have any effect in a complete organism. Although low concentrations of strontium can be found in soft tissues, approximately 99% of the total body burden is contained in the skeleton (see Appendix E). No studies have been done to investigate whether strontium would inhibit arsenic methylation in a whole animal model.

Toxicologic Significance - The toxicological significance of the interaction is not clear (C). Methylation of arsenic is generally considered a detoxification reaction because the methylation products, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), are less acutely toxic than inorganic arsenic, have a lower affinity for tissue constituents and proteins, and are excreted more rapidly (De Kimpe et al. 1999). However, MMA and DMA are not without toxic effects themselves. Studies of MMA and DMA have shown effects on the respiratory tissues, gastrointestinal tract, liver, kidney, reproduction, development, and genetic material (ATSDR 2000). There is some evidence that DMA is a cancer promoter (Yamamoto et al. 1995).

Modifying Factors - The only data available regarding the effect of strontium on arsenic toxicity are from an *in vitro* test system that may not be representative of *in vivo* exposure (b).

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

Table 7. Matrix of BINWOE Determinations for Simultaneous Exposure to Chemicals of Concern

		ON TOXICITY OF				
		Jet fuels	Hydrazines	Trichloroethylene	Arsenic	Strontium-90
EFFECT OF	Jet fuels		? (0)	**=IIIC (0)**[a] ? (0)[b]	? (0)	? (0)
	Hydrazines	? (0)		? (0)	? (0)	? (0)
	Trichloroethylene	**=IIIC (0)**[a] ? (0)[b]	? (0)		? (0)	? (0)
	Arsenic	? (0)	? (0)	? (0)		? (0)
	Strontium-90	? (0)	? (0)	? (0)	**>IIICb (+0.08)**	

[a] Neurological effects
[b] Effects on targets other than the nervous system

2.4 Recommendations for Data Needs

Neither *in vivo* data from human or animal studies nor *in vitro* data examining the toxicity of the 5-component mixture, or for 4- or 3-component submixtures, are available. Similarly, PBPK models describing the behavior of the 5-component mixture, or for 4- or 3-component submixtures, are not available. In the absence of direct interaction data, 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 toxicity data available for any of the 10 component pairs of the mixture. Data on the potential mechanistic interactions between the component pairs are also scarce.

For the individual components, oral MRL/RfDs are available only for arsenic and trichloroethylene. Jet fuels, hydrazines, and strontium-90 are all known to produce noncancer effects by oral exposure, so the lack of oral health guidance values for these materials is problematic. Inhalation MRL/RfCs are available for all three of the chemicals in the mixture for which this route of exposure is expected to potentially contribute to exposure of offsite receptors at rocket launch sites (jet fuels, hydrazines, and trichloroethylene), although a chronic value is not available for hydrazines. Oral slope factors and inhalation unit risks are available for all of the mixture components, except jet fuels, for which there is no evidence of carcinogenicity.