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 strontium, cobalt, cesium, PCBs, and trichloroethylene. 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 in humans or 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 Strontium and Cobalt

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between stable or radioactive strontium and cobalt compounds in affecting health-related endpoints in humans or animals. No PBPK models for co-exposure to strontium and cobalt were located.

Shared targets of toxicity following internal exposure to radiostrontium and exposure to radiocobalt compounds include hematological effects (alterations in erythrocyte number), reproductive effects, immunological effects, and an increased incidence of cancer.

Because calcium-related mechanisms have been demonstrated for chemical uptake and toxicity for stable forms of both strontium and cobalt, several *in vitro* studies have been performed using stable strontium and stable cobalt as regulators of calcium during excitation of cardiac (Mentrard et al. 1984) and neuronal

(Krnjevic et al. 1979a, 1979b; Mellow et al. 1978) cells. In isolated neuronal cells, stable cobalt (0.2 μmole) is capable of blocking stable strontium-mediated (1.0 μmole) neurotransmitter release, though sufficiently high stable strontium levels (3.5 μmole) can surmount the effect (Mellow et al. 1978). However, as relatively high levels of each compound were utilized, and neither compound presents with significant neurotoxicity following *in vivo* exposure, the relevance of this mechanism to potential toxic interactions between stable cobalt and stable strontium compounds is unclear.

No other *in vitro* or *in vivo* studies were located examining the possible modes of joint toxic action between stable or radioactive strontium and cobalt in producing hematological, immunological, developmental, reproductive, or cancer effects.

2.2.2 Strontium and Cesium

In 1948, the USSR's first weapons-grade plutonium separation facility was put in operation. Technological flaws and lack of expertise led to the radioactive contamination of large populations in the Ural Mountains. Over the period of 1949–1956, large discharges [~$3x10^6$ Curies (Ci)] of radionuclides, consisting primarily of strontium-90, cesium-137, zirconium, niobium (also known as columbium), and rubidium, were discharged into the water of the Techa river (Akleyev et al. 1995). In 1957, a thermo-chemical explosion of a waste tank released a large amount (~$2x10^7$ Ci) of radioactive material, primarily cesium-137 (66% of the total) into the air, again exposing a portion of this population to radiation doses sufficient to potentially cause health effects. In 1967, about 600 Ci of radioactivity, consisting entirely of strontium and cesium, were dispersed by the wind from the drying shores of a contaminated lake. Exposure analysis revealed that the exposures had the character of a combined exposure of external gamma-irradiation and internal irradiation from strontium-90 (^{90}Sr) and cesium-137 (^{137}Cs), occurring over a chronic duration. The exposed populations were compared to a reference group of people living nearby, but who were remote from the Techa river, and therefore were not exposed to the radioactive contamination. Estimated maximal dose levels for the exposed populations were 3-4 Sieverts (Sv) for the initial exposure, 0.9 Sv for the 1957 release, and 0.003 Sv for the 1967 release.

While it was impossible to accurately determine the early health effects due to these exposures, Akleyev et al. (1995) reported that 0.5–19% of the population of villages along the Techa river exposed to the first (1949-1956) radiation incident reported, upon examination 2–4 years following the incident, chronic radiation sickness (CRS), characterized by the following signs: hematologic syndrome (e.g., leukopenia, granulocytopenia, thrombocytopenia), neurological disturbances (e.g., asthenia, vegetative dysfunction,

microorganic central nervous system infection), ostealgia, immunity changes (e.g., inhibition of nonspecific immunity, autoallergy), and cardiovascular syndrome (e.g., tachycardia, hypotonia). Recovery from these symptoms depended on exposure rates, with greater exposure leading to longer recovery times; a complete recovery from the hematological and neurological functions occurred within 13–16 and 14–20 years following the beginning of the exposure, respectively. Immunity disorders persisted for 30 years and longer after the beginning of the exposure. The incidence of leukemia was increased in this population 5–20 years after the exposure. Significant increases in long-term morbidity and mortality, both general and cancer-specific, were also observed (Akleyev et al. 1995; Kossenko et al. 2000).

The incidence of spontaneous abortions, stillbirths, and ectopic pregnancies was not different among exposed women, relative to the control population, nor did exposure induce a significant effect on birth rate or on cancer mortality in the offspring (Akleyev et al. 1995; Kossenko et al. 1994). However, subjects who were initially exposed *in utero* or at the age of 1–2 years showed the greatest changes in immune system parameters (e.g., inhibition of nonspecific immunity and autoallergy).

No other *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between stable or radioactive strontium and cesium in affecting health-related endpoints in humans or animals. Studies of the Techa River population exposed to radiation from radioactive strontium and cesium identified shared targets of toxicity for co-exposure to strontium and cesium radionuclides as effects on the bone marrow (bone marrow depression), both acute radiation sickness (ARS, described in ATSDR 1999) and chronic radiation sickness outlined above, and increased likelihood of cancer, especially leukemia. However, no information was provided regarding the potential modes of joint action between strontium and cesium in producing these effects.

2.2.3 Strontium and Trichloroethylene

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between stable or radioactive strontium and trichloroethylene in affecting health-related endpoints in humans or animals. No studies were located in which treatment with stable or radioactive strontium before trichloroethylene exposure or treatment with trichloroethylene before strontium exposure was examined. The primary shared target of toxicity of strontium radionuclides and trichloroethylene following oral exposure is the immune system (decreased immune response) (see Appendices A and D). The potential mode(s) of joint action of strontium and trichloroethylene on the immune system cannot be reliably predicted, owing

primarily to a lack of understanding of the mode(s) of action of trichloroethylene-induced immunological effects. No other shared targets of toxicity for strontium and trichloroethylene were identified. Trichloroethylene has been shown in several animal studies to result in an increase in tumor incidence, but the target organ(s) have not been consistent across studies, and the effects have generally been seen only at levels that also caused increased mortality (Appendix D). Additional data will therefore be required to assess any potential joint toxic action between strontium and trichloroethylene on carcinogenic endpoints.

Exposures to either radiostrontium or trichloroethylene have been shown to result in a decreased immune response in animal studies (Appendices A and D), but the mechanisms of trichloroethylene-induced effects are not sufficiently well-studied to allow for reliable mechanistic inferences as to possible joint toxic actions of trichloroethylene and radiostrontium. Other effects of radiostrontium (musculoskeletal and hematological effects and cancer) have not been demonstrated as sensitive targets of trichloroethylene, and plausible modes of joint action on these strontium targets are not obvious (see Appendices A and D). Similarly, other effects of trichloroethylene (neurological, hepatic, and renal effects, see Appendix D) are not believed to be sensitive targets of radiostrontium (see Appendix A). No data were located to indicate how exposure to radiostrontium might influence neurological effects from trichloroethylene itself or hepatic and/or renal effects involving metabolites of trichloroethylene. Information on the carcinogenic effects of trichloroethylene is inconclusive, with conflicting data on critical organs and concentrations in available studies (Appendix D).

2.2.4 Strontium and PCBs

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between strontium radionuclides and PCBs in affecting health-related endpoints in humans or animals. No studies were located in which treatment with strontium before PCB exposure or treatment with PCBs before strontium exposure was examined. Potential shared targets of toxicity for strontium and PCBs include effects on the immune system (see Appendices A and E). Both strontium radionuclides and PCBs have been shown to cause cancer; however, the primary target organs of carcinogenesis for the two are not the same (bone, bone marrow, and leukemia for strontium; liver and thyroid for PCBs).

While both PCBs and radiostrontium have been shown to cause reductions in the immune response, possible differences in mechanism between the two and a lack of joint action data prevent reliable mechanistic inferences as to the effect of radiostrontium on PCB-induced immunotoxicity. Exposure to PCBs has been shown to have effects on neurodevelopment (see Appendix E), though the precise

mechanisms of these effects (i.e., Ah-receptor-mediated or not) have not been fully elucidated. As discussed above (see Tables 3 and 5), studies of radiostrontium have not shown effects on neuro-development, though it is possible that radiation from strontium that crosses the placenta may be able to influence the development of the fetus. Due to incomplete understanding of the potential mechanisms of developmental effects of strontium and PCBs, no reliable prediction of mode of joint actions can be made. While radiostrontium has been shown to cause a profound reduction in circulating erythrocytes, effects on erythrocyte number is not a sensitive endpoint for PCBs; in the absence of experimental data, therefore, no inference can be made regarding the potential action of PCBs on the hematological effects of radio-strontium. Perturbation of calcium-based mechanisms may be responsible for some of the effects of PCBs, but the potential effect of strontium, which substitutes for calcium in many physiologic processes, on these mechanisms cannot be reliably determined. Similarly, potential effects of PCBs on the actions of strontium cannot be determined from the available data. Radiostrontium acts as a genotoxic carcinogen, with ionization events leading to eventual cellular transformation. PCBs are capable of acting as tumor promoters, as well as complete carcinogens, and as such, it is possible that they may have an effect on radiostrontium carcinogenesis. However, available data are inadequate to assess whether this potential joint action would be additive, greater-than-additive, or less-than-additive.

2.2.5 Cobalt and Cesium

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between cobalt and cesium radionuclides in affecting health-related endpoints in humans or animals.

No data were located identifying sensitive shared targets specifically for oral exposures to radiocobalt and radiocesium compounds; however, data on effects of either radionuclide following oral exposure are lacking, perhaps due to the poor oral absorption of cobalt and rapid elimination (effective clearance half-life of 10 days for cobalt and 70 days for cesium) of the two radionuclides. Because both cobalt and cesium distribute uniformly throughout the body and both radionuclides emit beta and gamma radiation, sensitive shared targets of exposure to radioactive cobalt and radioactive cesium are expected to be similar, if not identical. Sensitive shared targets for exposure to cobalt and cesium radiations include effects on the developing organism (altered neurodevelopment, skeletal defects), radiation sickness (at very high doses), and cancer (see Appendices B and C). The database for effects of high-dose external cobalt radiation is considerably more extensive than that for radiation from radiocesium sources, but

given the similar character of the radiations, the effects of exposure to the two radionuclides would be expected to be similar.

2.2.6 Cobalt and Trichloroethylene

Allemand et al. (1978) pretreated groups of rats with subcutaneous injections of 30 mg/kg of stable cobalt chloride ($CoCl_2$) twice daily for 3 days. Twelve hours after the final pretreatment injection, animals were given an intraperitoneal injection of 1 mL/kg of trichloroethylene, and then were sacrificed at 3, 6, 9, or 12 hours post-injection. Animals pretreated with $CoCl_2$ showed significantly lower levels of serum glutamic pyruvic transaminase levels, measured as a marker for trichloroethylene hepatotoxicity, than controls. The study authors suggested that the cobalt pretreatment may have reduced the levels of cytochrome P450 enzymes, resulting in reduced metabolism of trichloroethylene to an active metabolite; however, P450 levels were not directly measured following cobalt pretreatment. No further mechanistic studies of potential interactions between cobalt and trichloroethylene were located.

No other *in vitro* or *in vivo* studies were located regarding possible joint toxic actions between stable or radioactive cobalt and trichloroethylene in affecting health-related endpoints in humans or animals. Shared targets following exposure to stable or radioactive cobalt and trichloroethylene toxicity are limited to effects on immunological endpoints (see Appendices B and D). While radiocobalt exposure may result in increased cancer incidence (Appendix B), the available data on the carcinogenic effects of trichloroethylene are inconclusive (Appendix D).

Exposures to either trichloroethylene or cobalt radiation have been shown to result in a decreased immune response in animal studies, but the effects for trichloroethylene are not well-studied (see Appendix D). Immunotoxicity from gamma and beta radiation from radiocobalt compounds is expected to involve early ionizing events that lead to toxic effects on cells of the immune system (Appendix B). Available mechanistic information is insufficient to reliably project whether or not trichloroethylene may influence radiocobalt immunotoxicity, or whether radiocobalt may influence trichloroethylene immunotoxicity. Other sensitive effects of trichloroethylene (neurological, hepatic, and renal effects; see Appendix D) are not believed to be sensitive targets of cobalt or cobalt radiation. No data were located to indicate how exposure to radiocobalt might influence neurological effects from trichloroethylene itself or hepatic and/or renal effects from metabolites of trichloroethylene. Allemand et al. (1978) noted that in rats pretreated with stable cobalt chloride, a decrease in markers for trichloroethylene hepatotoxicity was seen. The study authors suggested that the cobalt pretreatment may have reduced the levels of cytochrome P450

enzymes, resulting in reduced metabolism of trichloroethylene to active metabolites. However, in the absence of studies involving the possible influence of cobalt co-exposure on P450-mediated trichloroethylene metabolism and/or toxicity, reliable projections of how co-exposure to radiocobalt will affect trichloroethylene toxicity cannot be made. Similarly, no data were located to indicate how exposure to trichloroethylene might influence reproductive and neurodevelopmental effects from radiocobalt. Exposure to ionizing radiation from radioactive cobalt is expected to increase risk for development of cancer, but information on the carcinogenicity of trichloroethylene is inconclusive, with conflicting data on critical organs and concentrations in available studies (Appendix D). No mechanistic information as to the potential effects of trichloroethylene on the carcinogenic effects of radiocobalt was located. Thus, it is uncertain whether cancer is a common health hazard from trichloroethylene and radiocobalt.

2.2.7 Cobalt and PCBs

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions of cobalt radionuclides and PCBs in humans or animals. Shared targets of cobalt radionuclides and PCBs include impaired reproductive performance and altered neurodevelopment (see Appendices B and E).

Exposure to PCBs has been shown to have effects on neurodevelopment (see Appendix E), though the precise mechanism of these effects (i.e., Ah-receptor-mediated or not) has not been fully elucidated. Radiation from cobalt isotopes has also been shown to have profound effects on neurodevelopment (see Appendix B), presumably through ionization events resulting in germ cell alteration. However, available data are not adequate to reliably predict the potential joint toxic actions of radiocobalt and PCB coexposure on neurodevelopmental endpoints. Similarly, while both radiocobalt compounds and PCBs can cause decreases in immune function (and immune cells), differing mechanisms between the two and a lack of joint action data prevent reliable mechanistic inferences as to possible interactions. Both stable cobalt and radiation from cobalt isotopes can have a detrimental effect on the testes, resulting in decreased reproductive ability (see Appendix B). While PCBs also cause a decrease in reproductive ability, it is believed that the female, rather than the male as for cobalt-related reproductive effects, is more sensitive to PCB-induced changes in reproductive ability. The potential interaction(s) between radiocobalt and PCBs on reproductive endpoints cannot be predicted due to lack of data. Calcium-based mechanisms may be responsible for some of the effects of PCBs, but the potential effect of cobalt ions, which can function as a calcium channel blocker, on these mechanisms cannot be reliably determined. While both cobalt radiation and exposure to PCBs can cause an increased incidence of cancer,

understanding of the mechanism(s) of these processes is insufficient to allow for predictions of possible joint effects on carcinogenic endpoints.

2.2.8 Cesium and Trichloroethylene

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions of cesium radionuclides and trichloroethylene in humans or animals. Shared targets following exposure to cesium and trichloroethylene toxicity are restricted to effects on immunological endpoints (Appendices C and D). While radiocesium exposure may result in increased cancer incidence (Appendix C), the available data on the carcinogenic effects of trichloroethylene are inconclusive (Appendix D).

Exposures to either trichloroethylene or cesium radiation have been shown to result in a decreased immune response in animal studies, but the effects for trichloroethylene are not well-studied (see Appendix D). Mechanisms of trichloroethylene-induced immunotoxic effects are not sufficiently understood to allow for reliable mechanistic inferences of potential joint toxic actions of radiocesium and trichloroethylene on immune system endpoints. Other sensitive effects of trichloroethylene (neurological, hepatic, and renal effects, see Appendix D) are not believed to be sensitive targets of cesium radiation. No data were located to indicate how exposure to radiocesium might influence neurological effects from trichloroethylene itself or hepatic and/or renal effects from metabolites of trichloroethylene. Similarly, no data were located to indicate how exposure to trichloroethylene might influence reproductive and neurodevelopmental effects from radiocesium. Exposure to ionizing radiation from cesium is expected to increase risk for development of cancer, but information on the carcinogenicity of trichloroethylene is inconclusive, with conflicting data on critical organs and concentrations in available studies (Appendix D). No mechanistic information as to potential effects of trichloroethylene on the carcinogenic effects of radiocesium was located. Thus, it is uncertain whether cancer is a common health hazard from trichloroethylene and radiocesium.

2.2.9 Cesium and PCBs

No *in vitro* or *in vivo* studies were located regarding possible joint toxic actions of cesium radionuclides and PCBs in humans or animals. Sensitive shared targets of cesium radionuclides and PCBs include impaired reproductive performance and altered neurodevelopment (Appendices C and E).

Although radioactive cesium compounds and PCBs have been shown to have effects on reproductive endpoints, radiocesium exerts its chemical effects mainly on the male animal (testicular effects), while the data suggest that PCBs have greater effects in females (decreased fertility and embryonic death). Both cesium and PCBs have been shown to cause increases in cancer incidence, but the most sensitive target organs for carcinogenic effects differ for the two (Appendices C and E).

Exposure to PCBs has been shown to have effects on neurodevelopment (see Appendix E), though the precise mechanism of these effects (i.e., Ah-receptor-mediated or not) has not been fully elucidated. Radiation from cesium isotopes has also been shown to have effects on neurodevelopment (see Appendix C), presumably through ionization events resulting in germ cell alteration. However, available data are not adequate to reliably predict the effect of coexposure to radiocesium and PCB on developmental changes. Similarly, while both radiocesium compounds and PCBs can cause decreases in immune function (and immune cells), differing mechanisms between the two and a lack of joint action data prevent reliable mechanistic inferences as to possible joint toxic actions. Radiation from cesium isotopes can have a detrimental effect on the testes, resulting in decreased reproductive ability. While PCBs also cause a decrease in reproductive ability, it is believed that the female, rather than the male as for cesium-related reproductive effects, is more sensitive to PCB-induced changes in reproductive ability. Potential interaction(s) between radiocesium and PCBs on reproductive endpoints cannot be predicted due to lack of data. While both cesium radiation and exposure to PCBs may cause an increased incidence of cancer, understanding of the mechanism(s) of these processes is insufficient to allow for predictions of possible joint effects carcinogenic endpoints.

2.2.10 Trichloroethylene and PCBs

Greenland et al. (1994) examined a cohort of workers from a manufacturing plant that was exposed to trichloroethylene and Pyranol (a mixture of 45–80% PCBs and trichlorobenzene), as well as other chemicals, including benzene, asbestos, and mineral oils, for increases in cancer mortality. Logistic regression estimates detected an increased odds ratio for lymphomas associated with Pyranol exposure, but no changes in odds ratios associated with trichloroethylene exposure.

Moslen et al. (1977) pretreated male rats with 150 μmoles/kg (~49 mg/kg) of Aroclor 1254 (a mixture of PCB congeners) by gavage daily for 7 days. Animals were then anesthetized with airborne 1% trichloroethylene for 2 hours, and allowed to recover. Animals were sacrificed, and the histology of the liver, along with hepatic enzyme and metal levels, examined. Trichloroethylene anesthesia in rats that were not

pretreated resulted in a mean anesthesia recovery time of 81 minutes, with no effect on liver enzyme levels, metal levels, or histopathology. By contrast, pretreatment with Aroclor 1254 resulted in a mean anesthesia recovery time of 244 minutes, with significant increases in trichlorinated urinary metabolites, hepatic serum glutamic-oxaloacetic transaminase (SGOT) and cytochrome P450 activities, and hepatic sodium, potassium, and calcium levels. Anesthesia recovery time was positively correlated with mean SGOT levels. Animals pretreated with Aroclor 1254 showed necrotic bands of pyknotic hepatocytes, with calcium-rich necrotic cells in corresponding regions. No examination of effects of Aroclor 1254 alone was reported, thus limiting the ability of the study to describe whether the effects of Aroclor 1254 on trichloroethylene-induced hepatic and neurologic effects occurred in an additive, less-than-additive, or greater-than-additive manner. However, Carlson (1975) did not report hepatic toxicity as a result of exposure to 25 mg/kg Aroclor 1254 for 6 days in male rats. Thus, it appears that the effect of PCB pretreatment on trichloroethylene toxicity is greater than additive. The study authors proposed that the increase in cytochrome P450 activity induced by Aroclor 1254 was responsible for the increased hepatotoxicity and increased neurotoxicity of trichloroethylene in this study.

No further *in vitro* or *in vivo* studies were located regarding possible joint toxic actions of trichloroethylene and PCBs in humans or animals. Shared targets of trichloroethylene and PCBs include neurological and hepatic effects (see Appendices D and E).

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

Mixtures containing strontium, cobalt, cesium, trichloroethylene, and PCBs may be found together at hazardous waste sites, most notably those located at present or former Department of Energy (DOE) facilities. No studies examining a five-component mixture of these compounds were located, nor were studies of three- or four-component mixtures available in the literature. No PBPK models are available for the complete mixture, or for any of the three- or four-component submixtures.

In the absence of studies that examine relevant endpoints and describe dose-response relationships following oral exposures to mixtures that contain these five 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 (e.g., the hazard index approach or the target-organ toxicity dose modification of the hazard index approach). Likewise, a component-based approach assuming response additivity appears reasonable for assessment of cancer risks from oral exposure to mixtures of these five chemicals.

It is recommended that these approaches treat mixtures of PCB congeners (i.e., total PCBs) as a single component of concern. This approach is consistent with ATSDR's approaches to deriving oral MRLs for PCBs, which are based on data linking health effects with exposure to PCB mixtures (Appendix E; ATSDR 2000). The profile does not focus on a representative PCB congener (or congeners) or subclasses of PCBs to discuss interactions with the other components of the subject mixture, because it is likely that: (1) multiple mechanisms are involved in PCB-induced health effects; (2) different PCB congeners may produce effects by different and multiple mechanisms; and (3) humans are exposed to complex mixtures of PCB congeners with differing biological activities.

In the introduction to this document, Table 1 presented an overview of the potential effects of concern from oral exposure to strontium, cobalt, cesium, trichloroethylene, and PCBs. Each of the five compounds affects a variety of target organs and endpoints. There are a number of target organs in common across two or more of the components of the mixture. As shown in Table 2, however, the bases for oral MRLs for trichloroethylene and PCBs are different, and oral MRLs for radiostrontium, radio-cobalt, and radiocesium compounds have not been derived. Available data on possible binary interactions among these five 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 quaternary or ternary mixtures, or for any of the binary component pairs of the mixture. Tables 3 through 10 describe binary weight-of-evidence (BINWOE) evaluations for the pairs of the five chemicals of concern using the classification scheme summarized in Figure 1 and in ATSDR (2001a). 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 interactions between the following pairs of chemicals:

- Strontium and trichloroethylene;
- Strontium and PCBs;
- Cobalt and PCBs;

- Cesium and trichloroethylene; and
- Cesium and PCBs

Lack of interaction data, conflicting interaction data, and/or incomplete understanding of mechanisms of action also preclude projecting interactions for cobalt and trichloroethylene.

Table 2. Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern (see Appendices A, B, C, D, and E for More Details)

Duration of Exposure	Radiostrontium	Radiocobalt	Radiocesium	Trichloro-ethylene	PCBs
Acute	None derived, inadequate data	None derived, inadequate data	None derived, inadequate data	Neurobehavioral changes in young mice	None derived, inadequate data
Inter-mediate	None derived, inadequate data[a]	None derived, inadequate data[b]	None derived, inadequate data	None derived, inadequate data	Neuro-developmental changes in monkey offspring
Chronic	None derived, inadequate data	None derived, inadequate data	None derived, inadequate data	None derived, inadequate data	Immuno-suppression in monkeys

[a]An oral MRL for stable (nonradioactive) strontium was derived based on musculoskeletal effects.
[b]An oral MRL for stable (nonradioactive) cobalt was derived based on hematological effects.

Evidence of varying quality and quantity is available supporting projections of additive joint action (or no interactive effect) for the following:

- Strontium and cobalt (Tables 3 and 4);
- Strontium and cesium (Tables 5 and 6);
- Cobalt and cesium (Tables 7 and 8); and
- PCBs and Trichloroethylene (Tables 9 and 10)

In summary, evidence for greater-than-additive joint action was available only for the effects of PCBs on trichloroethylene; these effects were noted at high (~49 mg/kg/day) exposures to PCBs. 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. For the pairs of radionuclides, available mechanistic data

suggest additive joint action at shared targets of toxicity, while for two pairs (PCBs and cobalt and PCBs and cesium) additive joint action at shared targets is recommended as a public health protective assumption due to lack of joint toxic interaction data, and lack of mechanistic understanding to reliably project potential non-additive interactions.

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 3. Effect of **Strontium** on **Cobalt**

BINWOE: =IIC (0)
reproductive effects
BINWOE: =IIC (0)
immunological effects
BINWOE: =IIC (0)
neurodevelopmental effects
BINWOE: =IIC (0)
cancer

Direction of Interaction - The joint action may be additive for reproductive, immunological, and neurodevelopmental effects and for cancer based on similar mechanisms of action between the radiations emitted by strontium and cobalt radionuclides.

Mechanistic Understanding - Cobalt radionuclides emit both beta and gamma radiation, while strontium radionuclides emit primarily beta radiation. Ionization events from both types of radiation are thought to be key precursor events in the development of effects from exposure to strontium or cobalt radiations (ATSDR 1999, 2001d, 2001e). Cobalt is distributed throughout the body with a comparatively short (<10 days) effective clearance half-life, while strontium preferentially accumulates in bone with a long (18 years) effective clearance half-life. As strontium localizes in bone and beta radiation is not highly penetrating, radiation from strontium radionuclides are expected to add to the effects of radiation from cobalt only when the effect of concern is adjacent to strontium-containing tissues. Because gamma rays are highly penetrating, radiation from cobalt isotopes can affect the fetus, as has been demonstrated in a number of animal studies (ATSDR 2001d). Radiation from cobalt sources has also been shown to result in reduced fertility due to damaging action to the testes, while animal studies have demonstrated that stable cobalt can also have detrimental effects on the testes. While it is possible that strontium radiation will add to the reproductive and/or neurodevelopmental effects of cobalt, it could only do so if it were localized very near to the testes (for reproductive effects) or the fetus (developmental effects), owing to the short path length of beta radiation. Available data for strontium do not suggest that significant exposure to strontium radiation occurs to the testes following oral exposure (ATSDR 2001e). Strontium is capable of crossing the placenta; however, developmental effects from maternal radiostrontium, when seen at all, manifest as reduced fertility and increased carcinogenesis in the offspring (ATSDR 2001e), whereas following exposure to cobalt radiation neurodevelopmental alterations are the most sensitive endpoint. A confidence rating of "II" was assigned for mechanistic understanding due to the dispositional restrictions on strontium radiation adding to cobalt radiation to produce effects on the reproductive and immune systems, as well as on neurodevelopment. The mechanisms of carcinogenesis are expected to be similar between the two radiations (ionization events leading to cellular transformation), though as with the toxic effects, the key events will have to occur in sites very near the radiostrontium compounds (i.e., the bone) in order for strontium radiation to add to the effects of cobalt radiation. A confidence rating of "II" for mechanistic understanding was therefore assigned for carcinogenic effects.

Toxicological Significance - Relevant joint toxic action data on pertinent health effects from simultaneous oral exposure were not located. No studies were located in which treatment with strontium before cobalt exposure was examined. A confidence rating of "C" for toxicological significance was assigned for all sensitive targets based on dispositional restrictions on strontium radiation adding to cobalt radiation, discussed above.

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

Table 4. Effect of **Cobalt** on **Strontium**

BINWOE: ? (0)
hematological effects
BINWOE: =IIC (0)
immunological effects
BINWOE: =IIC (0)
cancer

Direction of Interaction - The joint action may be additive for immunological effects and cancer based on similar mechanisms of action between the radiations emitted by cobalt and strontium radionuclides. The direction of interaction for hematological effects cannot be predicted from the available data.

Mechanistic Understanding - One mechanism by which stable cobalt may elicit its effects is through its actions as a calcium channel blocker. As the disposition of strontium within the body is determined by its ability to substitute for calcium, cobalt-induced calcium blockade might alter the uptake or distribution of strontium compounds. No studies were located that examined this potential mechanism *in vivo*, though one *in vitro* study in isolated neurons showed that cobalt is capable of competitively blocking strontium-induced action potentials. Stable cobalt has been shown to increase the production of red blood cells, while cobalt radiation and strontium radiation both result in a reduction in erythrocyte counts. The net effect of the two radionuclides on the hematologic system is unknown. Cobalt radionuclides emit both beta and gamma radiation, while strontium radionuclides emit primarily beta radiation. Gamma radiation is highly penetrating, and thus radiation from systemic or external cobalt may result in exposure of sensitive targets of strontium radiation (i.e., bone and bone marrow). Cobalt is distributed throughout the body with a comparatively short (<10 days) effective clearance half-life, while strontium preferentially accumulates in bone with a long (18 years) effective clearance half-life. Radiostrontium has been shown to result in decreased numbers of lymphocytes, and studies in humans have shown a decrease in circulating lymphocytes following long-term, low-level external exposure to cobalt radiation. Ionization leading to cell death are expected to be similar for both strontium and cobalt radiations; it is plausible that additive joint action may occur on the immune system components, but lack of data limits confidence to a rating of “II”. Likewise, the mechanisms of carcinogenic effects for strontium and cobalt radiations (ionization events eventually leading to cellular transformation) are expected to be similar, also warranting a rating of “II”.

Toxicological Significance - Relevant joint toxic action data on pertinent health effects with simultaneous oral exposure were not located. No studies were located in which treatment with cobalt before strontium exposure was examined. Accordingly, a confidence rating of “C” for toxicological significance was assigned for all sensitive targets, including cancer.

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

Table 5. Effect of **Strontium** on **Cesium**

BINWOE: =IIB (0)
immunological effects
BINWOE: =IIC (0)
neurodevelopmental effects
BINWOE: =IIC (0)
reproductive effects
BINWOE: =IIB(0)
cancer

Direction of Interaction - The joint action may be additive for reproductive, immunological, and neurodevelopmental effects and for cancer based on similar mechanisms of action between the radiations emitted by cesium and strontium radionuclides and a co-exposure study in humans (Akleyev et al. 1995).

Mechanistic Understanding - Cesium radionuclides emit both beta and gamma radiation, while strontium radionuclides emit primarily beta radiation. Ionization events from both types of radiation are believed to be key precursors to development of effects from either radionuclide. Cesium is distributed throughout the body with a moderate (~70 days, though it may vary with age, with younger populations having faster clearance) effective clearance half-life, while strontium preferentially accumulates in bone with a long (~18 years) effective clearance half-life. As strontium localizes in bone and beta radiation is not highly penetrating, radiation from strontium radionuclides would be expected to influence the effects of cesium radionuclides only when the effect of concern is adjacent to strontium-containing tissues. Radiostrontium exposure has been shown to result in decreased numbers of lymphocytes, and a study in humans showed a decrease in circulating neutrophils, presumed to be due to bone marrow irradiation (ATSDR 2001c). The mechanisms of these effects (ionization events leading to cell death) are expected to be similar for both strontium and cesium radiations. Because gamma rays are highly penetrating, radiation from cesium isotopes can affect the fetus, as has been demonstrated in a number of animal studies (ATSDR 2001c). Cesium radiation has also been shown to result in reduced fertility due to damaging action to the testes. While it is possible that strontium radiation will add to the reproductive and neurodevelopmental effects of cesium, it could only do so if it were localized very near to the testes (for reproductive effects) or the fetus (developmental effects), owing to the short path length of beta radiation. Available data for strontium do not suggest that significant exposure to strontium radiation occurs to the testes following oral exposure (ATSDR 2001e). Strontium is capable of crossing the placenta; however, developmental effects from maternal radiostrontium, when seen at all, manifest as reduced fertility and increased carcinogenesis in the offspring, whereas following exposure to cesium radiation neurodevelopmental alterations are the most sensitive endpoint. This dispositional property of strontium limits the confidence that additive joint action will occur between strontium and cesium; therefore, a rating of “II” for mechanistic understanding was selected. The mechanisms of carcinogenesis are expected to be similar between the two radiations (ionization events leading to cellular transformation), though as with the toxic effects, the key events will have to occur in sites very near the radiostrontium compounds (i.e., the bone) in order for strontium radiation to add to the effects of cesium radiation. A rating of “II” was selected for mechanistic understanding of additive effects of strontium on the carcinogenic effects of cesium.

Table 5. Effects of **Strontium** on **Cesium** *(continued)*

BINWOE: =IIB (0)
immunological effects
BINWOE: =IIC (0)
neurodevelopmental effects
BINWOE: =IIC (0)
reproductive effects
BINWOE: =IIB(0)
cancer

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located and no studies were located in which treatment with strontium before cesium exposure was examined. Endpoints of concern have been identified based on studies of exposure to radiocesium, and a co-exposure study in humans (Akleyev et al. 1995), which identified changes in immunological effects and increased cancer incidence. A rating of "B" was therefore assigned for these endpoints, while "C" ratings were assigned for endpoints not affected or not described in the study (neurodevelopment, reproductive effects).

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

Table 6. Effect of **Cesium** on **Strontium**

BINWOE:=IIB (0)
hematological effects
BINWOE: =IIB (0)
immunological effects
BINWOE: =IIB(0)
cancer

Direction of Interaction - The joint action may be additive for immunological effects and cancer, based on similar mechanisms of action between the radiations emitted by cesium and strontium radionuclides.

Mechanistic Understanding - Cesium radionuclides emit both beta and gamma radiation, while strontium radionuclides emit primarily beta radiation. Ionization events from both types of radiation are expected to be key precursor events to development of effects from either radionuclide. Gamma radiation is highly penetrating, and thus radiation from systemic or external cesium may result in exposure of sensitive targets of strontium radiation (i.e., blood cell progenitor cells). Cesium is distributed throughout the body with a moderate effective half-life (<70 days), while strontium preferentially accumulates in bone with a long (18 years) effective half-life. Strontium radiation has been shown to cause a reduction in circulating red blood cells (ATSDR 2001e). While no data are available for this effect for cesium radiation directly, the radiations from other gamma emitting compounds (i.e., cobalt) result in decreased red blood cell numbers systemically (ATSDR 1999, 2001d). Hematological effects were also noted in the Techa River population, which was co-exposed to radiocesium and radiostrontium (Akleyev et al. 1995). The mechanisms of these effects (ionization events leading to cell death) are expected to be similar for both strontium and cesium radiations, and are expected to result in additive joint action. Accordingly, a rating of "II" was assigned for the confidence in additivity for both hematological and immunological effects. Similarly, the mechanisms of carcinogenic effects for strontium and cesium radiations (ionization events eventually leading to cellular transformation) are expected to be similar. A rating of "II" was therefore assigned.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located and no studies were located in which treatment with cesium before strontium exposure was examined. Endpoints of concern have been identified based on studies of exposure to radiostrontium and radiation from cesium sources, and a co-exposure study in humans (Akleyev et al. 1995). Accordingly, a rating of "B" was assigned for toxicological significance, owing to the report of these effects in an human population co-exposed to radiocesium and radiostrontium.

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

Table 7. Effect of **Cobalt** on **Cesium**

BINWOE: =IIB (0)
reproductive effects
BINWOE: =IIB (0)
immunological effects
BINWOE: =IIB (0)
neurodevelopmental effects
BINWOE: =IIB (0)
cancer

Direction of Interaction - The joint action may be additive for all radiation-induced health effects, including cancer, based on similar characteristics, and presumably mechanisms of action, between the radiations emitted by cobalt and cesium radionuclides.

Mechanistic Understanding - While both cobalt and cesium may elicit effects based on nonradioactive mechanisms, the most sensitive effects following oral exposure are likely to be from emission of radiation from these radionuclides (Appendices B and C). Cobalt is distributed throughout the body with a comparatively short (<10 days) effective clearance half-life, and cesium is distributed throughout the body with a moderate effective clearance half-life (~70 days, though it may vary with age, with younger populations having faster clearance). Both cobalt and cesium emit beta and gamma radiations, the latter of which is expected to be primarily associated with the health effects induced by these radionuclides, mainly due to its high penetrating ability relative to beta radiation (Appendices B and C, ATSDR 1999). The mechanisms of action of the radiation from these two radionuclides (ionization events leading to cellular damage) is therefore expected to be very similar. Thus, for reproductive effects (testicular degeneration), immunological effects (reduced numbers of circulating immune cells), and neurodevelopmental effects (altered neurobehavioral parameters after *in utero* exposure), the joint action is expected to be additive based on identical key precursor events. However, data directly examining this potential additivity are not available. A rating of "II" for mechanistic understanding for noncancer effects of cobalt on cesium was assigned. Similarly, carcinogenic effects of both cobalt and cesium radiations are believed to result from ionization of key cellular targets, which eventually leads to cellular transformation. A rating of "II" was therefore utilized for carcinogenic effects as well.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. No studies were located in which treatment with cobalt before cesium exposure was examined. However, due to the similar character of the emissions from radioactive forms of both cobalt and cesium, a rating of "B" was assigned for toxicological significance.

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

Table 8. Effect of **Cesium** on **Cobalt**

BINWOE: =IIB (0)
reproductive effects
BINWOE: =IIB (0)
immunological effects
BINWOE: =IIB (0)
neurodevelopmental effects
BINWOE: =IIB (0)
cancer

Direction of Interaction - The joint action may be additive for all radiation-induced health effects, including cancer, based on similar characteristics, and presumably mechanisms of action, between the radiations emitted by cesium and cobalt radionuclides.

Mechanistic Understanding - While both cesium and cobalt may elicit effects based on nonradioactive mechanisms, the most sensitive effects following oral exposure are likely to be from emission of radiation from these radionuclides (see Appendices B and C). Cobalt is distributed throughout the body with a comparatively short effective clearance half-life (<10 days), while cesium is distributed throughout the body with a moderate effective clearance half-life (~70 days, though it may vary with age, with younger populations having faster clearance). Both cobalt and cesium emit beta and gamma radiations, the latter of which is expected to be associated with the health effects induced by these radionuclides (see Appendices B and C). The mechanisms of action of the radiation from these two radionuclides (ionization events leading to cellular damage) is therefore expected to be very similar. Thus, for reproductive effects (testicular degeneration), immunological effects (reduced numbers of circulating immune cells), and neurodevelopmental effects (altered neurobehavioral parameters after *in utero* exposure), the joint action is expected to be additive based on identical key precursor events. However, data directly examining this potential additivity are not available. A rating of "II" was therefore assigned for mechanistic understanding for noncancer effects of cesium on cobalt. Similarly, carcinogenic effects of both cobalt and cesium radiations are believed to result from ionization of key cellular targets, which eventually leads to cellular transformation. A rating of "II" was therefore utilized for carcinogenic effects as well.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. No studies were located in which treatment with cesium before cobalt exposure was examined. However, due to the similar character of the emissions from radioactive forms of both cesium and cobalt, a rating of "B" was assigned for toxicological significance.

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

Table 9. Effect of **PCBs** on **Trichloroethylene**

BINWOE: >IIB2 (0.40)
for hepatic effects
BINWOE: >IIB2 (0.40)
for neurological effects

Direction of Interaction - Based on data presented in Moslen et al. (1977) and on mechanistic understanding of the actions of PCBs and trichloroethylene, a greater-than-additive effect of PCBs on trichloroethylene toxicity is predicted.

Mechanistic Understanding - An important step in trichloroethylene hepatotoxicity is metabolic activation by cytochrome P450 enzymes to a reactive intermediate. PCB exposure has been demonstrated to increase hepatic P450 activity, and pretreatment with PCBs has been shown to result in increased levels of urinary trichloroethylene metabolites. Additionally, a greater-than-additive effect of PCBs on trichloroethylene toxicity has been demonstrated by Moslen et al. (1977). While P450 metabolism is not the only factor in considering the hepatotoxicty of trichloroethylene, available data suggest that this mechanism will result in a greater-than-additive acute toxicity resulting from combined exposure to PCBs and trichloroethylene. It should be noted, however, that available studies have examined only the effects on acute trichloroethylene exposures. Trichloroethylene neurotoxicity is thought to result from an interaction of trichloroethylene or its metabolites with neuronal membranes; available studies with trichloroethylene and/or PCBs do not suggest a mechanistic or toxic interaction between the two. However, Moslen et al. (1977) reported an enhancement of the trichloroethylene-induced anesthesia time in PCB-pretreated animals, suggesting that the cytochrome P450 mechanism described above may also play a role in modulating the neurotoxic effects of trichloroethylene. Information on the carcinogenic effects of trichloroethylene is inconclusive, with conflicting data on critical organs and concentrations in available studies; thus, reliable projections of the effects of PCBs on the potential carcinogenesis of trichloroethylene cannot be made.

Toxicological Significance - Relevant interaction data on pertinent health effects with simultaneous oral exposure were not located. Pretreatment with PCBs resulted in an increased hepatic toxicity of trichloroethylene following a single inhalation exposure (Moslen et al. 1977). While the Moslen et al. (1977) study did not directly examine the effect of PCB exposure alone (trichloroethylene and PCB+trichloroethylene were reported), data from other studies using the same PCB mixture (Carlson 1975) have not demonstrated hepatic or neurologic effects of PCBs. Data on intermediate or chronic exposures to PCBs and trichloroethylene, or data on co-exposure by the oral route for any duration, are lacking.

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

Table 10. Effect of **Trichloroethylene** on **PCBs**

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 trichloroethylene will influence PCB toxicity or carcinogenicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between trichloroethylene and PCBs.

Mechanistic Understanding - While both trichloroethylene and PCBs have been demonstrated to elicit neurological effects in animal studies (Appendices D and E), it is believed that they do so by different mechanisms. Understanding of these mechanisms is insufficient to allow for reliable predictions of the effect of trichloroethylene on PCB neurotoxicity. Similarly, while both have been shown to cause hepatotoxicity, both Ah-receptor-dependent and Ah-receptor-independent mechanisms are believed to be responsible for PCB-induced hepatic effects (Appendix E). The potential effects of trichloroethylene on the hepatic toxicity of PCBs cannot be reliably predicted from the available data. No mechanistic information as to potential effects of trichloroethylene on the carcinogenic effects of PCBs was located. Thus, it is uncertain whether cancer is a common health hazard from trichloroethylene and PCBs.

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

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 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 data available for any of the 10 component pairs of the mixture. Data on the potential mechanistic interactions between the component pairs are also lacking.

For the individual components, a chronic oral MRL is available only for PCBs. While intermediate oral MRLs for stable strontium and stable cobalt are available, no MRLs have been derived for internal exposure of any duration to radionuclides of strontium, cobalt, or cesium. MRLs for external exposure to ionizing radiation have been derived, and were deemed applicable for external exposures to cobalt and cesium radiations. MRLs for external exposure to strontium radiation have not been derived.