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. In Section 2.2, summary tables are provided at the end of each section on the binary mixtures. The tables are designed to provide an overview of the direction of interaction. The organization of the tables is by route, duration, and endpoint of toxicity so that all data for an endpoint of concern for a given route and duration are grouped together. The organization of the summary tables is designed to promote a synthesis of the data across studies and an understanding of the potential route, duration, and endpoint-specificity of the direction of interaction.

The absorption of lead and cadmium, and sensitivity to the effects of lead, cadmium, and possibly arsenic, is affected by the adequacy of essential metals, such as calcium, zinc, iron, and selenium, and also other nutrients, in the diet. Less is known about the dependence of chromium(VI) on such factors. In the following summaries of studies of joint toxic action, the use of animal diets or exposure conditions known to be inadequate or marginal in nutrients is noted. Where no such limitations are described, there was no indication in the study report of dietary insufficiency. Similarly, studies of populations whose diets may differ from the general U.S. population are also noted.

2.1 Mixture of Concern

The only study located regarding the toxicity of the complete mixture was a preliminary report of a study of the cytotoxicity of the mixture in human keratinocytes (Campain et al. 2000). Three immortal keratinocyte cell lines and normal epidermal keratinocytes were exposed to lead, arsenic, cadmium, and chromium (1:1 mixture of chromium(III):chromium(VI)) separately in order to characterize dose-response relationships for the individual metals. The mixture of all four metals was prepared at the cytotoxicity LC_{50} concentrations of arsenic, cadmium, and chromium, and using a high level of lead, for which no substantial cell killing could be determined at any of the concentrations tested. Statistical analysis of the data using an additivity response surface indicated that in two of the immortal cell lines,

the responses were antagonistic at high concentration (0.3X), but synergistic at the middle concentrations (0.1X and 0.03X). In the normal cell line, and in an immortal line with growth characteristics similar to normal cells, the responses were synergistic at the 0.3X and 0.1X concentrations. Implications of these findings to human health are uncertain. The keratinocyte is a target for arsenic toxicity and carcinogenicity, but not for the toxicity or carcinogenicity of the other metals in this mixture. For arsenic, the mechanism of dermal lesions may in part be related to cytotoxicity, but another suggested mechanism, particularly for carcinogenic effects on the skin, is that chronic, low-level exposure to arsenic stimulates keratinocyte secretion of growth factors, thus increasing cellular division along with DNA replication, allowing greater opportunities for genetic damage.

No physiologically based pharmacokinetic (PBPK) models were found for mixtures of lead, arsenic, cadmium, and chromium.

2.2 Component Mixtures

No PBPK models were found for the trinary or binary mixtures of these metals.

Studies of interactions or toxicity of two trinary mixtures were located and are reviewed in the following subsections. Studies relevant to the joint action of all possible binary mixtures are then evaluated. Human studies are discussed first, followed by animal studies. For data-rich mixtures, preference is given to simultaneous oral exposure studies. For data-poor mixtures, injection studies, sequential exposure studies, or *in vitro* studies may be included.

Some studies that are judged of inadequate quality or of less relevance due to exposure route are discussed because they are cited in the published literature, and it may be important to have an explanation of their limitations, or because they give information about an endpoint not covered in the more adequate studies. Studies of the impact of one metal on the tissue levels of another are included because interactions may be occurring during absorption and distribution that will impact critical tissue levels. This is particularly important with regard to levels of cadmium in the kidney.

At the end of each binary section, the *in vivo* data are summarized by exposure duration and endpoint in tables. These summary tables are designed to give an overview of the pattern of interactions across durations, endpoints, and studies. For chemical pairs with large databases, the information for the

influence of each chemical on the tissue concentrations is presented in a separate table. For pairs with smaller databases, the tissue concentration data are included in the toxicity/carcinogenicity table.

Many of the interactions studies reviewed in the following sections employed a design in which the dose of each metal in the mixture is the same as when given individually. Consider, for example, a study in which the treated groups received 1 mg/kg/day of chemical A alone, 2 mg/kg/day of B alone, or a mixture of 1 mg/kg/day chemical A plus 2 mg/kg/day of chemical B. The total dose of A and B in the mixture is 3 mg/kg/day. Results from this study design may be interpretable if both A and B caused responses when tested alone at their individual doses, because those responses can be used to determine whether the response to the mixture differs from that predicted by additivity. Also, if only one chemical caused the response, and the response from the mixture is less than the response from that chemical alone, the joint action may tentatively be classified as less than additive. Nevertheless, certain types of results from this study design are uninterpretable with regard to mode of joint action. If neither chemical alone caused the response at the dose tested individually, but the mixture caused the response, the result could be due to the higher total dose of metals in the mixture. In this case, the observed response cannot be classified as reflecting additivity or less-than or greater-than-additive joint action, because the data do not provide a basis for predicting the response due to additivity. This type of result is useful, however, because it demonstrates that subthreshold doses of the individual chemicals can, when administered in combination, result in a response, and suggests that assessment of exposure to each chemical separately may underestimate the effect of combined exposure.

2.2.1 Lead, Arsenic, and Cadmium

Lead, arsenic, and cadmium are often found at elevated concentrations in the environment near mining and smelting sites. Studies of biomarkers of exposure and clinical endpoints in populations living near such sites in the United States are available (e.g., ATSDR 1995a, 1995b; EPA 1998), but tend to focus on only one (lead) or two (lead and cadmium) of the contaminants, and do not investigate potential interactions. Studies using hair metal concentrations of lead, arsenic, and cadmium (and mercury and aluminum) as biomarkers of exposure have considered the impact of these three metals, singly and in binary combinations, on neurobehavioral endpoints in children (Marlowe et al. 1985a, 1985b; Moon et al. 1985). The studies that provide some information relevant to joint action will be evaluated in the appropriate sections on binary mixtures.

An intermediate-duration dietary study of a lead, cadmium, and arsenic mixture has been conducted in rats (Fowler and Mahaffey 1978; Mahaffey and Fowler 1977; Mahaffey et al. 1981). Dietary concentrations of these metals were chosen so as to produce slight to moderate effects and tissue concentrations for the individual metals. Young adult male rats (15/group) were fed nutritionally adequate purified diets containing 200 ppm lead from lead acetate (≈10 mg Pb/kg/day), 50 ppm cadmium from cadmium chloride (≈2.5 mg Cd/kg/day), and 50 ppm arsenic from sodium arsenate (≈2.5 mg As/kg/day) for 10 weeks. Diets containing binary mixtures of these metals and diets containing each of the individual metals at the same concentrations as in the trinary mixture also were tested. Endpoints included tissue levels of the metals, hematological endpoints, renal and hepatic histopathology, body weight, and food utilization. Differences between groups were assessed using analysis of variance; the model included main effects and interactions. Few changes in results were seen with the addition of a third metal to the binary combinations. Body weight gain was depressed to a comparable extent by the trinary mixture and the cadmium-arsenic mixture, as was food utilization (ratio of food consumption to weight gain). Body weight gain and food utilization were depressed to a greater extent with the trinary mixture than with the lead-cadmium mixture. A higher hemoglobin level (similar to controls) was seen for the trinary mixture as compared with the lead-cadmium mixture, but not as compared with the binary mixtures containing arsenic (which also were similar to controls). No specific mention was made of hepatic or renal histopathological changes in the rats that received the trinary mixture. The changes in the endpoints in the trinary versus the binary mixtures tended to be small in magnitude and inconsistent in direction across different endpoints. On the whole, the effects were explained by the binary combinations, which are discussed in subsequent sections on the binary mixtures.

2.2.2 Lead, Arsenic, and Chromium(VI)

Lead, arsenic, and chromium are common contaminants of groundwater near hazardous waste sites. In a study of a mixture of lead, arsenic, and chromium (equal parts chromium(III) and chromium(VI)), no stimulation of hepatocellular proliferation was seen in random field sections of livers of rats given this mixture in their drinking water for 7 days (Benjamin et al. 1999). Treatment with the mixture had no effect on the increased hepatocellular proliferation in diethylnitrosamine-initiated rats. Further testing for promotion of placental glutathione-S-transferase positive preneoplastic liver cell foci in rats after diethylnitrosamine initiation and partial hepatectomy showed an inhibitory effect on foci area and no effect on foci number. Thus, the mixture did not have promoting activity.

2.2.3 Lead and Arsenic

The data for this pair include a study of potential interactions on neurological effects in children, and several studies in animals. The animal studies investigated hematological, hepatic, renal, neurological, and carcinogenic effects. No studies investigated the potential impact of interactions on the arsenic effects of most concern for humans, dermal lesions and cancer. As mentioned in Section 1, there are no good animal models for the dermal toxicity and for the carcinogenicity of arsenic to humans.

Human and Animal Studies

Studies using concentrations of metals in children's hair as biomarkers of exposure to lead, arsenic, cadmium, mercury, and aluminum have investigated correlations with cognitive function, classroom behavior, and visual motor performance (Marlowe et al. 1985a, 1985b; Moon et al. 1985). The 60–80 children were selected randomly from grades 1–6 in one to three schools in similar communities in Wyoming. The hair was collected from an area close to the nape of the neck and washed with deionized water, non-ionic detergent, and organic solvent to remove topical contaminants. Based on hierarchical multiple regression analysis, and after accounting for confounding variables such as age of parents at subject's birth, parents' occupations and education, father's social class and presence in the home, child's birth weight and length of hospitalization, a significant association of lead with increased scores for maladaptive classroom behavior was found, with additional increases from the interaction of arsenic with lead (and cadmium with lead) (Marlowe et al. 1985a). Arsenic was significantly associated with decreased reading and spelling performance, with additional contributions to the variance from the interaction of lead with arsenic (Moon et al. 1985). (Aluminum was associated inversely with visual motor performance [Marlowe et al. 1985b; Moon et al. 1985]). Although these studies attempted to account for confounding variables, they did not include some significant covariates such as the care-giving environment (Home Observation for Measurement of the Environment [HOME] inventory) and nutritional status. The additional variance accounted for by the lead-arsenic interaction was 5% for reading, 7% for spelling, and 3% for behavior, and by the lead-cadmium interaction was 4% for behavior. This type of finding in a single study does not prove causation, but is suggestive.

Two case reports of poisoning from ethnic herbal medicines containing lead and arsenic, or lead, arsenic, and mercury, do not provide information on interactions of lead and arsenic (Mitchell-Heggs et al. 1990; Sheerin et al. 1994).

In a 10-week dietary study of 200 ppm lead (≈10 mg Pb/kg/day) and 50 ppm arsenic (≈2.5 mg As/kg/day) in young adult male rats, hemoglobin was slightly decreased and hematocrit was significantly decreased by arsenic alone, but not by lead alone or the lead-arsenic mixture, indicating a less-than-additive effect for the mixture (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Each of these two metals increased urinary coproporphyrin excretion, and the effect of the mixture was additive. Uroporphyrin excretion was increased by arsenic and not affected by lead; results from the mixture were the same as for arsenic alone (no apparent interaction) (Fowler and Mahaffey 1978; Mahaffey et al. 1981). Light and electron microscopic evaluation of renal tissue revealed cloudy swelling of the proximal tubules, intranuclear inclusion bodies, and mitochondrial swelling in the lead alone and the lead-arsenic treated groups, and mitochondrial swelling in the arsenic alone group. Light microscopic examination of the livers indicated that lead alone had no effect and that lead did not affect arsenic-induced hepatic parenchymal swelling (Mahaffey and Fowler 1977; Mahaffey et al. 1981). The investigators did not consider the electron or light microscopic results indicative of interactions, and the results were not presented in enough detail to support independent evaluation. Neither metal affected tissue distribution of the other (kidney, liver, brain, and bone concentrations), relative to distribution following dietary exposure to the single metal at the same dose level as in the mixture (Mahaffey et al. 1981). Lead was not detected in liver or brain.

A chronic oral study in rats comparing the effects of lead arsenate with those of lead carbonate and calcium arsenate, compounds with solubilities similar to lead arsenate, gives some insight into the lead-arsenic mixture (Fairhall and Miller 1941). Lead arsenate was fed to female rats for 1 or 2 years in the diet at a concentration providing a dose of 10 mg lead arsenate/day, equivalent to ≈18 mg Pb/kg/day and ≈6.3 mg As/kg/day. Additional groups were fed lead carbonate or calcium arsenate concentrations that provided the same amount of lead or arsenic as in the lead arsenate group. Mortality was highest in the calcium arsenate (67%) and lead arsenate (62%) groups and lower in lead carbonate and control groups (42% for both) at 2 years. During the first 8 months of the study, however, mortality was much higher in the calcium arsenate group than in the other three groups. The effects exclusively attributable to lead (the presence of intranuclear inclusion bodies in the kidney, and decreased hematopoietic activity in spleen) appeared less severe in the lead arsenate group than in the lead carbonate group. Similarly, the effects of arsenic (increased mortality, hemosiderin deposition in spleen) appeared less severe in the lead arsenate group than in the calcium arsenate group. The splenic effects of arsenic reflect destruction of red blood cells. Renal effects in common to both calcium arsenate and lead carbonate were swelling of the renal convoluted tubule cells, inclusion of brown granules in these cells, and hyaline casts in the collecting tubules and ducts of Bellini. The severity of tubular swelling and brown granules was greatest

in the lead carbonate group, less severe in the lead arsenate group, and least severe in the calcium arsenate group. The number of hyaline casts was greatest in the calcium arsenate group, less numerous in the lead arsenate group, and least numerous in the lead carbonate group. These results, and the data on renal intranuclear inclusion bodies, indicate a less-than-additive joint renal toxicity of the lead and arsenic components of lead arsenate. Markedly higher arsenic concentrations were seen in the kidneys of rats fed calcium arsenate as compared with those fed lead arsenate, and higher lead concentrations were seen in the kidneys and bone of rats fed lead carbonate as compared with those fed lead arsenate. Bone lead concentrations generally were an order of magnitude higher than kidney lead concentrations in the groups fed the lead compounds. No effects on tissue distribution were seen in liver.

The effects of lead and arsenic on each other's distribution to the brain and on levels of neurotransmitters and their metabolites were studied in mice (Mejia et al. 1997). Lead acetate at 116.4 mg/kg/day (74 mg Pb/kg/day) and sodium arsenite at 13.8 mg/kg/day (8.0 mg As/kg/day) were administered by gavage separately and together to adult male mice for 14 days. Six areas of the brain (hypothalamus, medulla, pons, midbrain, striatum, hippocampus, and cortex) were examined. Arsenic alone generally increased the concentration of dopamine and serotonin and their metabolites and decreased norepinephrine in the brain areas. The only significant effect of lead alone was an increase in 3,4-dihydroxyphenyl-acetic acid, a metabolite of dopamine, in the hypothalamus. The mixture produced effects similar to those of arsenic alone except for an increase in serotonin in the cortex and midbrain and a decrease in norepinephrine in hippocampus that were significant, and greater than the slight change in the same direction seen with either metal alone. These effects of the mixture on neurotransmitter levels did not appear to be greater than additive because the predicted change (the sum of the changes from 74 mg Pb/kg/day alone and 8.0 mg As/kg/day alone) was approximately the same as the observed change (produced by the mixture of 74 mg Pb/kg/day+8.0 mg As/kg/day). Blood lead levels, monitored only in the lead alone group, reached 79.3 μg/dL, but no signs of toxicity were seen in the animals. The concentrations of arsenic in the brain areas were decreased by coexposure to lead (significantly in four of the areas), and those of lead were increased by coexposure to arsenic (significantly in three of the areas), relative to concentrations resulting from exposure to that metal alone.

Another chronic feeding study compared the carcinogenicity and toxicity of sodium arsenate (soluble compound) and lead arsenate (insoluble), both at dietary levels of 100 ppm arsenic, corresponding to ≈7.8 mg As/kg/day (Kroes et al. 1974). The lead arsenate diet provided ≈22 mg Pb/kg/day. Rats were exposed shortly after birth, by feeding of the diets to their mothers, and at various intervals after

weaning, were fed the same diets as their mothers had received. The lead arsenate group was started after the control and sodium arsenate groups, and starting body weight for this group was much lower (≈34 g) than for the other groups (77–99 g). Hematological studies, conducted after 1 year on the diets, showed no consistent significant effects on hematological values, including hemoglobin, hematocrit, red count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) from arsenic or arsenic plus lead, as compared with controls. There were no histopathological effects in a wide range of tissues including heart, liver, kidney, spleen, brain, and testes in animals that died during the study or were terminated at 27 months. No differences in tumor incidences were noted among the treated and control groups. As no significant effects were seen in the arsenic or the lead-arsenic groups, no conclusions regarding joint action are possible. Reasons for the discrepancy in results for lead arsenate in this study as compared with that of Fairhall and Miller (1941) are not apparent. This study provides no evidence of greater-than-additive effects with regard to carcinogenicity (or toxicity) in rats at doses that can be tolerated for chronic exposure. The rat, however, is not a good model for health effects of arsenic in humans.

In a 14-day dietary study of metal interactions on tissue metal contents in young male rats, lead did not affect arsenic concentrations in liver, kidney, or small intestine, but decreased the concentrations of arsenic in bone in a dose-related manner (Elsenhans et al. 1987). The rats were coexposed to lead as the acetate at 20, 52, 89, 226, or 394 ppm lead (equivalent to ≈1.9, 4.9, 8.5, 21, or 37 mg Pb/kg/day) and to arsenic (as sodium arsenite) at 7 ppm arsenic (equivalent to arsenic to ≈0.76 mg As/kg/day). Coexposure of the rats to arsenic at 7, 16, 24, 56, or 89 ppm (equivalent to ≈0.67, 1.5, 2.3, 5.3, or 8.5 mg As/kg/day) and to lead at 20 ppm (≈1.9 mg/kg/day) did not result in detectible levels of lead in liver, kidney, and small intestine, so interactions could not be evaluated. Potential effects of arsenic coexposure on bone lead concentrations were not mentioned. The diets also included 9 ppm cadmium and 13 ppm nickel.

In vitro studies of genotoxicity in human lymphocytes reported that the increase in the frequency of aberrant cells from exposure to a mixture of lead acetate and sodium arsenite was additive as compared with the increases produced by each alone at the same concentration as in the mixture (Nordenson and Beckman 1984). Similar *in vitro* tests for sister chromatid exchange (SCE) in human lymphoctes *in vitro* found that the mixture produced significantly fewer SCEs than expected on the basis of additivity (Beckman and Nordenson 1986).

Potential Mechanisms of Interaction

Lead alters heme synthesis by stimulating mitochondrial delta-aminolevulinic acid synthetase (ALAS), directly inhibiting delta-aminolevulinic acid dehydratase (ALAD), which results in increased urinary delta-aminolevulinic acid (ALA) excretion, and by inhibiting the mitochondrial ferrochelatase-mediated insertion of iron into protoporphyrin, resulting in an elevation of zinc protoporphyrin in erythrocytes (ATSDR 1999b). At relatively high levels of exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Arsenic interferes with mitochondrial heme synthesis enzymes, resulting in increased urinary excretion of uroporphyrin, but not ALA (Fowler and Mahaffey 1978). Arsenic may have a toxic effect on the erythropoietic cells of the bone marrow, and increases hemolysis (ATSDR 2000a). There are potential points of interaction or additivity for arsenic and lead for hematological effects, but the direction is not clear, and might be predicted to be additive or greater than additive.

Lead did not affect the renal concentrations of arsenic in an intermediate-duration dietary study (Mahaffey et al. 1981), but renal arsenic concentrations were decreased in rats simultaneously exposed to lead in a chronic dietary study (Fairhall and Miller 1941). Renal lead concentrations were not affected in rats simultaneously exposed to arsenic in a chronic dietary study (Fairhall and Miller 1941). A 14-day study (Elsenhans et al. 1987) and an intermediate simultaneous oral study (Mahaffey 1981) reported that renal lead was below the detection limit both with and without coexposure of the rats to arsenic. Both lead and arsenic affect renal mitochondria (ATSDR 1999b, 2000a), but in general, mechanisms of toxicity for these two metals are different. No clear mechanistic foundation for joint action on the kidney is apparent.

Concentrations of arsenic in skin of humans exposed to background levels of arsenic were higher than in other “live” tissues except blood (Liebscher and Smith 1968). Arsenic accumulated in the skin of animals given long-term exposure (Lingren et al. 1982). Arsenic reacts with the sulfhydryl groups of proteins, inactivates enzymes, and interferes with mitochondrial function. Relatively high-dose intermediate-duration toxicity to the skin is considered to be due to cytotoxic effects. Chronic low-level exposure to arsenic is thought to stimulate keratinocyte secretion of growth factors. The resulting increase in cell division and DNA replication would afford greater opportunities for genetic damage (ATSDR 2000a). Lead also interferes with mitochondrial function and reacts with sulfhydryl groups. Lead does not appear to be accumulated in the skin (ATSDR 1999b). No data regarding the effects of

lead on concentrations of arsenic in skin were located; in general, oral coexposure to lead and arsenic decreased or did not affect levels of arsenic in soft tissue and bone (Elsenhans et al. 1987; Fairhall and Miller 1941; Mahaffey et al. 1981; Mejia et al. 1997). Mechanistic understanding indicates that there are possible points of interaction, but is insufficient to indicate a direction.

Following 14 days of gavage administration of this pair of metals, lead decreased the arsenic concentrations in the brain of adult mice, as compared with arsenic alone at the same dose as in the mixture (Mejia et al. 1997). In the same study, arsenic increased the lead concentrations in the brain of adult mice, as compared with lead alone at the same dose as in the mixture. Both metals have been reported to affect neurotransmitter levels in brain (ATSDR 1999b; Mejia et al. 1997), and both can bind to sulfhydryl groups of proteins and alter mitochondrial function. Thus, interactions are conceivable, but the potential direction is not clear.

Summary

Table 3 provides an overview of the interaction data regarding the effects of lead on the toxicity of arsenic, and Table 4 summarizes the data regarding the effects of lead on tissue concentrations of arsenic. Similarly, Tables 5 and 6 summarize the effects of arsenic on the toxicity and tissue concentrations of lead. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

Table 3. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Arsenic by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Hematological (RBC and hematocrit)			10 + 2.5 (r)	<additive	Mahaffey et al. 1981
Intermediate	Hematopoietic (urinary coproporphyrin)		10 + 2.5 (r)		additive	Fowler and Mahaffey 1978; Mahaffey et al. 1981
Intermediate	Hematopoietic (uroporphyrin)		10 + 2.5 (r)		additive	Fowler and Mahaffey 1978; Mahaffey et al. 1981
Intermediate	Renal (mitochondrial swelling)		10 + 2.5 (r)		additive	Mahaffey et al. 1981
Intermediate	Neurological (reading, spelling)	exposure biomarkers = hair Pb and As (hc)			>additive	Moon et al. 1985
Intermediate	Neurological (neurotransmitter levels)		74 + 8.0 (m)		additive	Mejia et al. 1997
Chronic	Hematological (splenic hemosiderosis indicating red cell destruction)			18 + 6.3 (r)	<additive	Fairhall and Miller 1941

Table 3. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Arsenic by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Chronic	Renal (hyaline casts in tubules)			18 + 6.3 (r)	<additive	Fairhall and Miller 1941
Chronic	Cancer		22 + 7.8 (r)		indeterminate: no effect of As or of As+Pb at same dose of As as in mixture; no Pb alone group	Kroes et al. 1974

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat, m = mouse, hc = human (child)

Table 4. Summary of Available Data on the Influence of Lead on Tissue Concentrations of Arsenic by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (14 days)	Renal, hepatic, small intestine As levels		1.9–3.7 + 0.67[a] (r)[b]		additive	Elsenhans et al. 1987
Acute (14 days)	Bone As levels			1.9–3.7 + 0.67 (r)	<additive	Elsenhans et al. 1987
Intermediate	Renal, hepatic, brain, bone As levels		10 + 2.5 (r)		additive (below detection limit in bone)	Mahaffey et al. 1981
Intermediate	Brain As levels			74 + 8 (m)	<additive	Mejia et al. 1997
Chronic	Renal As levels			18 + 6.3 (r)	<additive	Fairhall and Miller 1941
Chronic	Hepatic, bone As levels		18 + 6.3 (r)		additive	Fairhall and Miller 1941

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat, m = mouse

Table 5. Summary of Available Data on the Influence of Arsenic on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Hematopoietic (urinary coproporphyrin)		2.5 + 10[a] (r)[b]		additive	Fowler and Mahaffey 1978; Mahaffey et al. 1981
Intermediate	Renal (proximal tubular cloudy swelling, intranuclear inclusion bodies, mitochondrial swelling)		2.5 + 10 (r)		additive	Mahaffey et al. 1981
Intermediate	Neurological (classroom behavior)	Exposure biomarkers = hair As and Pb (hc)			>additive	Marlowe et al. 1985a
Intermediate	Neurological (neurotransmitter levels)		8.0 + 74 (m)		additive	Mejia et al. 1997
Chronic	Hematopoietic (splenic myelosis)			6.3 + 18 (r)	<additive	Fairhall and Miller 1941
Chronic	Renal (swollen convoluted tubule cells, intranuclear inclusion bodies)			6.3 + 18 (r)	<additive	Fairhall and Miller 1941

Table 5. Summary of Available Data on the Influence of Arsenic on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Chronic	Cancer		7.8 + 22 (r)		indeterminate: no effect of As or of As+Pb at same dose of As as in mixture; no Pb alone group	Kroes et al. 1974

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat, m = mouse, hc = human (child)

Table 6. Summary of Available Data on the Influence of Arsenic on Tissue Concentrations of Lead by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (14 days)	Hepatic, renal, small intestine As levels		1.9–3.7 + 0.67[a] (r)[b] (below detection limit)		additive?	Elsenhans et al. 1987
Intermediate	Bone, hepatic, renal, brain Pb levels		2.5 + 10 (r) (below detection limit)		additive?	Mahaffey et al. 1981
Intermediate	Brain Pb levels	8 + 74 (m)			>additive	Mejia et al. 1997
Chronic	Bone, renal Pb levels			6.2 + 18 (r)	<additive	Fairhall and Miller 1941
Chronic	Hepatic Pb levels		6.3 + 18 (r)		additive	Fairhall and Miller 1941

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat, m = house

2.2.4 Lead and Cadmium

The database for this pair is voluminous, consisting of studies of renal and other effects in workers exposed primarily by inhalation, studies of cardiovascular effects in adults and neurological endpoints in children exposed primarily orally, and studies of a wide variety of endpoints, including cardiovascular, renal, neurological, and testicular, in animals exposed orally (no interaction studies were located for animals exposed by inhalation). Some of the findings are reasonably congruent (cardiovascular effects) and others are conflicting (neurological). Injection studies provide supporting information for testicular effects. An injection study regarding teratogenicity is inadequate, but is discussed because it is cited in the literature.

Human Studies—Inhalation Exposure

A number of epidemiological studies are available for this binary mixture; some show significant associations or interactions. This type of finding in a single study does not prove causation, but is suggestive.

Renal dysfunction, measured as increased urinary clearance of β_2-microglobulin and albumin, in workers exposed to lead and cadmium was similar to that in workers exposed to cadmium alone, indicating a lack of interactive or additive effects (Roels et al. 1978). The workers exposed to lead alone did not have elevated indices of renal dysfunction as compared with controls. Exposed workers were defined as those with urinary cadmium (CdU) $\geq$2 μg/g creatinine, PbB $\geq$35 μg/dL, or both. Mean urinary and blood values for the four groups were as follows:

Controls (N = 77):	CdU = 0.81 μg/g creatinine, PbB = 16 μg/dL
Cadmium (N = 42):	CdU = 11.4 μg/g creatinine, PbB = 22.7 μg/dL
Cadmium + Lead (N = 17):	CdU = 6.57 μg/g creatinine, PbB = 43.5 μg/dL
Lead (N = 19):	CdU = 1.29 μg/g creatinine, PbB = 45.6 μg/dL

Additional evidence of lack of interactive or additive effects with regard to renal dysfunction was provided in a subsequent study of 62 workers exposed to lead and cadmium in lead or cadmium smelters (mean CdU = 7.08 μg/g creatinine, mean PbB = 38.7 μg/dL) and 88 control workers from the same smelters (mean CdU = 0.88 μg/g creatinine; mean PbB = 16.4 μg/dL) (Buchet et al. 1981). Correlation

analysis of the lead and cadmium group using levels of lead and cadmium in blood and urine as the independent variables showed that indices of renal damage correlated with cadmium only and indices of interference with heme synthesis (hematocrit, hemoglobin, free erythrocyte porphyrin, and urinary ALA) correlated with lead only. Two-way analysis of variance was used to investigate a possible interaction between lead and cadmium on kidney function, focusing on the endpoints that had shown an increased prevalence of abnormal values. The control and mixed exposure groups were pooled and then subdivided into three classes on the basis of cadmium in blood or urine; each class was further subdivided into two subclasses on the basis of lead in blood. No interaction effect was discerned; the indices of renal dysfunction were associated with cadmium.

Measurement of vitamin D_3 (cholecalciferol) metabolites in 19 workers, exposed to lead and cadmium for at least 5 years in a non-ferrous metal smelter, indicated that coexposure to these metals may perturb the conversion of 25-hydroxyvitamin D_3 to 1α,25-dihydroxyvitamin D_3 (Chalkley et al. 1998), the active form of vitamin D. CdU was significantly inversely correlated with plasma 24R,25-dihydroxyvitamin D_3. Neither CdU nor PbB showed significant correlations with plasma 1α,25-dihydroxyvitamin D_3. When workers were divided into three groups according to PbB and CdU, significant differences in the plasma 1α,25-dihydroxy-vitamin D_3 values were seen across groups. In comparison with the normal range of 15–40 pg/mL for this active form of vitamin D_3, the mean levels of 1α,25-dihydroxyvitamin D_3 in these exposed groups can be characterized as follows:

	Low Pb High Cd n = 7	Raised Pb Low Cd n = 7	High Pb High Cd n = 5
PbB μmol/dL	<1.9	>1.4 (30 μg/dL)	>1.9
CdU nmol/L	>8	<8	>8
1α,25-dihydroxyvitamin D	<normal	high normal	>normal

These results are suggestive of an interactive effect, but no additional details regarding PbB and CdU (such as the mean and range) were provided for each group, and the PbBs for the "Raised Pb Low Cd" group were not comparable with those in the "High Pb High Cd" group. None of the groups appeared to have truly low (comparable to general population) indices of exposure to lead or cadmium. Characterization of the findings for the "High Pb High Cd" group in terms of the effect of one metal on the toxicity of the other is problematic because neither metal alone correlated, either directly or inversely,

with plasma 1α,25-dihydroxyvitamin D_3 in this population. A decrease in plasma levels of this active form of vitamin is regarded as adverse; an increase may or may not be. Accordingly, this study is not included in the interaction summary tables for this pair.

A study comparing lead-exposed workers and lead plus cadmium-exposed workers with a healthy control group on immune parameters reported no differences in NK cytotoxicity or in the percentage of lymphocytes with CD4 phenotype (T-helper cells), but a slight but significant decrease in the percentage of B-lymphocytes (CD20) in the lead-cadmium group as compared with controls, whereas the lead-alone group had a smaller (nonsignificant) decrease in the percentage of B-lymphocytes (Yucesoy et al. 1977b). The lead-cadmium group, however, had somewhat higher mean PbB values, longer duration of exposure, and higher age than the other groups, which may have accounted for the results. Therefore, the study is not included in the summary table.

Human Studies—Oral Exposure

The potential association between cardiovascular-related mortality and tissue lead and cadmium were investigated in a study of 106 autopsies on persons who lived in an area of North Carolina with soft water and acidic, leached soil (Voors et al. 1982). Residents of this area were expected to have somewhat elevated exposure to these metals because soil cadmium is more available to plants when the soil is acidic and leached and when the water is soft, and because soft water leaches lead from lead-containing plumbing into drinking water. The aorta was chosen as the index tissue for the heart's exposure to lead and the liver was chosen as the index tissue for cadmium (it was not discussed why the aorta was not used for cadmium as well). Cases having cancer as the cause of death were eliminated due to increased variability of metal levels, and those lacking aorta or liver samples were eliminated, leaving 75 for analysis. A stepwise logistic regression analysis was performed with the cause of death as the dependent variable, and the log-transformed lead and cadmium tissue levels and age at death as independent variables. Tissue lead and cadmium each were significantly associated with the proportion of deaths resulting from cardiovascular disease. An exception was five cases where aortic lead was below detection limits (but liver cadmium levels were high for these, and these cases had multiple illnesses and other causative factors). Additional analysis indicated that the proportion of deaths related to cardiovascular disease was lowest when both lead and cadmium tissue levels were low and increased as the combined tissue levels increased in a manner that appeared compatible with additivity.

A multisite study of populations exposed to lead and cadmium in residential areas near National Priorities List (NPL) smelting and mining sites investigated correlations between exposure and biomarkers of exposure, and between biomarkers of exposure and clinical tests for hematopoietic, hepatic, renal, and immunological effects of the individual metals, but did not investigate potential interactions (ATSDR 1995b). A few correlations between PbB or CdU and hematological and immunological values were statistically significant, but hematological associations were not consistent across age groups or with related clinical values, or were not consistent with other reports, and immunological findings may have been due to respiratory illness. Although the study does not give information on joint toxic action, and therefore is not included in the summary table, it is mentioned here because it detected few indications of health effects from environmental exposure to lead and cadmium at residential areas near four hazardous waste sites. Limitations of the study, in terms of detecting associations with health effects, included the short minimum residency requirement (adequate for induction of hematopoietic effects but not for cadmium induction of renal effects), lack of data regarding recent or ongoing illness (which may impact immune results), low numbers of participants over 45 (in whom renal effects might be more likely), higher soil lead concentrations in control than in exposed residential areas, and lack of assessment for impact of other environmental contaminants associated with smelting and mining sites (such as arsenic).

As previously described in the section on lead and arsenic, studies using concentrations of metals in children's hair as biomarkers of exposure to lead, arsenic, cadmium, mercury, and aluminum have investigated correlations with cognitive function, classroom behavior, and visual motor performance (Marlowe et al. 1985a, 1985b; Moon et al. 1985). The 60–80 children were selected randomly from grades 1–6 in one to three schools in similar rural communities in Wyoming. The hair was collected from an area close to the nape of the neck and washed with deionized water, non-ionic detergent, and organic solvent to remove topical contaminants. Based on hierarchical multiple regression analysis, and after accounting for confounding variables such as age of parents at subject's birth, parents' occupations and education, father's social class and presence in the home, child's birth weight, and length of hospitalization, a significant association of lead with increased scores for maladaptive classroom behavior was found, with additional increases from the interaction of cadmium with lead (and arsenic with lead) (Marlowe et al. 1985a). Although these studies attempted to account for confounding variables, they did not include other significant covariates such as the care-giving environment (HOME inventory) and nutritional status. The additional variance in behavioral measures accounted for by the lead-cadmium interaction was 4%. (In the other studies, arsenic and lead-arsenic were inversely

correlated with cognitive function and aluminum and lead-aluminum were inversely correlated with visual motor performance [Marlowe et al. 1985b; Moon et al. 1985]).

A previous study focused on potential correlations between children's hair lead and cadmium concentrations and intelligence test results, school achievement scores, and motor impairment assessments in 149 children of ages 5–16 recruited from four counties in Maryland through newspaper ads (Thatcher et al. 1982). Hair samples were washed with hexane, alcohol, and deionized water prior to analysis. Hair lead and cadmium were much higher in the children from rural homes than in those from urban homes. Potential sources of higher lead and cadmium exposure in rural environments include pesticides. Arsenic exposure from pesticides also would be likely in rural environments, but was not taken into account. Using hierarchical regression analyses to adjust for potentially confounding variables (sex, age, race, socioeconomic status), the study found that lead and cadmium each were significantly inversely associated with intelligence test scores and achievement test scores, but not associated with gross motor movement scores. Additional analyses indicated that lead independently accounted for a significant amount of the performance IQ variance, whereas cadmium independently accounted for a significant amount of the verbal IQ variance. Analyses of variance did not reveal any significant interactions between these two metals for any of the test scores. This study accounted for fewer known confounders than did the studies by Marlowe et al. (1985a, 1985b) and Moon et al. (1985), and the population in this study appeared to be more diverse.

Animal Studies—Oral Exposure

Potential interactions on the cardiovascular system have been investigated extensively in female rats maintained in a low-metal environment and fed a rye-based diet low in toxic and essential metals (Kopp et al. 1980a, 1980b; Perry and Erlanger 1978; Perry et al. 1983). The administration of 0.1, 1.0, or 5.0 ppm of lead and cadmium separately and together in drinking water for 3–18 months to weanling rats (15/group) produced increases in systolic pressure relative to controls (N=45). These exposure levels correspond to doses of ≈0.016, 0.16, and 0.78 mg/kg/day for subchronic exposure and ≈0.013, 0.13, and 0.67 mg/kg/day for chronic exposure. No statistical analysis for interactions was performed, but at 3 months, the increase for the mixture appeared additive at the low and high dose and possibly slightly greater than additive at the middle dose, as compared with the increases for either metal alone. At 6 months, the increase appeared additive for the low dose and high dose, and was not reported for the middle dose of the mixture. Additional results, shown only for the high dose, indicated that the systolic

pressure increase for the mixture at 9 months was approximately additive compared with the increases for the individual metals. At 18 months, systolic pressure was significantly elevated above controls only in the cadmium alone group, and not in the lead alone or mixture group (Perry and Erlanger 1978). Additional similar studies by the same group of investigators, using smaller numbers of weanling rats (3–6/group), the 5 ppm exposure level, and monitoring blood pressure at 3–15 months of treatment gave results for systolic blood pressure throughout the dosing period that were indicative of an approximately additive effect for the metals in combination versus both alone at the same doses as in the mixture (Kopp et al. 1980a, 1980b). In another study by the same group, the administration of 1 ppm cadmium in drinking water for 2–16 months starting with young adult rats (13–14/group) resulted in significantly elevated systolic pressure within 2 months that appeared to gradually and slightly increase during the rest of the study. Administration of 1 ppm lead plus 1 ppm cadmium in drinking water did not increase systolic pressure over that observed after administration of cadmium alone. Lead alone was not tested (Perry et al. 1983). Thus, the results of these studies were variable, but on the whole, indicated an additive joint action for lead and cadmium on systolic blood pressure in this particular rat model over much of the lifespan.

The rat model used in the above blood pressure studies included the feeding of a rye-based diet abnormally low in toxic and essential metals, and housing that minimized exposure to these substances. The conditions were designed to duplicate those used by Schroeder and Vinton (1962). Calcium and potassium were low, and chromium(III) was later found to be less than optimal. Control rats in these studies have unusually low blood pressure, perhaps due to their low exposure to toxic metals. Although lead alone or cadmium alone at relatively low levels of exposure clearly caused hypertension in this rat model, the relevance of this result to human health is uncertain. At higher levels of cadmium exposure, some of the rye-based dietary studies showed decreases or no effect on blood pressure. Some other rat studies, employing commercial diets, have not reported hypertension from low or higher-level oral administration of cadmium (ATSDR 1999a, 1999b; Friberg et al. 1986).

Hematological effects were investigated in a 10-week dietary administration of 200 ppm lead (≈10 mg Pb/kg/day) and/or 50 ppm cadmium (≈2.5 mg Cd/kg/day) to young adult male rats (Fowler and Mahaffey 1978; Mahaffey and Fowler 1977; Mahaffey et al. 1981). Explanations of statistical analyses, and presentation of statistical significance in the data tables, are unclear and make interpretation of the data problematic for this pair of metals. When administered separately or together, lead and cadmium increased the numbers of circulating red blood cells to a similar extent. Lead did not affect hemoglobin

or hematocrit, but cadmium slightly decreased hematocrit. The mixture produced decreases in both hemoglobin and hematocrit, but the experimental design and reporting, and the lack of significant responses from either metal alone, do not support a determination as to whether the joint action was additive or greater- or less-than additive. Relative to control values, urinary ALA (measured as total excretion/24 hours) was greatly increased by lead alone, but was not affected by cadmium alone. The urinary ALA level resulting from the mixture was intermediate between the values for lead alone and cadmium alone, suggesting a less-than-additive interaction. Lead alone increased urinary coproporphyrin, and cadmium did not affect this endpoint or the response of this endpoint to lead.

In the same series of studies, coadministration of cadmium and lead caused a marked reduction in swelling of renal proximal tubule cells and intranuclear inclusion bodies as compared with lead alone, and, as mentioned previously, a marked reduction in renal Pb concentrations (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Cadmium alone had no effect on relative kidney weight, and no light or electron microscopic changes were mentioned in the kidneys of the cadmium-alone group. When administered with lead, cadmium did not affect the lead-induced increase in relative kidney weight. Renal cadmium content, an index of renal cadmium toxicity, was the same for the lead-cadmium mixture as for cadmium alone. Other tissue levels of cadmium also were not affected by lead. Coadministration of cadmium with lead resulted in a significant reduction in levels of lead in blood, kidneys, and femur relative to those seen with lead alone. Lead was not detectible in liver or brain.

Another study of hematological effects in rats investigated the impact of deficient versus normal dietary calcium and of no versus normal versus high vitamin D on interactions between lead and cadmium (and zinc) (Thawley et al. 1977). In this study, young male rats were fed lead carbonate (5,000 ppm Pb, ≈430 mg Pb/kg/day) and cadmium carbonate (90 ppm Cd, ≈7.7 mg Cd/kg/day) separately or as a mixture in the various diets for 42 days in order to evaluate the impact on red blood cell parameters and urinary ALA. Analysis of variance was performed for main effects and interactions. Because of the nature of this study, separating out interactions of lead and cadmium under conditions of normal calcium and vitamin D is problematic. Such interactions, according to inspection of the data tables and the investigators' conclusions, occurred under conditions of deficient calcium and no or high vitamin D. Additional limitations were a duration of exposure that may not have been sufficient for full expression of the hematological effects, the nonreporting of the study's data on hematocrit (a toxicologically significant endpoint), and the small number of animals/treatment (2 rats/treatment/replicate x 4 replicates = 8 rats). Inspection of the data obtained under normal dietary cadmium and vitamin D indicates little or

no change in blood hemoglobin from exposure to each metal alone versus slightly decreased blood hemoglobin from exposure to the mixture. Slight decreases in MCH, MCHC, and MCV were seen for each metal alone, and somewhat greater (apparently additive) decreases in each of these values were seen for the mixture. Urinary ALA (measured as mg/100 mg creatinine in grab sample from cage holding two rats—therefore only four samples/group), was too highly variable in the lead and cadmium—lead groups to support meaningful conclusions; the standard deviations were nearly as large as the means.

Gavage studies of tissue distribution of cadmium and lead used a less relevant dosing regimen. Male rats were gavaged twice a week with lead acetate at 70 mg Pb/kg, or once a week with cadmium chloride at 20 mg Cd/kg, for 7 weeks. A mixture group was given lead and cadmium "simultaneously"; no further detail regarding the treatment of this mixture group was provided. Controls received sodium acetate twice a week in equimolar concentration to the acetate in the lead acetate solution (Skoczynska and Smolik 1994; Skoczynska et al. 1994). Although the doses for the mixture group were not specified, it seems likely that they were the same as for each metal alone. Results of the tissue distribution studies included no significant effect of either metal on the concentrations of the other in blood, heart, or brain. The concentrations of both metals in liver and in kidney were decreased in the mixture group as compared with either metal alone (Skoczynska et al. 1994). In the second study, similar results were obtained for blood and liver, the only tissues analyzed (Skoczynska and Smolik 1994).

A study in mice investigated the effect of intermediate-duration oral coexposure to lead and cadmium on viral-induced mortality, tissue histopathology, and tissue distribution of the metals (Exon et al. 1979). Groups of mice were exposed to the following concentrations of lead (from lead acetate) and cadmium (from cadmium acetate) in their drinking water for 10 weeks:

13 ppm lead (≈3.25 mg Pb/kg/day) + 3 ppm cadmium (≈0.75 mg Cd/kg/day)
130 ppm lead (≈32.5 mg Pb/kg/day) + 30 ppm cadmium (≈7.5 mg Cd/kg/day)
1,300 ppm lead (≈325 mg Pb/kg/day) + 300 ppm cadmium (≈75 mg Cd/kg/day)
2,600 ppm lead (≈650 mg Pb/kg/day) + 600 ppm cadmium (≈150 mg Cd/kg/day).

Additional groups were exposed to lead alone or cadmium alone at the same concentrations as in the mixtures. Following 10 weeks of exposure, all mice were innoculated with encephalomyocarditis virus and observed for 16 days, at which time the experiment was terminated. Virus-related mortality was increased (relative to controls) in the lead alone group, decreased in the cadmium-alone group, and was

slightly lower than, but not significantly different from, controls in the lead-cadmium group. Histopathological analyses was performed on tissues from moribund mice. Renal lesions were seen in the kidneys of mice exposed to the metals either singly or in combination and consisted of moderate degeneration and necrosis of the tubular epithelial cells; whether these differed in severity among groups was not discussed, and incidence cannot be determined when only moribund mice are examined. Intranuclear inclusion bodies were seen only in the kidneys of mice exposed to lead. Lesions attributable to lead or cadmium toxicity were not seen in other tissues, including brain, testes, and liver. Coadministration of lead and cadmium resulted in increased renal lead and cadmium concentrations, compared with the same dose of lead alone or cadmium alone, except at the highest combined doses, at which decreases in renal lead and cadmium occurred. Concentrations of cadmium in testes and liver also appeared to be increased in groups coexposed to lead, except at the highest combined dose, in which they were decreased; whereas concentrations of lead in these tissues appeared to be decreased by coexposure to cadmium, except at the highest dose group, in which they were increased. No clear effects of combined exposure on brain lead or cadmium concentrations were seen. The tissue concentration data were based on pooled tissues from three mice/group, so the degree of variability and the significance of the results cannot be assessed. Tissue samples were taken after the viral infection and observation period.

Dietary studies in rats have investigated the effects of a lead-cadmium mixture on brain concentrations of these metals, on neurotransmitters, and on behavioral endpoints in rats. Lead acetate (500 ppm Pb, ≈43 mg Pb/kg/day) or cadmium chloride (100 ppm Cd, ≈8.6 mg Cd/kg/day), or the combination of the two metals at the same doses as given separately, were fed to adult male rats in the diet for 60 days. This treatment did not cause overt signs of toxicity (Nation et al. 1989, 1990). Body weight was not depressed in the 1989 study and was depressed only by the lead-cadmium diet in the 1990 study; analysis of variance did not indicate significant interaction with regard to this endpoint. Levels of the neurotransmitters serotonin and dopamine and their metabolites were analyzed in five areas of the brain (brain stem frontal cortex, nucleus accumbens, olfactory tubercle, and striatum). The pattern of effects on neurotransmitters was complex, but lead tended to have more marked effects than did cadmium, and the lead-induced perturbation of dopamine and serotonin turnover was attenuated by cadmium. Both lead alone and cadmium alone were associated with increased rates of lever pressing for food in schedule-controlled responding. Exposure to the mixture, however, resulted in a lever-pressing rate that was not different from that of controls (Nation et al. 1989). Monitoring of the animals' activity revealed that lead exposure resulted in a general increase in activity (increased movement, decreased rest time, and increased vertical activity, relative to controls), whereas cadmium exposure resulted in a general decrease

in activity. The activity of animals exposed to the mixture was not different from that of controls. Thus, the behavioral effects of each metal appeared to antagonize those of the other metal. The two metals did not affect each other's concentration in the brain, but cadmium decreased PbB levels (Nation et al. 1990).

An additional study of activity in rats exposed to much lower doses of lead and cadmium reported different results. This study was conducted on rats that were exposed to 5 ppm of lead (0.62 mg Pb/kg/day), 5 ppm cadmium (≈0.62 mg Cd/kg/day), or 5 ppm lead plus 5 ppm cadmium in their drinking water for 16 months (Lockett and Leary 1986). The activity levels, reported as activity units/hour at hourly intervals for 10 hours, were decreased by lead alone and to a greater extent by lead and cadmium, relative to controls. With cadmium alone, activity was similar to that of controls, although the time of peak activity appeared to be shifted. No statistical analysis for interactions was performed, the data were displayed graphically, and the area under the activity curve was not reported. The results appear to show a slight potentiation by cadmium of lead's depressive effect on activity, but this conclusion should be regarded as tentative, since only one dose of each metal alone was tested, and the dose of each metal in the mixture was the same as when tested singly, so the total metal dose was higher in the mixture. Cadmium concentrations in the brain were not affected by coadministration of lead; data relevant to an effect of cadmium on lead concentrations in the brain were not reported.

An intermediate-duration drinking water study of lead and cadmium focused on testicular toxicity (Saxena et al. 1989). Lead (50 ppm, 1.91 mg Pb/rat/day, ≈8.4 mg Pb/kg/day) and cadmium (50 ppm, 2.14 mg Cd/rat/day, ≈9.1 mg Cd/kg/day) were administered separately as the acetates to weanling male rats for 120 days. Additional groups received a mixture of cadmium (25 ppm, 1.015 mg Cd/rat/day, ≈5.3 mg Cd/kg/day) and lead (25 ppm, 1.1015 mg Pb/rat/day, ≈5.3 mg Pb/kg/day), or water without added metals. Thus, the total dose of metal was approximately the same for the mixture group (≈10.6 mg/kg/day) as for the single metal groups (8.4 and 9.1 mg/kg/day) (doses were estimated from the reported mg metal/rat/day based on water consumption and from estimated time-weighted average body weights). Final body weights were slightly depressed in the lead alone and cadmium alone groups, but were significantly depressed in the mixture group. Relative testes weights were slightly increased in the lead group, and significantly increased in the cadmium group, and further increased in the mixture group. Detrimental effects on sperm motility and seminiferous tubule diameter in the mixtures group were the same as with cadmium alone, and more severe than with lead alone. Sperm counts in the caudal epididymis were decreased significantly in all three groups, and the effect was significantly more severe in the mixture group as compared with either metal alone. The percentage of damaged seminiferous

tubules was significantly greater in all treatment groups and was markedly more severe in the mixtures group: control 5.4%, lead 18.4%, cadmium 37.6%, and the lead-cadmium mixture 67.0%. The investigators suggest that coexposure to lead may increase the accumulation of cadmium in the testes, based on a previous study in rats (Shukla and Chandra 1987), in which lead and cadmium were administered at lower doses and, for cadmium, by a different route: lead at 5 ppm in the drinking water and cadmium at 0.1 and 0.4 mg/kg/day intraperitoneally, simultaneously for 30 days. While this cotreatment resulted in higher concentrations of cadmium in the testes, cadmium was not administered by a natural route, and the cotreatment resulted in lower concentrations of lead in the testes.

A study of the developmental toxicity of cadmium and a cadmium-lead mixture, administered to rat dams in drinking water during gestation and early lactation, to the reproductive organs of their pups (Corpas and Antonio 1998) provides little information on potential interactions due to the lack of a group treated with lead alone. For example, when effects from the mixture were greater than from cadmium alone, it cannot be determined whether the joint action is additive or deviates from additivity. Additional limitations of this study include the small number of dams (four/group) and the use of individual pups rather than the litter as the unit for statistical analysis. Cadmium acetate (1.13 mg Cd/kg/day) and a mixture of cadmium acetate and lead acetate (1.14 mg Cd/kg/day and 34.47 mg Pb/kg/day) were administered to pregnant rats throughout gestation until the pups were born; additional groups were continued on treatment through postnatal day 5. The concentration of cadmium in the blood of the pups was lower and in testes was higher in the mixture group as compared with the cadmium alone group. Seminiferous tubule diameter was decreased, relative to controls, to the same extent in the cadmium and cadmium-lead groups. A reduction in the numbers of pro-spermatogonia was greater in the group exposed to the mixture than in the group exposed to cadmium alone. (Only four testes/group were examined histopathologically.)

In a 14-day dietary study of metal interactions on tissue metal contents in young male rats, lead did not affect cadmium concentrations in liver, kidney, small intestine, or bone (Elsenhans et al. 1987). The rats were coexposed to lead as the acetate at 20, 52, 89, 226, or 394 ppm lead (equivalent to ≈1.9, 4.9, 8.5, 21, or 37 mg Pb/kg/day) and to cadmium (as the chloride) at 9 ppm cadmium (equivalent to 0.86 mg Cd/kg/day). Coexposure of the rats to cadmium at 9, 19, 28, 73, or 181 ppm (equivalent to ≈0.86, 1,8, 2.7, 6.9, or 17 mg Cd/kg/day) and to 20 ppm lead (≈1.9 mg Pb/kg/day) did not result in detectible tissue levels of lead in liver and small intestine. Levels of lead in kidney were lower in the four higher-dose cadmium groups as compared with the lowest-dose cadmium group, but no dose-response relationship

was seen. Data for bone lead concentrations were not presented. Although the authors stated that as far as the analytical methods could determine, cadmium did not affect the levels of the other toxic metals in the other tissues, it is unclear whether or not lead was detectible in bone in this experiment. The diets in this study also supplied 7 ppm arsenic and 13 ppm nickel.

Animal Studies—Injection

The effects of lead and cadmium on the kidney, reproductive tissues, and bladder of the male rat were studied following intraperitoneal injection of 0.05 mg lead acetate (0.067 mg Pb/kg/day), 0.05 mg cadmium chloride (0.065 mg Cd/kg/day), or a mixture of 0.025 mg lead acetate (0.034 mg Pb/kg/day) and 0.025 mg cadmium chloride (0.032 mg Cd/kg/day) for 1 month (Fahim and Khare 1980). Note that the dose regimen for this experiment differs from most in that the total dose for the metals separately and for the mixture is constant (i.e., the doses of the metals in the mixture is half the dose given separately so that the total metal dose stays the same). Thus, a dose addition model can be used to evaluate whether interactions have occurred. The injections were into the lower abdomen near the prostate (and bladder); controls were injected with saline. No histological changes were observed in the kidney, but lead and cadmium each caused the formation of calcium oxalate stones in the kidney and bladder, and acted synergistically when injected together. The mixture also acted synergistically in causing calcification and histopathological changes in the bladder, including squamous metaplasia, fibrosis, and inflammation in the bladder. Other synergistic effects of the mixture were damage to the testicular seminiferous tubules, prostatic atrophy, and squamous metaplasia of the prostate. No significant changes were seen in the seminal vesicles and epididymis of any of the groups. The applicability of these results to a natural route of exposure is uncertain, because not only were the metals injected intraperitoneally, but in such a location as to have direct contact with some of the affected organs. Neither of these metals is readily absorbed through the digestive tract.

In an earlier study by the same laboratory, daily intraperitoneal injection of 0.025 mg of lead as the acetate (≈0.0625 mg Pb/kg/day) and daily intramuscular injection of 0.025 mg of Cd as the chloride (0.0625 mg Cd/kg/day) for 70 days produced marked testicular effects (seminiferous tubule damage and absence of spermatogenesis) in male rats. No testicular effects were observed in rats injected with 0.050 mg (≈0.125 mg/kg/day) or 0.25 mg (0.625 mg Pb/kg/day, 0.714 mg Cd/kg/day) of either metal alone (Der et al. 1976).

An intravenous study of developmental toxicity of lead acetate and cadmium sulfate in hamsters (Ferm 1969) suffers from deficiencies in design and in data analysis and reporting that preclude meaningful evaluation of interactions. Pregnant hamsters were injected with 2 mg/kg of cadmium alone or in combination with 25 or 50 mg/kg of lead. An additional group was injected with 50 mg/kg of lead alone. As compared with water-injected controls, the lead-alone group had an increase in resorptions, and the resorptions were further increased in the cadmium plus high lead group. The frequency and severity of cadmium-induced cleft lip and palate were decreased by the high dose of lead, but the frequency of cadmium-induced exencephaly appeared to be increased by the low dose of lead. The frequency and severity of lead-induced tail malformations appeared to be increased by cadmium. In addition, a severe caudal malformation of the lower extremities was seen in a substantial number of the fetuses treated with the cadmium-high lead combination. Limitations of the study, however, include the lack of any statistical analysis, and the presentation of data only for individual embryos/fetuses, with no indication of litter incidence. In epidemiological studies, lead has not been shown to be associated with congenital anomalies, and when administered to animals by natural routes of exposure, has not caused malformations (ATSDR 1999b). Cadmium, administered by natural routes, has caused malformations in animals, including dysplasia of facial bones and rear limbs and sharp angulation of the distal third of the tail in rats or mice, generally at relatively high maternal doses (ATSDR 1999a). The relevance of the results of this intravenous study are uncertain because lead does not cause malformations by natural routes of exposure and because the evidence from other studies suggests that cadmium also may affect the development of the tail and hind limbs, so cadmium may have been acting additively with lead rather than potentiating lead-induced posterior malformations. In addition, given that a larger percentage of embryos was resorbed following the combined cadmium-high lead treatment, and that a larger number of fetuses had exencephaly following the combined cadmium-low lead treatment, a conclusion that lead protected against the developmental toxicity of cadmium cannot be supported.

Potential Mechanisms of Interaction

Lead and cadmium appear to act on different components related to hematopoetic toxicity. Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, and inhibiting the insertion of iron into protoporphyrin, mediated by ferrochelatase. As a result of alterations in the activity of ALAS and ALAD, ALA accumulates in blood, urine, and soft tissues (ATSDR 1999b). Cadmium may inhibit heme synthesis indirectly by decreasing the absorption of iron from the gastrointestinal tract (ATSDR 1999a). Thus, potential additive or greater-than-additive effects of lead plus cadmium on

hematological parameters might be expected based on metal-specific mechanisms of inhibition of heme synthesis. The decrease in PbB in rats exposed to cadmium and lead, as compared with lead alone, may indicate an interference of cadmium with the absorption of lead, as further discussed below.

Mechanistic considerations for the joint action of lead and cadmium on the kidney include the possible interference of each metal on the absorption or kidney distribution of the other. The renal toxicity of cadmium is associated with the accumulation of cadmium in the kidney over chronic durations of exposure until a critical concentration is reached. One 14-day study (Elsenhans et al. 1987) and three intermediate-duration studies (Exon et al. 1979; Mahaffey et al. 1981; Skoczynska et al. 1994) of oral coexposure to lead and cadmium in rats and mice have investigated the impact of lead on renal cadmium concentrations. Taken together, the results do not define a logical dose-response pattern. The 14-day study (Elsenhans et al. 1987) and the most relevant intermediate-duration study (Mahaffey et al. 1981) indicate that lead does not affect the accumulation of cadmium in the kidney. Studies of the impact of cadmium on the absorption and distribution of lead also are not entirely consistent (Elsenhans et al. 1987; Exon et al. 1979; Mahaffey et al. 1981; Nation et al. 1990; Skoczynska et al. 1994), but the weight of evidence indicates that cadmium coexposure decreases lead concentrations in blood and a number of tissues, including the kidney. It has been suggested (Mahaffey and Fowler 1977) that cadmium may alter the surface of the gastrointestinal tract, causing malabsorption, as has been seen in Japanese quail. The lesions seen in the quail included shortening and thickening of the villi, marked shortening of the microvilli, and a dense cellular infiltrate in the lamina propria. These changes were considered similar to those seen in some malabsorption syndromes in humans.

With regard to neurological effects, cadmium and lead did not affect each other's concentrations in the brain (Mahaffey et al. 1981; Nation et al. 1990; Skoczynska et al. 1994), although cadmium decreased blood and tissue concentrations of lead in a number of studies, previously discussed. Both cadmium and lead have been reported to affect neurotransmitters in animals (ATSDR 1999b; Nation et al. 1989). As discussed by Nation et al. (1989), cadmium may inhibit calcium entry into neurons and the attendant release of catecholamines. Lead also is thought to inhibit the influx of calcium into neurons, inhibiting transmitter release, may act as a calcium agonist within the cell, and may activate protein kinase C and calmodulin. The complexity of the literature dealing with mechanisms pertinent to the neurological effects of lead (ATSDR 1999b) does not support a simple hypothesis regarding potential mechanisms of interactions between cadmium and lead.

Mechanisms underlying the observed synergistic interaction of lead and cadmium on the testes are not known. Because simultaneous dietary administration of zinc protected against the synergistic effects of the dietary lead-cadmium mixture on the testes (Saxena et al. 1989), the interaction may be mediated through effects on zinc-containing enzymes, including DNA and RNA polymerases. Both lead and cadmium interfere with zinc-enzyme complexes (ATSDR 1999a, 1999b).

Summary

Table 7 provides an overview of the interaction data regarding the effects of lead on the toxicity of cadmium, and Table 8 summarizes the data regarding the effects of lead on tissue concentrations of cadmium. Similarly Tables 9 and 10 summarize the effects of cadmium on the toxicity and tissue concentrations of lead, respectively. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

Table 7. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Cadmium by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation exposure (PbB μg/dL; CdU μg/g creatinine)						
Chronic	Renal (proteinuria)		43.5 + 6.57[a] (ha)[b] 38.7 + 7.08 (ha)			Roels et al. 1978 Buchet et al. 1981
Oral exposure (mg/kg/day)						
Intermediate	Cardiovascular (systolic blood pressure increase)	0.16 + 0.16 (r)	0.016 + 0.016 (r) 0.78 + 0.78 (r) 0.78 + 0.78 (r)		generally additive except at 0.16 (but no statistical analysis for interactions, large standard derivations)	Perry and Erlanger 1978 Kopp et al. 1980a Kopp et al. 1980b
Intermediate	Cardiovascular (systolic blood pressure increase)		0.67 + 0.67 (r)		additive? (blood pressure same as for 0.67 Cd alone; no Pb alone group)	Perry et al. 1983
Intermediate	Hematological (hemoglobin, hematocrit)		10 + 2.5 (r) 430 + 7.7		indeterminate: effects from mixture but not individual metals at same doses as in mixture	Mahaffey and Fowler 1977; Mahaffey et al. 1981 Thawley et al. 1977
Intermediate	Hematological (MCV, MCH, MCHC)		430 + 7.7		additive	Thawley et al. 1977
Intermediate	Neurological (IQ and achievement test scores)		exposure biomarkers = hair Cd and Pb (hc)		no interaction	Thatcher et al. 1982

Table 7. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Cadmium by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Neurological (schedule-controlled responding)			43 + 8.6 (r)	<additive	Nation et al. 1989
Intermediate	Neurological (activity)			43 + 8.6 (r)	<additive	Nation et al. 1990
Intermediate	Testicular (sperm count, seminiferous tubule damage)	5.3 + 5.3 (r)			>additive	Saxena et al. 1989
Intermediate	Developmental (seminiferous tubule diameter in pups)		34.47 + 1.14 (r)		additive? effect of mixture same as Cd alone but no Pb alone group	Corpas and Antonio 1998
Intermediate	Developmental (number of pro-spermatogonia in pups)		34.47 + 1.14 (r)		indeterminate: effect of mixture > Cd alone at same dose as in mixture, but no Pb alone group	Corpas and Antonio 1998
Chronic	Cardiovascular-related mortality		exposure biomarkers: aortic Pb, hepatic Cd (ha)		additive (?)	Voors et al. 1982

Table 7. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Cadmium by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Chronic	Cardiovascular (systolic blood pressure increase)		0.67 + 0.67 (r)	0.67 + 0.67 (r)	additive or <additive	Kopp et al. 1980a, 1980b Perry and Erlanger 1978
Chronic	Cardiovascular (systolic blood pressure increase)		0.67 + 0.67 (r)		additive? blood pressure same as for 0.4 Cd alone; no Pb alone group	Perry et al. 1983
Intraperitoneal injection (mg/kg/day)						
Intermediate	Prostate, bladder (calcification, squamous metaplasia, fibrosis)	0.034 + 0.032 (r)			>additive	Fahim and Khare 1980
Intermediate	Testicular (seminiferous tubule damage)	0.034 + 0.032 (r) 0.0625 + 0.0625[d] (r)			>additive	Fahim and Khare 1980 Der et al. 1976

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat, ha = human (adult), hc= human (child)
[c]70 mg Pb/kg twice a week and 20 mg Cd/kg once a week
[d]Intramuscular injection

Table 8. Summary of Available Data on the Influence of Lead on Tissue Concentrations of Cadmium by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (14 days)	Bone, hepatic, renal, small intestine Cd levels		1.9–37 + 0.86 (r)		additive	Elsenhans et al. 1987
Intermediate	Blood Cd levels		70 + 20[c]		additive	Skoczynska et al. 1994
Intermediate	Heart Cd levels		70 + 20[c] (r)		additive	Skoczynska et al. 1994
Intermediate	Bone Cd levels		10 + 2.5 (r)		additive? (below detection limit)	Mahaffey et al. 1981
Intermediate	Hepatic Cd levels	3.25–325 + 0.75–75[d] (m)	10 + 2.5 (r)	70 + 20[c] (r) 650 + 150[d] (m)	ambiguous: additive based on study with most relevant design (see footnotes)	Mahaffey et al. 1981 Skoczynska et al. 1994 Exon et al. 1979
Intermediate	Renal Cd levels	3.25–325 + 0.75–75[d] (m)	10 + 2.5 (r)	70 + 20[c] (r) 650 + 150[d] (m)	ambiguous: additive based on study with most relevant design (see footnotes)	Mahaffey et al. 1981 Skoczynska et al. 1994 Exon et al. 1979

Table 8. Summary of Available Data on the Influence of Lead on Tissue Concentrations of Cadmium by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Brain Cd levels		10 + 2.5 (r) (below detection limit) 43 + 8.6 (r) 70 + 20[c] (r)		additive	Mahaffey et al. 1981 Nation et al. 1990 Skoczynska et al. 1994
Intermediate	Testes Cd levels	3.25–325 + 0.75–75[d] (m)		650 + 150[d] (m)	>additive except <additive at high dose	Exon et al. 1979
Intermediate	Developmental (blood Cd levels in pups)			34.47 + 1.14 (r)	<additive	Corpas and Antonio 1998
Intermediate	Developmental (testes Cd levels in pups)	34.47 + 1.14 (r)			>additive	Corpas and Antonio 1998
Chronic	Brain Cd levels		0.62 + 0.62 (r)		additive	Lockett and Leary 1986

[a]First dose listed is for the chemical influencing the other chemical's tissue concentrations.
[b]Species code: r = rat, m = mouse
[c]70 mg Pb/kg twice a week and 20 mg Cd/kg once a week
[d]Tissue concentrations were determined following injection of encephalomyocarditis virus and 16 days of observation (without metal treatment).

Table 9. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation exposure (CdU µg/g creatinine; PbB µg/dL)						
Chronic	Hematological (hematocrit hemoglobin, free erythrocyte porphyrin, urinary ALA)		6.57 + 43.5 (ha) 7.08 + 38.7 (ha)		additive	Roels et al. 1978 Buchet et al. 1981
Oral exposure (mg/kg/day)						
Intermediate	Cardiovascular (systolic blood pressure increase)	0.16 + 0.16 (r)	0.016 + 0.016 (r) 0.78 + 0.78 (r) 0.78 + 0.78 (r)		generally additive except at 0.16 (but no statistical analysis for interactions, large standard derivations)	Perry and Erlanger 1978 Kopp et al. 1980a Kopp et al. 1980b
Intermediate	Hematological (hemoglobin, hematocrit)		2.5 + 10 (r) 7.7 + 430 (r)		indeterminate: effects from mixture but not individual chemicals at same doses as in mixture	Mahaffey and Fowler 1977; Mahaffey et al. 1981 Thawley et al. 1977
Intermediate	Hematological (MCV, MCH, MCHC)		7.7 + 430 (r)		additive	Thawley et al. 1977

Table 9. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Hematopoietic (urinary ALA)			2.5 + 10 (r)	<additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intermediate	Hematopoietic (urinary coproporphyrin)		2.5 + 10 (r)		additive	Fowler and Mahaffey 1978; Mahaffey et al. 1981
Intermediate	Renal (proximal tubular cloudy swelling; intranuclear inclusion bodies, mitochondrial swelling)			2.5 + 10 (r)	<additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intermediate	Renal (relative kidney weight)		2.5 + 10 (r)		additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intermediate	Renal (intranuclear inclusion bodies)			0.75–150 + 3.25–650[d] (m)	<additive	Exon et al. 1979
Intermediate	Immunological (virus-induced mortality)			0.75–150 + 3.25–650[d] (m)	<additive	Exon et al. 1979
Intermediate	Neurological (classroom behavior)	exposure biomarkers = hair Cd and Pb (hc)			>additive	Marlowe et al. 1985a

Table 9. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intermediate	Neurological (IQ and achievement test scores)		exposure biomarkers = hair Cd and Pb (hc)		no interaction	Thatcher et al. 1982
Intermediate	Neurological (serotonin and dopamine turnover)			8.6 + 43 (r)	<additive	Nation et al. 1989
Oral exposure (mg/kg/day)						
Intermediate	Neurological (schedule-controlled responding)			8.6 + 43 (r)	<additive	Nation et al. 1989
Intermediate	Neurological (activity)			8.6 + 43 (r)	<additive	Nation et al. 1990
Intermediate	Reproductive (sperm count, seminiferous tubule damage)	5.3 + 5.3 (r)			>additive	Saxena et al. 1989
Intermediate	Developmental (seminiferous tubule diameter in pups)		1.14 + 34.47 (r)		indeterminate: effect of mixture the same as Cd alone but no Pb alone group	Corpas and Antonio 1998
Intermediate	Developmental (number of pro-spermatogonia in pups)		1.14 + 34.47 (r)		indeterminate: effect of mixture greater than of Cd alone but no Pb alone group	Corpas and Antonio 1998

Table 9. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Chronic	Cardiovascular-related mortality		exposure biomarkers: aortic Pb, hepatic Cd (ha)		additive (?)	Voors et al. 1982
Chronic	Cardiovascular (systolic blood pressure increase)		0.67 + 0.67 (r)	0.67 + 0.67 (r)	additive or <additive	Kopp et al. 1980a, 1980b Perry and Erlanger 1978
Chronic	Neurological (activity)	0.62 + 0.62 (r)			>additive	Lockett and Leary 1986
Intraperitoneal injection (mg/kg/day)						
Intermediate	Prostate, bladder (calcification, squamous metaplasia, fibrosis)	0.032 + 0.034 (r)			>additive	Fahim and Khare 1980

Table 9. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intermediate	Testes (seminiferous tubule damage)	0.032 + 0.034 (r) 0.0625[e] + 0.0625 (r)			>additive	Fahim and Khare 1980 Der et al. 1976

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat, m = mouse, ha = human (adult), hc = human (child)
[c]20 mg Cd/kg once a week and 70 mg Pb/kg twice a week
[d]Tissue samples were taken from moribund animals following injection of encephalomyocarditis virus and up to 16 days of observation (without metal treatment).
[e]Intramuscular injection

Table 10. Summary of Available Data on the Influence of Cadmium on Tissue Concentrations of Lead by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (14 days)	Renal lead levels			0.86–17 + 1.9 (r)	<additive?	Elsenhans et al. 1987
Intermediate	Blood Pb levels		20 + 70[c] (r)	2.5 + 10 (r) 8.6 + 43 (r)	<additive for studies with more relevant dosing regimen	Mahaffey et al. 1981 Nation et al. 1990 Skoczynska et al. 1994
Intermediate	Heart Pb levels		20 + 70[c] (r)		additive	Skoczynska et al. 1994
Intermediate	Bone Pb levels			2.5 + 10 (r)	<additive	Mahaffey et al. 1981
Intermediate	Hepatic Pb levels	150 + 650[d] (m)	2.5 + 10 (r) (below detection limit)	20 + 70[c] (r) 0.75–75 + 3.25–325[d] (m)	dose dependent?	Mahaffey et al. 1981 Skoczynska et al. 1994 Exon et al. 1979
Intermediate	Renal Pb levels	0.75–75 + 3.25–325[d] (m)		2.5 + 10 (r) 20 + 70[c] (r) 150 + 650[d] (m)	ambiguous: <additive for study with more relevant design (Mahaffey et al. 1981)	Mahaffey et al. 1981 Skoczynska et al. 1994 Exon et al. 1979

Table 10. Summary of Available Data on the Influence of Cadmium on Tissue Concentrations of Lead by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Testes Pb levels	150 + 650[d] (m)		0.75–75 + 3.25–325[d] (m)	<additive >additive at high dose	Exon et al. 1979
Intermediate	Brain Pb levels		2.5 + 10 (r) (below detection limit) 8.6 + 43(r) 20 + 70[c] (r)		additive	Mahaffey et al. 1981 Nation et al. 1990 Skoczynska et al. 1994

[a]First dose listed is for the chemical influencing the other chemical's tissue concentrations.
[b]Species code: r = rat, m = mouse
[c]20 mg Cd/kg once a week and 70 mg Pb/kg twice a week
[d]Tissue concentrations were determined on pooled samples from 3 mice/group following injection of encephalomyocarditis virus and 16 days of observation (without metal treatment).

2.2.5 Lead and Chromium(VI)

The only information regarding potential interactions of these two metals is an *in vitro* genotoxicity study.

A study of chromosomal damage *in vitro* determined that chromosomal damage from lead chromate is attributable to the chromium(VI) content of the chemical (Wise et al. 1994). Using Chinese hamster ovary cells and suspensions of lead chromate particles, which generated solubilized chromium and lead, the investigators determined that exposure of the cells to sodium chromate at concentrations that produced similar time courses of intracellular concentrations of chromium resulted in a similar degree and type of chromosomal damage as from lead chromate. Exposure of the cells to lead glutamate at levels that resulted in intracellular lead levels 400-fold higher than those produced by lead chromate produced no chromosomal damage. A higher level of lead glutamate was weakly clastogenic, but produced a different spectrum of chromosomal effects than did lead chromate. A study of apoptic cell death induction by lead chromate determined that the mode of cell death in Chinese hamster ovary cells was similar for exposure to particulate lead chromate and for exposure to soluble sodium chromate under conditions that mimicked conditions of ionic chromate uptake after lead chromate exposure: all of the cells killed by either treatment underwent apoptosis (Blankenship et al. 1997). The results of these *in vitro* studies give no indication of interactions between the lead and the chromium constituents of lead chromate. Effects were attributable solely to the chromium(VI) content.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.6 Arsenic and Cadmium

Studies relevant to joint toxic action of arsenic and cadmium include a study of cancer mortality in workers, oral studies in animals regarding hematological, hepatic, and renal effects, and *in vitro* and intraperitoneal studies in animals of less direct relevance, included because the data for this pair are limited.

Studies in Humans and Animals

A study of lung cancer mortality in a cohort of workers exposed to arsenic and cadmium at a cadmium recovery plant in the United States gave ambiguous results (Sorahan and Lancashire 1997). A major purpose of the study was to determine the risk of lung cancer from exposure to various cadmium compounds and to investigate potential confounding by arsenic. The cohort consisted of 571 men first employed in the period 1926–1969 with follow up through 1982. Individual estimates of cumulative cadmium exposure were derived from detailed job histories. Arsenic trioxide exposures were categorized only as high or low. A significant positive trend for lung cancer risk with increasing cumulative exposure to cadmium was demonstrated and was more pronounced when the exposures were lagged by 10 or 20 years. When similar analyses were applied to subgroups with high or low arsenic exposure, a significant positive trend for lung cancer risk and cumulative exposure to cadmium was seen in the group coexposed to high arsenic, but not in the group with low or negligible arsenic exposure. Because the form of cadmium to which workers were exposed was different for the high arsenic exposures (cadmium oxide) as compared with the low arsenic exposures (cadmium sulfide and cadmium sulfate), no clear conclusions regarding causality or interactions can be drawn. As the investigators pointed out, the results were consistent with a number of hypotheses, including the following: cadmium oxide is carcinogenic to the lung in the presence of arsenic trioxide; both cadmium oxide and arsenic trioxide are lung carcinogens, but cadmium sulphate and cadmium sulfide are not (or are less potent); or arsenic trioxide is a lung carcinogen and cadmium oxide, sulfate, and sulfide are not. Thus, this study is not suitable for inclusion in the summary tables. As discussed in Appendix B to this profile, inorganic arsenic (particularly arsenic trioxide) is a known human carcinogen by the inhalation route, based on evidence from occupational exposure studies. As discussed in Appendix C, previous studies of a possible association between cadmium exposure and lung cancer in U.S. cohorts have given conflicting results, and in non-U.S. cohorts have shown some increases in lung cancer but without a clear relationship between exposure level and duration and cancer risk.

In a 10-week dietary study, coadministration of 50 ppm arsenic (≈2.5 mg As/kg/day) and 50 ppm cadmium (≈2.5 mg Cd/kg/day) to young adult male rats caused a more marked reduction in body weight gain and food utilization than either metal alone at the same dose as in the mixture, but the joint action was less than additive for weight gain and greater than additive for food utilization. Both arsenic and cadmium increased the red blood cell count, and arsenic decreased hematocrit (cadmium decreased hematocrit slightly but not significantly). Effects of the mixture on these endpoints were less than

additive (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Cadmium did not affect the arsenic-enhanced urinary excretion of coproporphyrin and uroporphyrin (Fowler and Mahaffey 1978; Mahaffey et al. 1981). Cadmium did not affect the arsenic-induced moderate mitochondrial swelling in renal proximal tubule cells detected by electron microscopy. Cadmium appeared to inhibit arsenic-induced increased SGOT and eliminated arsenic-induced swelling of hepatic parenchymal cells (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Neither metal had a significant effect on the accumulation of the other in kidney, liver, or brain (Mahaffey et al. 1981).

An *in vitro* study also investigated potential interactions of arsenic and cadmium with regard to the kidney (Keith et al. 1995). Rabbit renal cortical slices were incubated with sodium arsenite and/or cadmium chloride, and uptake was measured at 2 hours. Cadmium did not inhibit the uptake of arsenic, and arsenic had little inhibitory effect on the uptake of cadmium. Interpretation of these results is uncertain without additional information on the mechanism of uptake of these metals and their membrane or intracellular location in the tissue slices.

A dietary study of metal interactions on tissue metal concentrations in young female rats showed no effect of arsenic (as sodium arsenite) on the accumulation of cadmium in the kidney (Schmolke et al. 1992). Rats were fed control diets (with no additional "toxic" metals) or diets with a constant level of cadmium (11 ppm, equivalent to ≈0.95 mg Cd/kg/day) and three different levels of arsenic (7.5, 15, and 30 ppm equivalent to ≈0.65, 1.3, and 2.6 mg As/kg/day) for 15 weeks. There was no change in the accumulation of cadmium in the kidney with increasing dietary levels of arsenic. Lead (20 ppm) and nickel (12 ppm) were also present in the arsenic and cadmium-containing diets, but were not detectible in the kidney at any dietary level of arsenic. In a similar study conducted by the same group of investigators, arsenic did not affect cadmium concentrations in liver, kidney, or small intestine of young male rats following coexposure to arsenic at 7, 16, 24, 56, or 89 ppm (equivalent to ≈0.67, 1.5, 2.3, 5.3, or 8.5 mg As/kg/day) and to 9 ppm cadmium (equivalent to ≈0.86 mg Cd/kg/day) in the diet for 14 days (Elsenhans et al. 1987). In this same study, cadmium did not affect the arsenic concentrations in the same tissues following coexposure to cadmium at 9, 19, 28, 73, or 181 ppm (equivalent to ≈0.86, 1.8, 2.7, 6.9, or 17 mg Cd/kg/day) and to 7 ppm arsenic (equivalent to ≈0.67 mg As/kg/day) in the diet for 14 days. The diets also included 20 ppm lead and 13 ppm nickel.

Two acute intraperitoneal studies from the same group of investigators reported that the joint toxic action of arsenic (as sodium arsenite) and cadmium (as cadmium chloride) in rats was greater than additive with

regard to lethality, but was inconsistent across target organs (Diaz-Barriga et al. 1990; Yanez et al. 1991). LD_{50} studies indicated synergism of arsenic and cadmium with regard to acute lethality. These studies included the effect of constant doses of one metal on the LD_{50} of the other (effect of 10 mg sodium arsenite/kg [5.8 mg As/kg] on 1.6–26 mg cadmium chloride/kg [0.98–16 mg Cd/kg] for cadmium LD_{50}; effect of 2.6 mg cadmium chloride/kg [1.6 mg Cd/kg] on 5–20 mg sodium arsenite/kg [2.9–11 mg As/kg] for arsenic LD_{50}) and a comparison of the lethality of the mixture of 1.6 mg Cd/kg and 5.8 mg As/kg with each chemical separately at the same dose (Yanez et al. 1991). An exception was the observation of no apparent effect of a lower constant dose of cadmium (0.98 mg Cd/kg with arsenic at 4.6–11 mg As/kg) versus apparent potentiation of arsenic lethality at the higher dose of cadmium (1.6 mg Cd/kg and arsenic at 2.9–8.7 mg As/kg) (Diaz-Barriga et al. 1990). Both dose levels of cadmium were within the 95% confidence limits of the LD_0.

In addition, following administration of 1.6 mg Cd/kg, 5.8 mg As/kg, and a mixture of the two chemicals at the same doses as administered separately, the kidney, liver, and testes were examined histopathologically and for glutathione content (Diaz-Barriga et al. 1990); tissue levels of these metals were measured; and cardiac levels of glutathione, lipid peroxidation, and metallothionein were determined (Yanez et al. 1991). The histopathological examinations (Diaz-Barriga et al. 1990) indicated that arsenic protected against the testicular hemorrhage produced by cadmium. Renal congestion was observed, particularly of the glomerular capillaries, following cadmium alone, and also in the renal cortex of rats injected with arsenic alone. In the rats injected with the mixture, a generalized congestion of the glomeruli was seen and the capsular space was absent in many glomeruli. Thus, renal toxicity appeared more severe following injection with the mixture, but whether the effects differed from additivity and if so, in which direction, cannot be determined from these data. Ascites was found in many of the rats that were injected with cadmium, their livers were very friable, and congestion with enlargement of the sinusoids was seen. In the arsenic-treated rats, the liver sinusoids were enlarged. In rats treated with the mixture, light congestion of the liver was seen, indicating that arsenic may have ameliorated the hepatic toxicity of cadmium. Glutathione levels in these tissues did not appear to correlate with the degree of damage or the apparent joint toxic action. Taken together, the histopathological findings did not account for the apparent synergistic effect on acute lethality (Diaz-Barriga et al. 1990). Additional experiments (Yanez et al. 1991) revealed no significant changes in tissue metal concentrations in the kidney or testis. Coadministration of arsenic reduced the hepatic concentration of cadmium, and coadministration of cadmium increased the cardiac levels of arsenic. In the heart, glutathione concentration and lipid peroxidation were increased by both chemicals and by the

mixture (to about the same extent as with the more potent chemical, arsenic) and metallothionein levels were increased by cadmium alone and to the same extent by the mixture (Yanez et al. 1991). The relevance of interactions data regarding target organs in animals dying of acute toxicity to the exposure scenario of concern for humans residing near hazardous waste sites may be questionable.

The following sequential injection studies are less relevant to determining the mode of joint action, and therefore are not included in the summary tables, but are reviewed in the text because the database for this chemical pair is sparse, and these studies provide some information relevant to mechanisms in rats.

Pretreatment of male rats with a non-toxic dose of arsenic (22.5 μmole sodium arsenite/kg, subcutaneously) followed 24 hours later by cadmium (10, 20, or 30 μmole cadmium chloride/kg, subcutaneously) markedly reduced mortality, hepatotoxicity (SGOT), and testicular hemorrhagic necrosis as compared with cadmium alone (Hochadel and Waalkes 1997). The adverse effects of cadmium and protection by arsenic were seen at the highest dose of cadmium. Cadmium pretreatment (3 μmole cadmium chloride/kg) in the same manner did not affect the lethality of arsenic (68, 79, 84, or 90 μmole sodium arsenite/kg) and no increases in SGOT were seen with arsenic alone or with cadmium followed by arsenic. Both cadmium and arsenic pretreatments increased hepatic metallothionein levels, with cadmium being the more potent inducer, but no further increase was seen with the sequential treatment.

In mice, an 8-day pretreatment with cadmium chloride (intraperitoneal injections of 2, 3, 4, 8, 12, or 18 μmole/kg on days 2, 4, 6, and 8, with 1/4 dose given on day 1) protected against the lethality of 12.9 mg/kg of arsenic trioxide injected subcutaneously on day 9 (Kreppel et al. 1988). The protection was apparent at ≥4 μmole/kg. The cadmium pretreatment produced decreased body weight gains at 12 and 18 μmole/kg. Cadmium levels in the liver were dose-related, and an increase in the metallothionein content of the liver was seen. Zinc pretreatment was much less effective in protecting against arsenic lethality.

Potential Mechanisms of Interaction

Arsenic induces metallothionein, a protein which binds and sequesters cadmium, protecting cellular components from the toxicity of free cadmium. In parenteral administration studies, pretreatment of animals with low doses of cadmium (Goering and Klassen 1984) or with arsenic (Hochadel and Waalkes 1997) or other inducers of metallothionein (ATSDR 1999a) protected against the lethality and acute

hepatoxicity of cadmium (renal toxicity was not investigated). On the other hand, the cadmium-metallothionein complex (CdMT), when released from the liver or administered by injection, is directly and indirectly toxic to the kidney. Direct toxicity of CdMT to the brush border membrane of the proximal convoluted tubules has been reported (Cherian 1985; Suzuki and Cherian 1987). In addition, CdMT is filtered by the glomerulus and reabsorbed by the proximal convoluted tubules. The metallothionein is then degraded, releasing free cadmium intracellularly, which may cause tissue damage unless the capacity of the kidney to produce intracellular metallothionein to bind the cadmium is sufficient (ATSDR 1999a). MT-null mice (mice that lack the ability to synthesize MT) are unusually susceptible to the renal, hepato-, immuno-, and hematotoxicity and to the lethality of subcutaneously injected cadmium (Habeebu et al. 2000; Liu et al. 1998, 1999a). MT-null mice also are unusually susceptible to the renal toxicity of subcutaneously injected CdMT (Liu et al. 1999b). These findings indicate the importance of intracellular MT in protecting against cadmium toxicity, and that the toxicity of cadmium to the kidney is not mediated solely through CdMT. Single-dose oral studies in normal and MT-1 transgenic mice (which carried extra copies of a MT gene and have higher constitutive levels of MT in their tissues, particularly in the liver) indicate that at a relatively high dose of cadmium (300 μmole/kg [34 mg/kg], close to the maximum tolerated dose), cadmium retention in the whole body, liver, and kidney 1 week after dosing are approximately double those seen in normal mice, and (induced) MT levels are approximately triple the levels in normal mice. At lower doses of cadmium, differential retention generally did not occur, even though levels of MT were much higher in the MT-1 transgenic mice than in the normal mice. Levels of MT in the intestine are also higher in the MT-1 transgenic mice, but did not appear to impair absorption of cadmium. The relevance of these results to intermediate or chronic exposure is uncertain. Predicting the consequences of concurrent oral exposure to arsenic and cadmium is problematic, because the outcome would depend on the balance between release of the toxic CdMT complex from liver versus induction of renal intracellular MT to bind (detoxify) cadmium. In addition, retention of cadmium in the kidney (and other tissues) is associated with binding of cadmium to intracellular MT. When the concentration of cadmium in the kidney reaches a critical concentration, renal dysfunction ensues (ATSDR 1999a; IRIS 2001). Therefore, MT induction may provide some short-term protection against renal damage, but could conceivably contribute to an increased accumulation of cadmium in the kidney and the subsequent development of chronic renal toxicity.

Summary

The studies considered more relevant to the evaluation of the joint toxic action of arsenic and cadmium are summarized in Table 11 for the interaction data regarding the effects of arsenic on the toxicity and tissue concentrations of cadmium, and Table 12 for the interaction data regarding the effects of cadmium on the toxicity and tissue concentrations of arsenic. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

Table 11. Summary of Available Data on the Influence of Arsenic on Toxicity/Carcinogenicity of Cadmium by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (14 days)	Renal, hepatic, small intestine Cd levels		0.67–8.5 + 0.86 (r)		additive	Elsenhans et al. 1987
Intermediate	Renal Cd levels		0.65–2.6 + 0.95 (r) 2.5 + 2.5 (r)		additive	Schmolke et al. 1992 Mahaffey et al. 1981
Intermediate	Hepatic, brain Cd levels		2.5 + 2.5 (r)		additive	Mahaffey et al. 1981
Intermediate	Hematological (RBC, hematocrit)			2.5 + 2.5 (r)	<additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intraperitoneal injection (mg/kg/day)						
Acute	LD_{50} Lethality	5.8 + 0.98–16 (r) 5.8 + 1.6 (r)			>additive	Yanez et al. 1991
Acute	Hepatic Cd levels			5.8 + 1.6 (r)	<additive	Yanez et al. 1991
Acute	Renal, testicular Cd levels		5.8 + 1.6 (r)		additive	Yanez et al. 1991

Table 11. Summary of Available Data on the Influence of Arsenic on Toxicity/Carcinogenicity of Cadmium by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal injection (mg/kg/day)						
Acute	Renal (congestion of glomerular capillaries)		5.8 + 1.6 (r)		indeterminate: effects more severe from mixture than from individual metals at same doses as in mixture	Diaz-Barriga et al. 1990
Acute	Testicular (hemorrhage)			5.8 + 1.6 (r)	<additive	Diaz-Barriga et al. 1990
Acute	Hepatic (histopathology, ascites, friability)			5.8 + 1.6 (r)	<additive	Diaz-Barriga et al. 1990

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat

Table 12. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Arsenic by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (14 days)	Renal, hepatic, small intestine As levels		0.86–17 + 0.67 (r)		additive	Elsenhans et al. 1987
Intermediate	Renal, hepatic and brain As levels		2.5 + 2.5 (r)		additive	Mahaffey et al. 1981
Intermediate	Renal (mitochondrial swelling)		2.5 + 2.5 (r)		additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intermediate	Hepatic (SGOT, mild histopathology)			2.5 + 2.5 (r)	<additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intermediate	Hematological (RBC, hematocrit)			2.5 + 2.5 (r)	<additive	Mahaffey and Fowler 1977; Mahaffey et al. 1981
Intermediate	Hematopoietic (urinary coproporphyrin and uroporphyrin)		2.5 + 2.5 (r)		additive	Fowler and Fowler 1978; Mahaffey et al. 1981
Intraperitoneal injection (mg/kg/day)						
Acute	LD_{50} Lethality	1.6 + 2.9–11 (r) 1.6 + 5.8 (r)			>additive	Yanez et al. 1991

Table 12. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Arsenic by Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal injection (mg/kg/day)						
Acute	LD_{50}	1.6 + 2.9–8.7 (r)	0.98 + 4.6–11 (r)		additive at 0.98 Cd >additive at 1.6 Cd	Diaz-Barriga et al. 1990
Acute	Renal, hepatic, testicular As levels		1.6 + 5.8 (r)		additive	Yanez et al. 1991
Acute	Cardiac As levels	1.6 + 5.8 (r)			>additive	Yanez et al. 1991
Acute	Hepatic (enlarged sinusoids)			1.6 + 5.8 (r)	<additive	Diaz-Barriga et al. 1990
Acute	Renal (cortical congestion)		1.6 + 5.8 (r)		indeterminate: glomerular effects more severe from mixture than from individual chemicals at same doses as in mixture	Diaz-Barriga et al. 1990

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat

2.2.7 Arsenic and Chromium(VI)

Few data are available regarding the interactions of this pair of chemicals. An oral study of the effect of chromium(VI) on the absorption of arsenic (Gonzales et al. 1995) is available. Two acute toxicity studies were performed because of concern for the impact on human health of lumber treated with combinations of inorganic salts of chromium(VI), arsenic, and copper (Mason and Edwards 1989; Mason et al. 1989). These studies were designed to identify hazard, rather than to elucidate the mode of joint toxic action of chromium and arsenic, and their relevance is limited by the use of intraperitoneal injection as the route of administration and the lack of appropriate statistical analysis. These studies are described in the following paragraphs.

The effect of chromium(VI) (from potassium dichromate) on the absorption of arsenic (from arsenic pentoxide) was studied in intact rats and in *in situ* perfused rat intestines (Gonzalez et al. 1995). The rats were fasted before metal administration. Coadministration of these metals to the rats by gavage in buffered solution (2 mL of 40 or 80 μg Cr(VI)/mL, equivalent to 0.27 and 0.53 mg Cr(VI)/kg, and 3 or 30 μg As/mL, equivalent to 0.02 or 0.2 mg/kg) resulted in a greater absorption of arsenic than with administration of the same doses of arsenic alone. Results from the intestinal perfusion experiments also indicated greater absorption of arsenic in the presence of chromium(VI). Excretion of arsenic in urine and feces by 48 hours postadministration was decreased in the rats that received chromium(VI) with the arsenic, as compared with those that received arsenic alone. The investigators' explanations for the effect of chromium(VI) on absorption are that it modified intracellular pH, providing an adequate H^+ gradient for As absorption, or that it was caustic to the microvilli, allowing free diffusion of arsenic through the damaged membrane. The decreased fecal excretion of arsenic in the mixture-treated rats was suggested to be a function of increased absorption, and the decreased urinary excretion, possibly due to chromium favoring conditions that increase tubular reabsorption of arsenic.

An acute intraperitoneal study in male rats studied the effect on mortality, growth, and the kidney (relative kidney weight and serum creatinine levels) of simultaneous single injection of low or high doses of sodium dichromate (5 mg/kg, equivalent to 0.87 mg Cr(VI)/kg; and 35 mg/kg, equivalent to 6.1 mg Cr(VI)/kg) with low or high doses of sodium arsenate (commonly called disodium arsenate; 25 mg/kg/day, equivalent to 10 mg As/kg; and 90 mg/kg, equivalent to 36 mg As/kg) (Mason and Edwards 1989). The observation period was four days. Statistical analyses were limited to comparison with controls; no analyses for interactions were reported. Simultaneous administration of the low doses

of arsenic and chromium appeared to antagonize the renal effects (increased relative kidney weight and increased serum creatinine) that resulted from the administration of each metal alone at the same dose as in the mixture. The increase in body weight gain was similar among the low arsenic, low chromium(VI), and low arsenic-low chromium groups (final body weights 121.7, 123.8, and 125.7%, respectively, of starting body weights, versus 112.4% for controls). There were no deaths at the low dose of each metal separately or together.

Additional combinations of high doses of one metal with low doses of the other were studied. Low arsenic with high chromium(VI) did not significantly alter mortality (25–33%), kidney weight, or serum creatinine, relative to high chromium(VI) alone (no mortality with low arsenic alone). Low chromium(VI) with high arsenic resulted in significant mortality as compared with no mortality with high arsenic alone or low chromium(IV) alone. Kidney weight and serum creatinine were approximately the same for the high dose arsenic-low dose chromium(VI) mixture as for each chemical alone at the same dose as in the mixture. The renal effects of each chemical alone, however, were not dose-related and in one instance, showed an inverse relationship with dose (serum creatinine at low arsenic was 96 μmole/L, but at high arsenic was 39.5 μmole/L, similar to controls). Thus, conclusions regarding interactions on renal endpoints at the high doses of either metal are problematic. In addition, conclusions regarding interactions on mortality are uncertain due to experimental design and reporting, and not particularly germane to the expected exposure at hazardous waste sites. Therefore, only the simultaneous low-dose part of the study is included in the summary table.

The data of Mason and Edwards (1989) suggest that chromium and arsenic antagonized each other's acute toxicity at the "low" intraperitoneal doses. Interpretation of results from the other dose combinations is problematic, and given the mortality, may not be particularly relevant.

In an acute intraperitoneal study of developmental toxicity, rats were injected on day 8 of gestation with 2 mg Cr(VI)/kg (from sodium dichromate), 5 mg As/kg (from sodium arsenate), or mixtures of the two ranging from 0.25 mg Cr(VI)/kg plus 0.63 mg As/kg to 2 mg Cr(VI)/kg plus 5 mg As/kg (Mason et al. 1989). No effects on maternal body weight gain, number of implants, live fetuses, resorptions, fetal weight, or fetal abnormalities were seen in the group treated with chromium(VI) alone. The only significant effect in the group treated with arsenic alone was an increase in percent of fetuses with ectrodactyly. In the groups given the mixtures, effects were seen only at the highest dose of both metals, which resulted in decreased maternal body weight gain, increased resorptions, decreased fetal body

weight, and increased percentages of fetuses with skeletal abnormalities, including retardation, delayed ossification of vertebrae, shortening of the ribs, and ectrodactyly. Limitations of the study design preclude determining whether this outcome reflects greater-than-additive, additive, or even less-than-additive joint action.

An *in vitro* study also investigated potential interactions of arsenic and chromium(VI) with regard to the kidney (Keith et al. 1995). Rabbit renal cortical slices were incubated with sodium arsenite and/or potassium dichromate and uptake was measured at 2 hours. Chromium(VI) slightly inhibited the uptake of arsenic, and arsenic inhibited the uptake of chromium(VI). Interpretation of these results is uncertain without additional information on the mechanism of uptake of these metals and their membrane or intracellular location in the tissue slices.

Table 13 summarizes the data regarding the effects of arsenic on the toxicity of chromium(VI) and Table 14 summarizes the data regarding the effects of chromium(VI) on the toxicity of arsenic.

Table 13. Summary of Available Data on the Influence of Arsenic on Toxicity/Carcinogenicity of Chromium(VI) by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal injection (mg/kg/day)						
Acute	Renal (relative weight, serum creatinine)			10 + 0.87 (r)	<additive	Mason and Edwards 1989
Acute	Maternal body weight gain		5 + 2 (r)		indeterminate: effects from mixture but not single metals at same dose as in mixture	Mason et al. 1989
Acute	Developmental		5 + 2 (r)		indeterminate: effects from mixture but not single metals at same dose as in mixture	Mason et al. 1989

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat

Table 14. Summary of Available Data on the Influence of Chromium(VI) on Toxicity/Carcinogenicity of Arsenic by Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute	Absorption of arsenic (blood concentrations 5–70 minutes)	(0.27 or 0.53) + (0.02 or 0.2) (r)			>additive	Gonzalez et al. 1995
Intraperitoneal injection (mg/kg/day)						
Acute	Renal (relative weight, serum creatinine)			0.87 + 10 (r)	<additive	Mason and Edwards 1989
Acute	Maternal body weight gain		2 + 5 (r)		indeterminate: effects from mixture but not single metals at same doses as in the mixture	Mason et al. 1989
Acute	Developmental		2 + 5 (r)		indeterminate: effects from mixture but not single metals at same doses as in the mixture	Mason et al. 1989

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat

2.2.8 Cadmium and Chromium(VI)

The database relevant to the joint toxic action is limited, consisting of a single animal study not designed to investigate joint toxic action, and two *in vitro* studies.

A study that included the effects of subcutaneous injection of chromium(VI) (a renal toxicant) on the tissue levels and urinary excretion of cadmium in 1-month-old female rats pretreated with cadmium in their drinking water was designed to investigate the mechanism of the increased excretion of cadmium in urine that occurs concomitantly with cadmium-induced renal damage (Bernard and Lauwerys 1981). The study provides little information regarding joint action for this pair of metals. In this study, rats pretreated with 100–200 ppm cadmium in their drinking water for 1 or 4 months as follows: the cadmium-treated groups received 100 ppm the first week, 150 ppm the second week, 200 ppm from the third week on. Approximate doses were 23 mg/kg/day for 1-month exposure and 27 mg/kg/day for 4-month exposure.

Pretreatment with cadmium in drinking water (to "load" the kidneys with cadmium), followed by 2 weeks without cadmium, and then a single subcutaneous injection of 10 or 20 mg/kg sodium chromate (3.2 or 6.4 mg Cr(VI)/kg), resulted in dose-related increased excretion of cadmium in the urine. That is, the cadmium excretion was higher for the longer cadmium pretreatment and for the higher chromium(VI) dose, and higher in all combined chromium(VI)-cadmium groups than in the cadmium-alone groups. Cadmium alone did not result in abnormal levels of protein or amino acids in the urine, but did increase urinary excretion of cadmium. Kidney concentrations of cadmium, both metallothionein-bound and free, were decreased by chromium(VI) in a dose-related manner; hepatic concentrations of cadmium were not affected. Loss of cadmium from the kidney and increased excretion in the urine was attributed to the renal damage caused by chromium(VI). Pretreatment of rats by intraperitoneal injection of 1 mg Cd/kg/day, 5 days/week for 2 weeks followed by subcutaneous injection with 10 mg sodium chromate/kg 3 times at 2-day intervals resulted in increased urinary excretion of cadmium and increased proteinuria and amino aciduria (relative to treatment with cadmium alone, which did not cause abnormal excretion of protein or amino acids). Urinary excretion data returned to normal within 10 days after chromium(VI) treatment. An additional subcutaneous administration of 10 mg sodium chromate/kg 3 weeks after the first chromate treatment caused a lesser increase in urinary excretion of cadmium, protein, and amino acids. Thus, the degree of renal damage following chromium(VI) injection appeared to be related to the amount of cadmium remaining in the rats, suggesting that cadmium contributed to the effects. The lack

of a group treated with chromium(VI) alone limits further interpretation. Whether the increased renal damage is a result of additivity or of greater (or less) than additive joint action cannot be determined from these data.

An *in vitro* study also investigated potential interactions of cadmium and chromium(VI) with regard to kidney (Keith et al. 1995). Rabbit renal cortical slices were incubated with cadmium chloride and/or potassium dichromate and uptake was measured at 2 hours. Each metal inhibited the uptake of the other. Interpretation of these results is uncertain without additional information on the mechanism of uptake of these metals and their membrane or intracellular location in the tissue slices.

Another *in vitro* study reported that cadmium alone did not induce apoptosis in Chinese hamster ovary cells, but chromium-induced apoptosis was markedly inhibited by cadmium in a dose-related manner (Shimada et al. 1998). It was hypothesized that cadmium's ability to suppress apoptosis might be an aspect of its carcinogenic mechanism.

Tables 15 and 16 summarize the limited data from the *in vivo* study of renal effects in rats.

Table 15. Summary of Available Data on the Influence of Cadmium on Toxicity/Carcinogenicity of Chromium(VI) by Sequential Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Injection or mixed injection/oral exposure (mg/kg/day)						
Intermediate pretreatment Cd; acute Cr(VI)	Renal (proteinuria, amino aciduria)		23 (1 month) or 27 (4 months) (o) + 3.2 or 6.4 (sc) (r)		indeterminate: renal damage greater from combined treatment than from Cd alone; dose-related for Cr and Cd	Bernard and Lauwerys 1981
Acute pretreatment Cd; acute Cr(VI)	Renal (proteinuria, amino aciduria)		(3.2 (sc), recovery, 3.2 (sc)) + 1 (ip) (r)		indeterminate: renal damage greater from combined treatment, and greater with1st than 2nd Cr(VI) treatment (for which renal Cd burden lower)	Bernard and Lauwerys 1981

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat
[c]Route code: sc = subcutaneous, ip = intraperitoneal, o = oral

Table 16. Summary of Available Data on the Influence of Chromium(VI) on Toxicity/Carcinogenicity of Cadmium by Sequential Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Injection or mixed injection/oral exposure (mg/kg/day)						
Acute Cr(VI); Intermediate pretreatment Cd	Renal (proteinuria, amino aciduria)		3.2 or 6.4 (sc) + 23 (1 month) or 27 (4 months) (o) (r)		indeterminate: renal damage greater from combined treatment than from Cd alone; dose-related for Cr and Cd	Bernard and Lauwerys 1981
Acute Cr(VI); acute Cd pretreatment	Renal (proteinuria, amino aciduria)		1 (ip) + (3.2 (sc), recovery, 3.2 (sc)) (r)		indeterminate: renal damage greater from combined treatment, and greater with1st than 2nd Cr(VI) treatment (for which renal Cd burden lower)	Bernard and Lauwerys 1981

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat
[c]Route code: sc = subcutaneous, ip = intraperitoneal, o = oral

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

Lead, arsenic, cadmium, and chromium frequently occur together in the soil of hazardous waste sites; the exposure scenario of greatest concern for this mixture is long-term, low-level oral exposure. No adequate epidemiological or toxicological studies of the quaternary mixture are available. A preliminary report of an *in vitro* study provided some results regarding the joint cytotoxic action of the four metals to human keratinocytes (Campain et al. 2000), but the relevance of this study to human health is uncertain. A few studies have addressed trinary mixtures of these metals.

An intermediate-duration study of dietary exposure of rats to lead, arsenic, and cadmium singly and as binary and trinary mixtures, provides relevant information (Fowler and Mahaffey 1978; Mahaffey and Fowler 1977; Mahaffey et al. 1981). In this study, the changes in the hematological and clinical chemistry values that resulted from exposure to the trinary mixture, as compared with the binary mixtures, tended to be small in magnitude and inconsistent in direction across different endpoints. On the whole, the effects were explained by the binary mixtures. This suggests that components-based approaches that focus on the binary mixtures may be useful in predicting the toxicity of the mixture.

A drinking water study of a mixture of lead, cadmium, and chromium(VI+III) in diethylnitrosamine-initiated rats gave no evidence of promoting activity for the mixture (Benjamin et al. 1999).

No PBPK models are available for the complete mixture or for any of the submixtures.

Data regarding potential interactions of pairs of these metals are voluminous for the lead-cadmium mixture, and fairly extensive for the lead-arsenic mixture. Many of the studies for these two binary mixtures are highly relevant in terms of route, sequence, and duration, but they have other limitations, as discussed in Section 2.2. The data indicate that the joint toxic action of these two pairs of metals may not be consistent across endpoints. Results also are not always consistent across studies for the same endpoint or target organ. The data for the other binary mixtures are less extensive, and sometimes less relevant in terms of route, sequence, duration, and endpoint. For these reasons, the weight-of-evidence approach for the assessment of interactions through the preparation of binary weight-of-evidence determinations (BINWOEs) is advisable for this mixture (ATSDR 2001a, 2001b).

In the introduction to this document, Table 1 presented an overview of the potential effects of concern from oral exposure to the lead, arsenic, cadmium, and chromium(VI). Each of the four metals affects a wide range of target organs and endpoints. There are a number of target organs in common across two or more of the metals. As shown in Table 17, however, the bases for the MRLs (critical effects) of lead, arsenic, and cadmium are different, and for chromium(VI), have not been defined. According to ATSDR (2001b) guidance, BINWOE determinations should be target-organ specific. There are at least some data pertinent to a number of target-organ specific BINWOE determinations for some of the pairs of metals, as indicated previously in Table 2. BINWOE determinations for the effects of the other metals on the toxicity of arsenic are problematic due to the lack of interactions data on the critical effect (dermal lesions) and on cancer, an effect of concern for oral exposure to arsenic.

Table 17. Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern. See Appendices A, B, C, and D for More Details.

Duration of Exposure	Lead	Arsenic	Cadmium	Chromium
Acute	none derived for any duration because of lack of clear threshold and need to consider multi-media exposure	none derived, inadequate data	none derived, inadequate data	none derived for any duration because cannot establish NOAELs and LOAELs for reproductive and developmental effects
Intermediate	effect of concern is neurological, particularly in children	none derived, inadequate data	none derived, inadequate data	upper range of the estimated safe and adequate daily dietary intake in humans (NRC 1989) is to be used as provisional guidance for Cr(VI) and (III); chronic RfD available for Cr(VI) (but no critical effect)
Chronic	slope factor approach is to be used to predict PbB; Centers for Disease Control (CDC) level of concern is 10 µg/dL	dermal lesions in humans	renal damage in humans	

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. Any pertinent endpoints for which the data indicate synergistic effects may need to be considered.

The recommended approach for a mixture to which significant exposure is occurring, for which no suitable physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model exists, and whose components have different critical effects, is to use the target-organ toxicity dose (TTD) modification to the hazard index method to assess joint toxic action. This approach involves the estimation of endpoint-specific hazard indexes for the endpoints of concern for a particular mixture. The BINWOEs are then used to qualitatively predict the impact of interactions on the endpoint-specific hazard indexes. Thus, the BINWOEs must be appropriate for those endpoints.

Endpoints of concern for this mixture are neurological, dermal, and renal effects (the critical or sensitive effects of lead, arsenic, and cadmium). In addition, cardiovascular and hematological effects are sensitive effects of at least two of these three chemicals (Table1), and synergistic interactions have been reported for testicular effects of lead and cadmium. These endpoints appear to be significant for chromium(VI) as well, although no MRL or determination of critical effects has been derived for chromium(VI).

BINWOE development was undertaken for these endpoints. The BINWOE classification scheme (Figure 1) and the rationales for the BINWOE determinations (Tables 18–45) are presented at the end of this section. During this endeavor, it became apparent that because mechanistic considerations for the metals are exceedingly complex, for pairs of metals lacking any toxicologically relevant interaction data, the mechanistic understanding was unlikely to be sufficiently clear to support a judgment of direction of interaction with any confidence. Therefore, the effort was refocused on pairs with some toxicologically relevant interaction data. As a consequence, not all the indeterminate BINWOE ratings summarized in this section and in Chapter 3 will have tables in this Section (2.3) explaining the rationale for the indeterminate rating.

The BINWOE determinations are presented for each pair of metals in the same order as the pairs were considered in Section 2.2. BINWOEs for the critical effects are presented first, followed by BINWOEs for the other relevant effects.

For lead and arsenic, BINWOEs have been developed for neurological (Tables 18 and 19), dermal (Table 20), renal (Tables 21 and 22), cardiovascular (Tables 23 and 24), and hematological (Tables 25 and 26) effects. Oral exposure to lead does not cause dermal effects and to arsenic is not known to cause testicular effects, so BINWOEs were not considered for these metals and effects. The BINWOEs for

neurological toxicity were greater than additive (low to moderate confidence, >IIIB and >IIB), for renal and hematological were less than additive (<IIIB), and for cardiovascular and dermal were indeterminate. The BINWOE for the effect of arsenic on the testicular toxicity of lead also was considered indeterminate; the rationale is not presented in a table.

For lead and cadmium, the binary mixture with the largest database on joint toxic action, BINWOEs have been developed for neurological (Tables 27 and 28), renal (Tables 29 and 30), cardiovascular (Tables 31 and 32), hematological (Tables 33 and 34), and testicular effects (Tables 35 and 36). Dermal effects were not included because the skin is not a target for the oral toxicity of these two metals. As with the lead-arsenic pair, inconsistencies in predicted direction of interaction are seen across endpoints, particularly for the effects of cadmium on the toxicity of lead: greater than additive for neurological (>IIIB) and testicular (>IIA) effects, less than additive for renal (<IIA) and hematological (<IIIB) effects, and additive for cardiovascular (=IIIA) effects. Thus, the confidence, as reflected in the alphanumeric scores, is higher for testicular and renal effects than for the other effects. For the effects of lead on the toxicity of cadmium, the BINWOEs were more consistent: indeterminate for neurological; additive for renal (=IIAii), cardiovascular (=IIIA), and hematological (=IIC); greater than additive for testicular (>IIA). Further discussion and comparison of BINWOEs for the lead-arsenic and lead-cadmium pairs are presented in Chapter 3.

For lead and chromium(VI), the only available study of interactions was an *in vitro* genotoxicity study. Therefore, BINWOEs for lead and chromium(VI) are considered indeterminate for most endpoints, and not applicable for dermal, because oral exposure to these metals is not dermally toxic. (Although oral exposure to chromium(VI) has been reported to exacerbate dermatitis due to dermal contact with chromium(VI), this is an immunological effect.) The rationales for the indeterminate ratings are not presented in tables in this section.

For arsenic and cadmium, BINWOEs have been developed for renal (Tables 37 and 38), dermal (Table 39), hematological (Tables 40 and 41), and testicular effects (Table 42). No BINWOE was provided for the effect of arsenic on the dermal toxicity of cadmium because the skin is not a target organ for ingested cadmium, or for the effect of cadmium on the testicular toxicity of arsenic, because the testes are not known to be a target of arsenic toxicity. BINWOE ratings were indeterminate or additive for dermal and renal effects, and less than additive for hematological effects of either metal (moderate confidence, <IIB) and for testicular effects of cadmium (low confidence, <IIIB2ii). BINWOEs for the

remaining effects (neurological and cardiovascular) were indeterminate; the rationales are not presented in tables in this section.

For arsenic and chromium(VI), greater-than-additive BINWOEs (low confidence, >IIIC) were derived for the effect of chromium(VI) on the dermal toxicity and other non-renal toxicities (neurological, cardiovascular, hematological, and carcingenic) of arsenic (Table 43). In addition, less-than-additive BINWOEs (low confidence, <IIIB2ii and <IIIC2ii) were developed for the effects of arsenic and chromium(VI) on each other's renal toxicity (Tables 44 and 45) and for the effect of arsenic on other non-renal toxicities (neurological, hematologic, and testicular) of chromium(VI) (Table 44).

For cadmium and chromium(VI), the only available study of joint exposure was not designed to investigate interactions, although it does investigate renal endpoints. BINWOEs for this pair are considered indeterminate and rationales are not presented in tables in this section.

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 18. Effect of **Lead** on **Arsenic:** Neurological Toxicity for Oral Exposure

BINWOE: >IIIB (+1 x 0.32 + 0.71 = +0.23)

Direction of Interaction - The interaction is predicted to be greater than additive based on effects of combined exposure on reading and spelling in children (Moon et al. 1985). Additional corroborating information is not available, and mechanistic data are unclear.

Mechanistic Understanding - Following 14 days of gavage administration of this pair of metals, lead decreased the arsenic concentrations in the brain of adult mice, as compared with arsenic alone at the same dose as in the mixture (Mejia et al. 1997), indicating possible inhibition of arsenic neurotoxicity. Both metals have been reported to affect neurotransmitter levels in brain (ATSDR 1999b; Mejia et al. 1997). The study of joint action of lead and arsenic on neurotransmitters indicated no apparent influence or additivity (Mejia et al. 1997; further detail provided under toxicological significance). Both lead and arsenic can bind to sulfhydryl groups of proteins and alter mitochondrial function (ATSDR 1999b, 2000a; Goyer 1995). The mechanisms for mitochondrial effects are not identical, although there is some overlap: lead stimulates ALAS through feedback derepression and inhibits ferrochelatase (ATSDR 1999b) and may affect mitochondrial respiration and phosphorylation (Goyer 1995); arsenic inhibits succinic dehydrogenase, uncouples oxidative phosphorylation (ATSDR 2000a; Goyer 1995), and may affect mitochondrial heme synthesis enzymes (Fowler and Mahaffey 1978). Because these mechanisms are deleterious, joint action is expected to be additive or greater than additive. Thus, interactions are conceivable, but the mechanistic data are ambiguous with regard to direction of interaction. Brain concentration data indicate a protective effect of lead on arsenic distribution to brain, but other potential mechanisms are consistent with additive or greater-than-additive joint action, and the human data on neurobehavioral effects reviewed under toxicological significance indicate greater-than-additive interaction. Thus, a rating of III is appropriate for mechanistic understanding.

Toxicological Significance - In children, studies using hair lead and arsenic concentrations as biomarkers of exposure have reported a potentiating interaction of lead on arsenic-associated decreases in reading and spelling (Moon et al. 1985). Gavage dosing of adult mice with a lead-arsenic mixture in a 14-day study resulted in changes in neurotransmitter concentrations which tended to be the same as for arsenic alone, or in a few instances, additive as compared with the slight changes seen with either metal alone at the same dose as in the mixture. Lead alone had little effect on neurotransmitters (Mejia et al. 1997). The human data on neurological effects suggest a greater-than-additive interaction, whereas the animal data on neurotransmitter levels and on brain concentrations of arsenic (Mejia et al. 1997) do not. It is unclear, however, whether changes in brain neurotransmitter levels are responsible for the neurobehavioral effects of these metals. The human data are given higher priority in predicting the direction of interaction. The known neurological effects of arsenic for low-level, long-term exposure include both peripheral and central nervous system effects (ATSDR 2000a). Because of limitations in the human data, lack of support from the animal neurotransmitter data, and the ambiguous mechanistic data, confidence in the assessment is intermediate to low. A classification of B is appropriate.

Additional Uncertainties - The human study accounted for many potential confounding variables, but not for the care-giving environment and nutritional status.

Table 19. Effect of **Arsenic** on **Lead:** Neurological Toxicity for Oral Exposure

BINWOE: >IIB (+1 x 0.71 x 0.71 = +0.50)

Direction of Interaction - The interaction is predicted to be greater than additive based on a study of maladaptive classroom behavior in children (Marlowe et al. 1985a). Supporting data are lacking, and mechanistic information is not clear.

Mechanistic Understanding - Following 14 days of gavage administration of this pair of metals, arsenic increased the lead concentrations in the brain of adult mice, as compared with lead alone at the same dose as in the mixture (Mejia et al. 1997), suggesting the possibility of a potentiation of lead neurotoxicity. Both metals have been reported to affect neurotransmitter levels in brain (ATSDR 1999b; Mejia et al. 1997). The study of joint action of lead and arsenic on neurotransmitters indicated no apparent influence or additivity (Mejia et al. 1997; further detail provided under toxicological significance). Both lead and arsenic can bind to sulfhydryl groups of proteins and alter mitochondrial function (ATSDR 1999b, 2000a; Goyer 1995). The mechanisms for mitochondrial effects are not identical, although there is some overlap: lead stimulates ALAS through feedback derepression and inhibits ferrochelatase (ATSDR 1999b) and may affect mitochondrial respiration and phosphorylation (Goyer 1995); arsenic inhibits succinic dehydrogenase, uncouples oxidative phosphorylation (ATSDR 2000a; Goyer 1995), and may affect mitochondrial heme synthesis enzymes (Fowler and Mahaffey 1978). Because these mechanisms are deleterious, joint action is expected to be additive or greater than additive. Thus, the mechanistic data, while not clear as to direction, do not indicate that arsenic will be protective, but rather that joint action may be additive or greater than additive. A rating of II is, therefore, appropriate for mechanistic understanding.

Toxicological Significance - In children, studies using hair lead and arsenic concentrations as biomarkers of exposure have reported a potentiating interaction of arsenic on lead-associated maladaptive classroom behavior (Marlowe et al. 1985a). Gavage dosing of adult mice with an arsenic-lead mixture in a 14-day study resulted in neurotransmitter concentrations which tended to be the same as for arsenic alone, or in a few instances, additive as compared with the slight changes seen with either metal alone at the same dose as in the mixture. Lead alone had little effect on neurotransmitters (Mejia et al. 1997). The human data on neurological effects (Marlowe et al. 1985a) suggest a greater-than-additive interaction, as do the animal data showing an increase in brain concentrations of lead from co-exposure to arsenic (Mejia et al. 1997), but the animal data on neurotransmitter levels suggest additivity (Mejia et al. 1997). It is unclear, however, whether changes in brain neurotransmitter levels are responsible for the neurobehavioral effects of these metals. The human data are given higher priority in predicting the direction of interaction. The endpoint is relevant to lead's neurobehavioral effects on children (ATSDR 1999b). Because of limitations in the human data and the support from the animal brain lead data but lack of support from the animal neurotransmitter data, confidence in the assessment is intermediate. A classification of B is appropriate.

Additional Uncertainties - The human study accounted for many potential confounding variables, but not for the care-giving environment and nutritional status.

Table 20. Effect of **Lead** on **Arsenic:** Dermal Toxicity for Oral Exposure

BINWOE: ? (0)

Direction of Interaction - The direction of interaction cannot be predicted due to the lack of clear mechanistic understanding and pertinent toxicological data.

Mechanistic Understanding - Concentrations of arsenic in skin of humans exposed to background levels of arsenic were higher than in other "live" tissues except blood (Liebscher and Smith 1968). Arsenic accumulated in the skin of animals given long-term exposure (Lingren et al. 1982). Arsenic reacts with the sulfhydryl groups of proteins, inactivates enzymes, and interferes with mitochondrial function. Relatively high-dose intermediate-duration toxicity to the skin is considered to be due to cytotoxic effects. Chronic low-level exposure to arsenic is thought to stimulate keratinocyte secretion of growth factors. The resulting increase in cell division and DNA replication would afford greater opportunities for genetic damage (ATSDR 2000a). Lead also interferes with mitochondrial function and reacts with sulfhydryl groups. Lead does not appear to be accumulated in the skin (ATSDR 1999b). No data regarding the effects of lead on concentrations of arsenic in skin were located; in general, oral coexposure to lead and arsenic decreased or did not affect levels of arsenic in soft tissue and bone (Elsenhans et al. 1987; Fairhall and Miller 1941; Mahaffey et al. 1981; Mejia et al. 1997). Mechanistic understanding indicates that there are possible points of interaction, but is insufficient to indicate a direction.

Toxicological Significance - No studies of the effect of lead on the dermal toxicity or dermal carcinogenicity of arsenic were located, and the mechanistic data do not support further assessment. The available data regarding interactions on other target organs in the rat indicate no effect or an inhibitory effect of lead on arsenic's hematological and renal toxicity (Fowler and Mahaffey 1978; Mahaffey et al. 1981); and in the mouse, no effect on brain neurotransmitter effects of arsenic (Mejia et al. 1997). In children, a potentiating effect of lead on arsenic-induced reading and spelling decrements has been reported (Moon et al. 1985). Thus, the direction of interaction is not consistent across these other endpoints. In addition, the applicability of this information to arsenic's dermal effects is uncertain.

Table 21. Effect of **Lead** on **Arsenic**: Renal Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction is predicted to be less than additive based on the apparent protective effect of lead against the renal effects of arsenic in a chronic oral study in rats (Fairhall and Miller 1941). Mechanistic data do not offer clear support.

Mechanistic Understanding - Lead did not affect the renal concentrations of arsenic in an acute (14-day) dietary study (Elsenhans et al. 1987) or an intermediate-duration dietary study (Mahaffey et al. 1981) in rats, but renal arsenic concentrations were increased in rats simultaneously exposed to lead in a chronic dietary study (Fairhall and Miller 1941), indicating possible potentiation by lead of distribution of arsenic to the kidney. Both lead and arsenic affect renal mitochondria (ATSDR 1999b, 2000a; Goyer 1995; Mahaffey et al. 1981). The mechanisms for mitochondrial effects are not identical, although there is some overlap: lead stimulates ALAS through feedback derepression and inhibits ferrochelatase (ATSDR 1999b) and may affect mitochondrial respiration and phosphorylation (Goyer 1995); arsenic inhibits succinic dehydrogenase, uncouples oxidative phosphorylation (ATSDR 2000a; Goyer 1995), and may affect mitochondrial heme synthesis enzymes (Fowler and Mahaffey 1978). Because these mechanisms are deleterious, joint action is expected to be additive or greater than additive. Thus, tissue distribution and mitochondrial mechanists suggest a possible additive or greater-than-additive joint action, which is not in clear agreement with the renal toxicity data, discussed under toxicological significance. Therefore, a rating of III is appropriate due to ambiguity.

Toxicological Significance - In an intermediate-duration dietary study, lead alone and a lead-arsenic mixture caused similar renal effects—cloudy swelling of the proximal tubules, intranuclear inclusion bodies, and mitochondrial swelling. Mitochondrial swelling was the only renal effect seen with arsenic alone. Doses of each metal in the mixture were the same as when given alone. The investigators did not consider these results indicative of an interaction (Mahaffey et al. 1981), and detail to support an independent assessment was not provided. In a chronic dietary study in rats, lead alone and arsenic alone both caused hyaline casts in the renal collecting tubules and ducts of Bellini; these effects were more marked in the lead alone group. Feeding of both lead and arsenic (as lead arsenate) at the same doses as when administered alone produced effects on this endpoint that were less severe than for arsenic alone (Fairhall and Miller 1941), indicating a less-than-additive joint toxicity. Again, sufficient detail for independent assessment was not reported. Doses in the chronic study were higher than in the intermediate study, and the higher doses and longer duration may account for the difference in outcome. The results of the chronic study are toxicologically relevant to arsenic renal toxicity, but because they are not supported by other toxicity data or by the mechanistic data, and the findings were not reported in detail, an intermediate rating of B is chosen.

Table 22. Effect of **Arsenic** on **Lead**: Renal Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction is predicted to be less than additive based on the apparent protective effect of arsenic against the renal effects of lead in a chronic oral study in rats (Fairhall and Miller 1941). Mechanistic data do not offer clear support.

Mechanistic Understanding - Renal lead concentrations were not affected in rats simultaneously exposed to arsenic in a chronic dietary study (Fairhall and Miller 1941). A 14-day study (Elsenhans et al. 1987) and an intermediate simultaneous oral study (Mahaffey et al. 1981) reported that renal lead was below the detection limit both with and without coexposure of the rats to arsenic. Both lead and arsenic affect renal mitochondria (ATSDR 1999b, 2000a; Goyer 1995; Mahaffey et al. 1981). The mechanisms for mitochondrial effects are not identical, although there is some overlap: lead stimulates ALAS through feedback derepression and inhibits ferrochelatase (ATSDR 1999b) and may affect mitochondrial respiration and phosphorylation (Goyer 1995); arsenic inhibits succinic dehydrogenase, uncouples oxidative phosphorylation (ATSDR 2000a; Goyer 1995), and may affect mitochondrial heme synthesis enzymes (Fowler and Mahaffey 1978). Because these mechanisms are deleterious, joint action is expected to be additive or greater than additive. Thus, tissue distribution and mitochondrial mechanists suggest a possible additive or greater-than-additive joint action, which is not in clear agreement with the renal toxicity data, discussed under toxicological significance. Therefore, a rating of III is appropriate due to ambiguity.

Toxicological Significance - In an intermediate-duration dietary study, lead alone and a lead-arsenic mixture caused similar renal effects—cloudy selling of the proximal tubules, intranuclear inclusion bodies, and mitochondrial swelling. Mitochondrial swelling was the only renal effect seen with arsenic alone. Doses of each metal in the mixture were the same as when given alone. The investigators did not consider these results indicative of an interaction (Mahaffey et al. 1981), and detail to support an independent assessment was not provided. In a chronic dietary study in rats, lead alone and arsenic alone both caused swelling of the renal convoluted tubule cells; effects were more marked in the lead alone group. Feeding of both lead and arsenic (as lead arsenate) at the same doses as when administered alone produced effects on this endpoint that were similar in severity to arsenic alone (Fairhall and Miller 1941), possibly indicating a less-than-additive joint action. In addition, lead alone resulted in intranuclear inclusion bodies in the kidney, arsenic alone did not, and this effect was less severe in the lead arsenate group than in the lead alone group. Again, sufficient detail for independent assessment was not reported. Doses in the chronic study were higher than in the intermediate study. The results of the chronic study are toxicologically relevant to lead renal toxicity, but because they are not supported by other data, including the mechanistic data, and the findings were not reported in detail, an intermediate rating of B is chosen.

Table 23. Effect of **Lead** on **Arsenic**: Cardiovascular Toxicity for Oral Exposure

BINWOE: ? (0)

Direction of Interaction - The direction cannot be predicted due to a lack of mechanistic understanding and pertinent toxicological data.

Mechanistic Understanding - Potential mechanisms for cardiovascular effects of lead include an impact on the renin-angiotensin system, increases in intracellular calcium, and activation of protein kinase C branch of the calcium messenger system (ATSDR 1999b). Discussion of a mechanistic basis for cardiovascular effects of arsenic was not encountered.

Toxicological Significance - No studies toxicologically relevant to the potential interactions of lead and arsenic on cardiovascular endpoints were available, and the mechanistic data do not support further assessment. The available data regarding interactions on other target organs in the rat indicate no effect or an inhibitory effect of lead on arsenic's hematological and renal toxicity (Fowler and Mahaffey 1978; Mahaffey et al. 1981); and in the mouse, no effect on brain neurotransmitter effects of arsenic (Mejia et al. 1997). In children, a potentiating effect of lead on arsenic-induced reading and spelling decrements has been reported (Moon et al. 1985). Thus, the direction of interaction is not consistent across these other endpoints. In addition, the applicability of this information to arsenic's dermal effects is uncertain.

Table 24. Effect of **Arsenic** on **Lead**: Cardiovascular Toxicity for Oral Exposure

BINWOE: ? (0)

Direction of Interaction - The direction cannot be predicted due to a lack of mechanistic understanding and pertinent toxicological data.

Mechanistic Understanding - Potential mechanisms for cardiovascular effects of lead include an impact on the renin-angiotensin system, increases in intracellular calcium, and activation of protein kinase C branch of the calcium messenger system (ATSDR 1999b). Discussion of a mechanistic basis for cardiovascular effects of arsenic was not encountered.

Toxicological Significance - No studies toxicologically relevant to the potential interactions of arsenic and lead on cardiovascular endpoints were available, and the mechanistic data do not support further assessment. The available data regarding interactions on other target organs in the rat indicate no effect or an inhibitory effect of arsenic on lead's hematological and renal toxicity (Fowler and Mahaffey 1978; Mahaffey et al. 1981; Fairhall and Miller 1941); and in the mouse, no effect on brain neurotransmitter effects of lead (Mejia et al. 1997). In children, a potentiating effect of arsenic on lead-induced maladaptive classroom behavior has been reported (Marlowe et al. 1985a). Thus, the direction of interaction is not consistent across these other endpoints. In addition, the applicability of this information to arsenic's dermal effects is uncertain.

Table 25. Effect of **Lead** on **Arsenic**: Hematological Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction on hematological effects is predicted to be less than additive based on the apparent protection by coexposure to lead against arsenic-induced decreases in hematocrit and hemoglobin in an intermediate dietary study in rats (Mahaffey and Fowler 1977;Mahaffey et al. 1981) and hemosiderosis (reflecting red cell destruction) in an chronic dietary study in rats (Fairhall and Miller 1941). The mechanistic data do not clearly support this conclusion.

Mechanistic Understanding - Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, which results in increased urinary ALA excretion, and by inhibiting the mitochondrial ferrochelatase-mediated insertion of iron into protoporphyrin, resulting in an elevation of zinc protoporphyrin in erythrocytes (ATSDR 1999b). At relatively high levels of exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Arsenic interferes with mitochondrial heme synthesis enzymes, resulting in increased urinary excretion of uroporphyrin, but not ALA (Fowler and Mahaffey 1978). Arsenic may have a toxic effect on the erythropoietic cells of the bone marrow, and increases hemolysis (ATSDR 2000a). There are potential points of interaction or additivity for lead and arsenic, but the direction is not clear, and might be predicted to be additive or greater-than-additive. Thus, the mechanistic data do not support the toxicological significance data, and are given a rating of III to reflect ambiguity.

Toxicological Significance - In an intermediate-duration dietary study in rats, hematocrit was significantly decreased and hemoglobin was slightly decreased by arsenic alone, but not by lead alone or the lead-arsenic mixture. The dose of each metal in the mixture was the same as when the metal was given alone (Mahaffey and Fowler 1977; Mahaffey et al. 1981). This finding indicates that coexposure to lead decreased the hematological toxicity of arsenic. Other endpoints related to arsenic's hematopoietic effects (urinary uroporphyrin and coproporphyrin excretion) indicated additivity or no effect of lead (Fowler and Mahaffey 1978; Mahaffey et al. 1981). In a chronic dietary study in rats, splenic hemosiderosis (an indication of red cell destruction) was less severe in rats coexposed to lead and arsenic than in rats exposed to arsenic alone (Fairhall and Miller 1941, indicating a protective effect of lead. Arsenic causes anemia in humans, so the toxicological data on hematocrit, hemoglobin, and hemosiderosis are clearly relevant, but limitations of study design and analysis precluded the full evaluation of interactions. Accordingly, an intermediate rating of B is appropriate.

Table 26. Effect of **Arsenic** on **Lead**: Hematological Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction on hematological effects is predicted to be less than additive based on the apparent protection by coexposure to arsenic against lead-induced decreases in hematopoiesis in a chronic dietary study in rats (Fairhall and Miller 1941). The mechanistic data so not clearly support this conclusion.

Mechanistic Understanding - Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, which results in increased urinary ALA excretion, and by inhibiting the mitochondrial ferrochelatase-mediated insertion of iron into protoporphyrin, resulting in an elevation of zinc protoporphyrin in erythrocytes (ATSDR 1999b). At relatively high levels of exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Arsenic interferes with mitochondrial heme synthesis enzymes, resulting in increased urinary excretion of uroporphyrin, but not ALA (Fowler and Mahaffey 1978). Arsenic may have a toxic effect on the erythropoietic cells of the bone marrow, and increases hemolysis (ATSDR 2000a). There are potential points of interaction or additivity for arsenic and lead, but the direction is not clear, and might be predicted to be additive or greater-than-additive. Thus, the mechanistic data do not support the toxicological significance data, and are given a rating of III to reflect ambiguity.

Toxicological Significance - In an intermediate-duration dietary study in rats, both arsenic and lead increased urinary coproporphyrin excretion, and the effect of the arsenic-lead mixture on this endpoint was additive (Fowler and Mahaffey 1978; Mahaffey et al. 1981). In a chronic dietary study in rats, lead-induced splenic myelosis (decreased splenic hematopoiesis) was less severe in rats coexposed to arsenic and lead than in rats exposed to lead alone at the same dose as in the mixture (Fairhall and Miller 1941), indicating a protective effect of arsenic. Lead inhibits heme synthesis and can cause anemia in humans. The toxicological data on decreased hematopoiesis are considered more directly relevant, but limitations of study design and analysis precluded the full evaluation of interactions. Accordingly, an intermediate rating of B is appropriate.

Additional Uncertainties - Supporting data were lacking.

Table 27. Effect of **Lead** on **Cadmium**: Neurological Toxicity for Oral Exposure

BINWOE: ? (0)

Direction of Interaction - The direction cannot be determined. The available studies of interactions are not in agreement, and confidence in the studies is low.

Mechanistic Understanding - Lead did not affect cadmium concentrations in the brain of adult rats following dietary (Nation et al. 1990; Skoczynska et al. 1994) or drinking water (Lockett and Leary 1986) coexposure. Lead and cadmium both have been reported to affect neurotransmitters in animals (ATSDR 1999b; Nation et al. 1989). It is not clear, however, that the neurotoxicity of these chemicals is due to effects on neurotransmitter levels. Both cadmium (Nation et al. 1989) and lead (ATSDR 1999b) may inhibit calcium entry into neurons, and lead may act as a calcium agonist within the cell. Thus, additive or greater-than-additive joint action is plausible, but the complexity of the literature regarding potential mechanism for the neurological effects of lead (ATSDR 1999b) does not support a simple hypothesis regarding potential mechanisms of interactions between cadmium and lead. Mechanistic understanding is not adequate to predict the joint action of these metals on neurological endpoints.

Toxicological Significance - A study in children, using hair cadmium and lead levels as biomarkers of exposure, reported no effect of lead on cadmium-associated verbal IQ decrements (Thatcher et al. 1982). Confidence in this study is low because it accounted for very few potentially confounding variables. Some neurobehavioral findings in adult rats indicate less-than-additive interactions. Although both lead and cadmium increased the rates of lever pressing in schedule-controlled responding, the mixture did not. Lead increased, cadmium decreased, and the mixture did not affect the activity levels of the rats (Nation et al. 1989, 1990). Because these studies in animals did not support the findings of a study in children (Marlowe et al. 1985a) that suggested a greater-than-additive effect of cadmium on lead-associated maladaptive classroom behavior (a measure more related to the endpoints in the rat study), confidence in the rat studies is not high.

Additional Uncertainties - A possible explanation for the discrepancy in results is that there is no interaction at low exposure levels (as in the children studied by Thatcher et al. 1982), but that the joint action is antagonistic at high exposure levels (as in the rats studied by Nation et al. 1989, 1990).

Table 28. Effect of **Cadmium** on **Lead**: Neurological Toxicity for Oral Exposure

BINWOE: >IIIC (+1 x 0.32 x 0.32 = +0.10)

Direction of Interaction - The direction is greater than additive, based on a study of maladaptive classroom behavior in children (Marlowe et al. 1985a). The data are not consistent across studies in children or studies in animals; greater weight is given the higher quality study in children. Mechanistic data are ambiguous.

Mechanistic Understanding - Cadmium did not affect lead concentrations in the brain of adult rats following dietary coexposure, but decreased PbB (Mahaffey et al. 1981; Nation et al. 1990; Skoczynska et al. 1994). Lead and cadmium both have been reported to affect neurotransmitters in animals (ATSDR 1999b; Nation et al. 1989). In adult rats treated with cadmium and lead in the diet for an intermediate duration, cadmium attenuated the lead-induced perturbation of dopamine and serotonin turnover (Nation et al. 1989). Both cadmium (Nation et al. 1989) and lead (ATSDR 1999b) may inhibit calcium entry into neurons, and lead may act as a calcium agonist within the cell. The interference with calcium may indicate the possibility of additive or greater-than-additive joint action. Because mechanistic understanding is ambiguous, a rating of III is appropriate.

Toxicological Significance - In children, studies using hair cadmium and lead levels as biomarkers of exposure have reported a potentiating interaction of cadmium on lead-associated maladaptive classroom behavior (Marlowe et al. 1985a), but not on lead-induced performance IQ decrements (Thatcher et al. 1982). In adult rats treated with cadmium and lead in the diet for an intermediate duration, cadmium attenuated the lead-induced perturbation of dopamine and serotonin turnover (Nation et al. 1989). Although both cadmium and lead increased the rates of lever pressing in schedule-controlled responding, the mixture did not (Nation et al. 1989). Cadmium decreased, lead increased, and the mixture did not affect the activity levels of the rats (Nation et al. 1990). These endpoints in rats may be related to the classroom behavior endpoint in children, but the effect in these rat studies appears to be an antagonism. A chronic drinking water study in rats reported an apparent potentiation by cadmium of a depressive effect of lead on activity levels (Lockett and Leary 1986) at dose levels lower than tested by Nation et al. (1989, 1990). More weight is given to the human data, particularly because children (and immature animals) are more sensitive than adults, and to the lower-dose animal data (Lockett and Leary 1986). The finding of a lack of interaction with regard to performance IQ does not negate the possibility of an interaction on classroom behavior. In addition, the study on performance IQ accounted for confounding variables far less well than did the study on classroom behavior. Confidence in the assessment is low because the results of Marlowe et al. (1985a) are not supported by other human data and the animal data are not consistent across studies. Therefore, a ranking of C is chosen for toxicological significance.

Additional Uncertainties - The study reporting an interaction on classroom behavior accounted for many potential confounding variables, but not for the care-giving environment and nutritional status. It is possible that at lower exposure levels (as in the children studied by Marlowe et al. 1985a and the rats studied by Lockett and Leary 1986), the interaction is potentiating, and at higher exposure levels (as in the rats studied by Nation et al. 1989, 1990), the interaction is antagonistic.

Table 29. Effect of **Lead** on **Cadmium**: Renal Toxicity for Oral Exposure

BINWOE: = IIAii (0 x 0.71 x 1 x 0.79 = 0)

Direction of Interaction - The direction of interaction for renal effects, the critical effect of cadmium by the oral route, is predicted to be additive, based primarily on human occupational exposure data. (Buchet et al. 1981; Roels et al. 1978). Toxicological interaction data for this endpoint by the oral route are not available for humans and are inadequate for animals. Mechanistic data regarding the accumulation of cadmium in the kidney are conflicting, but the study with the most relevant design indicates that lead does not affect accumulation of cadmium in the kidney.

Mechanistic Understanding - The accumulation of cadmium in the kidney is associated with renal effects. Four studies of oral coexposure to lead and cadmium in rats and mice investigated the impact of lead on renal cadmium concentrations. The most relevant of the three studies (Mahaffey et al. 1981) indicates that lead does not affect the accumulation of cadmium in the kidney (thus, additive). Mechanistic understanding was therefore assigned an intermediate classification of II to reflect intermediate confidence in the mechanistic data.

Toxicological Significance - In two studies of smelter workers exposed to lead or lead and cadmium, renal dysfunction (proteinuria) correlated with cadmium exposure only (Buchet et al. 1981; Roels et al. 1978). Further analysis for potential interactions of lead and cadmium on kidney function revealed none (Buchet et al. 1981). Thus, lead did not affect the renal toxicity of cadmium. Renal dysfunction is the critical effect of cadmium for the chronic MRL; lead also can cause renal effects, but this is a relatively insensitive effect of lead. Two intermediate-duration oral studies of potential interactions of lead and cadmium in animals have investigated renal histopathology, but cadmium itself apparently did not cause renal effects in rats in one study (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Cadmium alone, lead alone, and the mixture caused renal damage in mice in the other study, which did not provide any comparative data regarding incidence or severity among the groups (Exon et al. 1979). Indices of renal damage in the animal studies, however, may not have been as sensitive as in the human studies, and a 10-week oral study may be too short for reasonable doses of cadmium to cause renal histopathology. A classification of A was selected to indicate the clear toxicological significance of the two human studies, and support from the mechanistic data.

Modifying Factors - A modifying factor for a different route of exposure (ii) is applied to account for application of interaction data from the inhalation route to an oral exposure scenario. The effects of cadmium and lead on the kidney are not route-specific, but some uncertainties are associated with the extrapolation from inhalation to oral.

Additional Uncertainties - The BINWOE determination is based primarily on intermediate- and chronic-duration data. It is less certain that it would apply to acute exposure, or that acute oral exposures associated with hazardous waste sites would be sufficient to result in renal effects of cadmium. The lead exposures in the occupational studies were not associated with any indices of renal damage. It is possible that the potential for interactions would be greater with higher lead exposures.

Table 30. Effect of **Cadmium** on **Lead**: Renal Toxicity for Oral Exposure

BINWOE: <IIA (-1 x 0.71 x 1.0 = -0.71)

Direction of Interaction - The predicted direction is less than additive, based on intermediate-duration dietary, drinking water, and gavage studies in rats and mice, which indicate that simultaneous administration of cadmium protected against renal lead accumulation, lead-induced renal histopathological effects, and intranuclear inclusion bodies. Mechanistic understanding indicates that cadmium may reduce the levels of lead in the kidney, possibly by interfering with absorption.

Mechanistic Understanding - Cadmium may interfere with the absorption of lead. In 14-day and intermediate-duration oral studies in animals, lead concentrations in blood and a number of tissues including the kidney were decreased by coexposure to cadmium (Elsenhans et al. 1987; Exon et al. 1979; Mahaffey et al. 1981; Nation et al. 1990; Skoczynska et al. 1994). The data were not entirely consistent, but the weight of evidence indicates a decrease. It has been suggested (Mahaffey and Fowler 1977) that cadmium may alter the surface of the gastrointestinal tract, causing malabsorption, as has been seen in Japanese quail. While this hypothesis may be plausible, there are little data, other than the decreased blood and tissue levels of lead, to support it. Therefore, an intermediate rating of II is selected for mechanistic understanding.

Toxicological Significance - No oral studies regarding potential impact of cadmium on lead's renal toxicity in humans are available. Occupational studies of exposure to cadmium and lead determined that indices of renal dysfunction correlated with cadmium and not with lead exposure (Buchet et al. 1981; Roels et al. 1978). This may be because occupational standards designed to protect against sensitive lead effects may protect against renal damage. In orally exposed animals, however, cadmium coexposure protected against the renal accumulation and toxicity of lead. This conclusion is based on the elimination of lead-induced renal histopathological effects following intermediate-duration simultaneous dietary exposure of rats to cadmium (Mahaffey and Fowler 1977; Mahaffey et al. 1981), the decrease or elimination of lead-containing intranuclear inclusion bodies in the renal tubular cells of rats by simultaneous dietary exposure to cadmium (Mahaffey and Fowler 1977; Mahaffey et al. 1981) and by drinking water coexposure to cadmium in mice (Exon et al. 1979), and decreased renal concentrations of lead in rats coexposed to cadmium in the diet (Mahaffey et al. 1981) or by gavage (Skoczynska et al. 1994). Renal effects of lead are similar in animals and humans, so the interactions are expected to be applicable to humans. The appropriate classification for toxicological significance is A.

Table 31. Effect of **Lead** on **Cadmium**: Cardiovascular Toxicity for Oral Exposure

BINWOE: =IIIA (0 x 0.32 x 1 = 0)

Direction of Interaction - The predicted direction is additive, based on a study of associations between tissue lead and cadmium and cardiovascular-related mortality in humans (Voors et al. 1982). This conclusion is supported by a series of intermediate-chronic drinking water studies in rats, which, overall, also indicate additivity of cadmium and lead effects on systolic blood pressure.

Mechanistic Understanding - Potential mechanisms for cardiovascular effects of lead include an impact on the renin-angiotensin system, increases in intracellular calcium, and activation of protein kinase C branch of the calcium messenger system (ATSDR 1999b). Discussion of a mechanistic basis for cardiovascular effects of cadmium was not encountered. Accordingly, the rating for mechanistic understanding is III.

Toxicological Significance - A study of cardiovascular-related deaths in an area of the United States where oral exposure to cadmium and lead was expected to be elevated indicated that tissue lead and cadmium each were significantly association with the proportion of deaths from cardiovascular disease, and that combined impact was compatible with additivity (Voors et al. 1982). Drinking water studies of lead and cadmium coexposure in rats generally indicated additive effects of the two metals on systolic blood pressure (Kopp et al. 1980a, 1980b; Perry and Erlanger 1978).

Additional Uncertainties - These studies of hypertension in rats used special low-metal housing and diets; their relevance to humans is uncertain.

Table 32. Effect of **Cadmium** on **Lead**: Cardiovascular Toxicity for Oral Exposure

BINWOE: =IIIA (0 x 0.32 x 1 = 0)

Direction of Interaction - The predicted direction is additive, based on a study of associations between tissue lead and cadmium and cardiovascular-related mortality in humans (Voors et al. 1982). This conclusion is supported by a series of intermediate-chronic drinking water studies in rats, which, overall, also indicate additivity of cadmium and lead effects on systolic blood pressure.

Mechanistic Understanding - Potential mechanisms for cardiovascular effects of lead include an impact on the rein-angiotensin system, increases in intracellular calcium, and activation of protein kinase C branch of the calcium messenger system (ATSDR 1999b). Discussion of a mechanistic basis for cardiovascular effects of cadmium was not encountered. Accordingly, the rating for mechanistic understanding is III.

Toxicological Significance - A study of cardiovascular-related deaths in an area of the United States where oral exposure to cadmium and lead was expected to be elevated indicated that tissue lead and cadmium each were significantly association with the proportion of deaths from cardiovascular disease, and that combined impact was compatible with additivity (Voors et al. 1982). Drinking water studies of lead and cadmium coexposure in rats generally indicated additive effects of the two metals on systolic blood pressure (Kopp et al. 1980a, 1980b; Perry and Erlanger 1978).

Additional Uncertainties - These studies of hypertension in rats used special low-metal housing and diets; their relevance to humans is uncertain.

Table 33. Effect of **Lead** on **Cadmium**: Hematological Toxicity for Oral Exposure

BINWOE: =IIC (0 x 0.71 x 0.32 = 0)

Direction of Interaction - The direction of interaction on hematopoietic effects is predicted to be additive based on apparent additive effects on erythrocyte size and hemoglobin content (Thawley et al. 1977).

Mechanistic Understanding - Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, and inhibiting the insertion of iron into protoporphyrin, mediated by ferrochelatase (ATSDR 1999b). Cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract (ATSDR 1999a). Thus, potential additive or greater-than-additive effects of lead plus cadmium on hematological parameters might be expected based on metal-specific mechanisms of inhibition of heme synthesis. Because the mechanistic data do not clearly indicate the mode of joint action, an intermediate rating of II is chosen.

Toxicological Significance - In intermediate-duration dietary studies in rats, decreased hematocrit and hemoglobin were seen in rats exposed to lead and cadmium in the diet, but not in those exposed to either alone at the same doses as in the mixture (Mahaffey and Fowler 1977; Mahaffey et al. 1981; Thawley et al. 1977). This finding indicates that subthreshold exposures to these metals can, in combination, result in hematological effects, but does not define whether joint action is additive, less than additive, or greater than additive. Decreases in erythrocyte size and hemoglobin content (MCV, MCH, MCHC) resulting from exposure to the mixture appeared additive as compared with exposure to each metal alone at the same dose as in the mixture (Thawley et al. 1977). Cadmium exposure by the oral or inhalation route causes anemia in humans, so the toxicological data are relevant. Although the data of Thawley et al. (1977) indicate an additive joint action on erythrocyte size and hemoglobin content, the decreased values seen with each metal alone were not statistically significant, and duration of this study may have been insufficient to allow full expression of effects on these hematological endpoints, so confidence in the conclusion of additivity is low. A classification of C is appropriate.

Additional Uncertainties - Limitations of study design and analysis precluded the full evaluation of interactions.

Table 34. Effect of **Cadmium** on **Lead**: Hematological Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction on hematopoietic effects is predicted to be less than additive, based on decreased PbB and decreased urinary ALA (delta-aminolevulinic acid) in animals coexposed to cadmium and lead through the diet for intermediate durations (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Mechanistic understanding is ambiguous.

Mechanistic Understanding - Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, and inhibiting the insertion of iron into protoporphyrin, mediated by ferrochelatase. As a result of alterations in the activity of ALAS and ALAD, ALA accumulates in blood, urine and soft tissues (ATSDR 1999b). Cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract (ATSDR 1999a). Thus, potential additive or greater-than-additive effects of cadmium plus lead on hematological parameters might be expected based on metal-specific mechanisms of inhibition of heme synthesis. Interference of cadmium with absorption of lead, however, may be indicated by the decreased PbB in rats exposed to cadmium and lead, as compared with lead alone. This mechanism might be expected to result in an apparent decrease in lead's hematopoietic toxicity. Thus, mechanistic data are ambiguous, and are given a rating of III.

Toxicological Significance - In an intermediate-duration dietary study in rats, cadmium inhibited the lead-induced increase in urinary ALA (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Increased excretion of urinary ALA is a result of the effects of lead on heme synthesis. Cadmium's amelioration of this effect of lead indicates that cadmium may inhibit lead's hematopoietic effects. Decreased hematocrit and hemoglobin were seen in rats exposed to both metals, but not in those exposed to either alone at the same doses as in the mixture (Mahaffey and Fowler 1977; Mahaffey et al. 1981; Thawley et al. 1977). This finding indicates that subthreshold exposures to these metals can, in combination, result in hematological effects, but does not define whether joint action is additive, less than additive, or greater than additive. Decreases in erythrocyte size and hemoglobin content (MCV, MCH, MCHC) resulting from exposure to the mixture appeared additive as compared with exposure to each metal alone at the same dose as in the mixture (Thawley et al. 1977), but the effects of each metal alone were not statistically significant and duration of this study may have been insufficient to allow full expression of effects on these hematological endpoints. More confidence is placed in the urinary ALA results, which indicate the possibility of a less than additive interaction on lead's inhibition of heme synthesis. Because mechanistic considerations are ambiguous, overall confidence in this assessment is medium to low, leading to a classification of B.

Additional Uncertainties - Limitations of study design and analysis precluded the full evaluation of interactions.

Table 35. Effect of **Lead** on **Cadmium**: Testicular Toxicity for Oral Exposure

BINWOE: >IIA (+1 x 0.71 x 1 = +0.71)

Direction of Interaction - The predicted direction is greater than additive, based on synergistic effects in an intermediate dietary study (Saxena et al. 1989) and two injection studies in rats. Mechanistic data, while not conclusive, support the plausibility of the interaction.

Mechanistic Understanding - Mechanistic understanding is incomplete. Because simultaneous administration of zinc protected against the synergistic effects of lead and cadmium on the testes (Saxena et al. 1989), the interaction may be mediated through effects on zinc-containing enzymes, including DNA and RNA polymerases. Both lead and cadmium interfere with zinc-enzyme complexes (ATSDR 1999a, 1999b). Thus, additive or greater-than-additive joint action is plausible, and an appropriate classification is II.

Toxicological Significance - In an intermediate-duration drinking water study in which the total dose of metal was kept constant (such that doses of lead and cadmium when given together were half the doses of each metal given separately), the effects of lead and cadmium on sperm counts and on seminiferous tubule damage in rats were synergistic (Saxena et al. 1989). Similar results were seen in intermediate-duration intraperitoneal and intramuscular injection studies in rats (Der et al. 1979; Fahim and Khare 1980). The toxicological significance is clear, and the results are consistent across studies, so the appropriate rating is A.

Table 36. Effect of **Cadmium** on **Lead**: Testicular Toxicity for Oral Exposure

BINWOE: >IIA (+1 x 0.71 x 1 = +0.71)

Direction of Interaction - The predicted direction is greater than additive, based on synergistic effects in an intermediate dietary study (Saxena et al. 1989) and two injection studies in rats. Mechanistic data, while not conclusive, support the plausibility of the interaction.

Mechanistic Understanding - Mechanistic understanding is incomplete. Because simultaneous administration of zinc protected against the synergistic effects of lead and cadmium on the testes, the interaction may be mediated through effects on zinc-containing enzymes, including DNA and RNA polymerases. Both lead and cadmium interfere with zinc-enzyme complexes (ATSDR 1999a, 1999b). Thus, additive or greater-than-additive joint action is plausible, and an appropriate classification is II.

Toxicological Significance - In an intermediate-duration drinking water study in which the total dose of metal was kept constant (such that doses of lead and cadmium when given together were half the doses of each metal given separately), the effects of lead and cadmium on sperm counts and on seminiferous tubule damage in rats were synergistic (Saxena et al. 1989). Similar results were seen in intermediate-duration intraperitoneal and intramuscular injection studies in rats (Der et al. 1979; Fahim and Khare 1980). The toxicological significance is clear, and the results are consistent across studies, so the appropriate rating is A.

Table 37. Effect of **Arsenic** on **Cadmium**: Renal Toxicity for Oral Exposure

BINWOE: ? (0)

Direction of Interaction - The direction of interaction for renal effects, the critical effect of cadmium by the oral route, cannot be predicted. The available toxicological data are inadequate, and mechanistic data, although voluminous, are ambiguous.

Mechanistic Understanding - The accumulation of cadmium in the kidney above a critical concentration is associated with renal effects (ATSDR 1999a; IRIS 2001). In intermediate-duration oral studies in rats, coexposure to arsenic did not affect concentrations of cadmium in the kidney (Mahaffey et al. 1981; Schmolke et al. 1992), indicating additivity (no interaction). Arsenic induces MT, a protein which binds and sequesters cadmium, protecting cellular components from the toxicity of free cadmium. On the other hand, the CdMT complex retains cadmium within the kidney and other tissues (ATSDR 1999a; Habeebu et al. 2000; Liu and Klassen 1996; Liu et al. 1998, 1999a, 1999b). If released into the circulation by the liver (or administered by injection), CdMT is toxic to the renal proximal convoluted tubules, both directly to the brush border membrane (Cherian 1985; Suzuki and Cherian 1987), and indirectly through reabsorption, followed by release of free cadmium intracellularly, which may cause tissue damage unless the capacity of the kidney to produce intracellular metallothionein to bind the cadmium is sufficient (ATSDR 1999a; Liu et al. 1999b). Thus, predicting the consequences of concurrent oral exposure to arsenic and cadmium is problematic, because the outcome may depend on the balance between release of the toxic CdMT complex from liver versus induction of renal intracellular MT to bind (detoxify) cadmium. In addition, higher MT levels in the kidney may result in greater retention of cadmium in the kidney. Therefore, MT induction may provide some short-term protection against renal damage, but could conceivably increase the renal accumulation of cadmium, resulting in exceedance of the critical concentration and the development of chronic renal toxicity. Thus, the mechanistic understanding is ambiguous.

Toxicological Significance - Renal dysfunction is the critical effect of cadmium for the chronic MRL. Arsenic also can cause renal effects, but this is a relatively insensitive and uncommon effect of arsenic (ATSDR 2000a). A 10-week oral study of potential interactions of arsenic and cadmium in rats reported no renal effects from cadmium alone, and only ultrastructural effects (mitochondrial swelling) in the kidneys of rats exposed to arsenic alone. The effects of the mixture, which contained the same dose of each metal as when they were given individually, were the same as those of arsenic alone (Mahaffey and Fowler 1977; Mahaffey et al. 1981). For cadmium, 10 weeks of oral exposure may not be long enough for renal histopathological effects to develop when reasonable doses are used. In an acute intraperitoneal study of lethality, the effects of the mixture on congestion of the glomerulus were more severe than from either metal alone at the same dose as in the mixture (Diaz-Barriga et al. 1990), but the study design and reporting of the data were not adequate to determine whether joint action was additive or different from additive, and intraperitoneal injection is not a good model for oral administration for cadmium. In sequential parenteral studies, pretreatment of animals with arsenic (Hochadel and Waalkes 1997), with low doses of cadmium (Goering and Klaassen 1984), or with other inducers of metallothionein (ATSDR 1999a) protected against the lethality and acute hepatoxicity of cadmium. The applicability of this acute, sequential, parenteral data on non-renal endpoints to simultaneous oral exposure and renal effects is questionable. Thus, the toxicological data do not indicate whether or not an interaction affecting the renal toxicity of cadmium is likely.

Table 38. Effect of **Cadmium** on **Arsenic**: Renal Toxicity for Oral Exposure

BINWOE: =IIB (0 x 0.71 x 0.71 = 0)

Direction of Interaction - The direction of interaction for renal effects, a relatively insensitive effect for arsenic, is predicted to be additive based on the lack of effect of cadmium on the renal toxicity and renal concentrations of arsenic in an intermediate-duration oral study in rats (Mahaffey and Fowler 1977; Mahaffey et al. 1981).

Mechanistic Understanding - Intermediate-duration oral studies in rats indicate that coexposure to cadmium did not affect renal arsenic concentrations, as compared with arsenic alone at the same dose (Mahaffey et al. 1981; Schmolke et al. 1992). This would indicate additivity (no interaction). Additional potential mechanistic impacts could come from the induction of metallothionein by cadmium, and the potential protective antioxidant effect of metallothionein on the toxicity of arsenic. Metallothionein is induced by chemicals that produce oxidative stress and protects against oxidative damage. Metallothionein would not be expected to sequester arsenic, as the affinity of arsenic for metallothionein is low (NRC 1999). Thus, mechanistic understanding could support either additive or less-than-additive joint action, and is accordingly classified as II.

Toxicological Significance - Renal dysfunction is the critical effect of cadmium for the chronic MRL. Arsenic also can cause renal effects, but this is a relatively insensitive and uncommon effect of arsenic (ATSDR 2000a). A 10-week oral study of potential interactions of arsenic and cadmium in rats reported no renal effects from cadmium alone, and only ultrastructural effects (mitochondrial swelling) in the kidneys of rats exposed to arsenic alone. The effects of the mixture, which contained the same dose of each metal as when they were given individually, were the same as those of arsenic alone (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Thus, cadmium did not affect the renal toxicity of arsenic. Arsenic is known to disrupt mitochondrial function, so the effect is toxicologically significant. In an acute intraperitoneal study of lethality, the effects of the mixture on congestion of the glomerulus were more severe than from cadmium alone; arsenic alone caused cortical congestion. The study design and reporting of the data were not adequate to determine whether joint action was additive or different from additive, and intraperitoneal injection is not a good model for oral administration for cadmium. The data from the oral study of renal effects indicate the likely direction is additive. Because there are no supporting data, other than the lack of effect of cadmium on renal arsenic levels, confidence in this assessment is not high; a rating of B is selected.

Additional Uncertainties - For cadmium, 10 weeks of oral exposure may not be long enough for renal histopathological effects to develop when reasonable doses are used. Whether a longer duration coexposure to cadmium and arsenic would be more likely to result in an interaction is uncertain.

Table 39. Effect of **Cadmium** on **Arsenic:** Dermal Toxicity for Oral Exposure

BINWOE: ? (0)

Direction of Interaction - The direction of interaction cannot be predicted due to the lack of clear mechanistic understanding and pertinent toxicological data.

Mechanistic Understanding - Concentrations of arsenic in skin of humans exposed to background levels of arsenic were higher than in other "live" tissues except blood (Liebscher and Smith 1968). Arsenic accumulated in the skin of animals given long-term exposure (Lingren et al. 1982). Arsenic reacts with the sulfhydryl groups of proteins, inactivates enzymes, and interferes with mitochondrial function. Relatively high-dose intermediate-duration toxicity to the skin is considered to be due to cytotoxic effects. Chronic, low-level exposure to arsenic is thought to stimulate keratinocyte secretion of growth factors. The resulting increase in cell division and DNA replication would afford greater opportunities for genetic damage (ATSDR 2000a). Arsenic induces metallothionein, but only a small percentage of administered arsenic is bound to metallothionein. The affinity of arsenic for metallothionein is much lower than that of cadmium. It has been suggested that metallothionein might protect against arsenic toxicity by acting as an antioxidant against oxidative injury produced by arsenic (ATSDR 2000a). Cadmium was a more potent inducer of metallothionein in an intraperitoneal study (Hochadel and Waalkes 1997). A single pretreatment with cadmium (to induce metallothionein) did not protect against the lethality of arsenic in rats in a subcutaneous injection experiment (Hochadel and Waalkes 1997), but 8-day pretreatment with cadmium did protect against arsenic lethality in mice in another injection study (Kreppel et al. 1988).

Toxicological Significance - No studies of the effect of cadmium on the dermal toxicity or dermal carcinogenicity of arsenic were located, and the mechanistic data do not support further assessment. The available data regarding interactions on other target organs in the rat indicate no effect or an inhibitory effect of cadmium coexposure on arsenic's hematological, hepatic, and renal toxicity (Fowler and Mahaffey 1978; Mahaffey and Fowler 1977; Mahaffey et al. 1981). In general, coadministration of cadmium tended to decrease or have no effect on tissue levels of coadministered arsenic (Mahaffey et al. 1981). The acute intraperitoneal lethality of arsenic was increased by simultaneous injection of cadmium, as were cardiac arsenic levels (Diaz-Barriga et al. 1990; Yanez et al. 1991), but lethality is of questionable relevance. The direction of interaction is not consistent across these other endpoints, although it tends to be additive or less than additive for the more relevant endpoints. The applicability of even the more relevant endpoints to arsenic's dermal effects is uncertain.

Table 40. Effect of **Arsenic** on **Cadmium**: Hematological Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction on hematological effects is predicted to be less than additive based on the apparent protection by coexposure to arsenic against changes in red cell count and hematocrit in an intermediate dietary study in rats (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Mechanistic understanding is ambiguous.

Mechanistic Understanding - Arsenic interferes with mitochondrial heme synthesis enzymes, resulting in increased urinary excretion of uroporphyrin (Fowler and Mahaffey 1978). Arsenic may have a toxic effect on the erythropoietic cells of the bone marrow, and increases hemolysis (ATSDR 2000a). Cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract (ATSDR 1999a). Thus, potential additive or greater-than-additive effects of arsenic plus cadmium on hematological parameters might be expected based on metal-specific mechanisms of inhibition of heme synthesis. Because the mechanistic data do not clearly indicate the mode of joint action, and conflict with the toxicological data, a rating of III is chosen.

Toxicological Significance - In an intermediate-duration dietary study in rats, both arsenic and cadmium increased the red blood cell count, and arsenic decreased the hematocrit (cadmium decreased hematocrit slightly but not significantly). Effects of the mixture were less than additive on these endpoints (Mahaffey and Fowler 1977; Mahaffey et al. 1981). The toxicological data are relevant to the hematological toxicity of cadmium. Limitations of study design and analysis precluded the full evaluation of interactions and supporting data are lacking. Accordingly, an intermediate rating of B is appropriate.

Table 41. Effect of **Cadmium** on **Arsenic**: Hematological Toxicity for Oral Exposure

BINWOE: <IIIB (-1 x 0.32 x 0.71 = -0.23)

Direction of Interaction - The direction of interaction on hematological effects is predicted to be less than additive based on the apparent protection by coexposure to cadmium against changes in red cell count and hematocrit in an intermediate dietary study in rats (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Mechanistic understanding is ambiguous.

Mechanistic Understanding - Arsenic interferes with mitochondrial heme synthesis enzymes, resulting in increased urinary excretion of uroporphyrin (Fowler and Mahaffey 1978). Arsenic may have a toxic effect on the erythropoietic cells of the bone marrow, and increases hemolysis (ATSDR 2000a). Cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract (ATSDR 1999a). Thus, potential additive or greater-than-additive effects of arsenic plus cadmium on hematological parameters might be expected based on metal-specific mechanisms of inhibition of heme synthesis. Because the mechanistic data do not clearly indicate the mode of joint action, and conflict with the toxicological data, a rating of III is chosen.

Toxicological Significance - In an intermediate-duration dietary study in rats, both arsenic and cadmium increased the red blood cell count, and arsenic decreased the hematocrit (cadmium decreased hematocrit slightly but not significantly). Effects of the mixture were less than additive on these endpoints (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Cadmium did not affect the arsenic-enhanced urinary excretion of coproporphyrin and uroporphyrin (Fowler and Mahaffey 1978; Mahaffey et al. 1981). The toxicological data on red cell count and hematocrit are considered more directly relevant to health concerns, and are relevant to the hematological toxicity of arsenic. Limitations of study design and analysis precluded the full evaluation of interactions and supporting data are lacking. Accordingly, an intermediate rating of B is appropriate.

Table 42. Effect of **Arsenic** on **Cadmium**: Testicular Toxicity for Oral Exposure

BINWOE: <IIIBii (-1 x 0.32 x 0.71 x 0.79 = -0.16) for acute exposure
BINWOE: <IIIB2ii (-1 x 0.32 x 0.71 x 0.79 x 0.79 = -0.14) for intermediate or chronic exposure

Direction of Interaction - The direction of interaction is predicted to be less than additive, based on the antagonism of testicular toxicity observed in an acute simultaneous intraperitoneal study in rats (Diaz-Barriga et al. 1990). The mechanistic data are ambiguous.

Mechanistic Understanding - Both cadmium and arsenic induce metallothionein and both have oxidant properties (ATSDR 1999a; 2000a). Although induction of metallothionein is a potential protective mechanism, because cadmium binds to metallothionein and is thereby prevented from damaging cellular constituents, the result of this process is chronic retention of cadmium. Data regarding the consequences of long-term coexposure to cadmium and a metallothionein-inducer were not encountered. Arsenic is not known to have reproductive effects (ATSDR 2000a). Cadmium has male reproductive effects. The testicular effects of cadmium may be due to cadmium interference with zinc-protein complexes that control DNA transcription, subsequently leading to apoptosis (ATSDR 1999a). Thus, mechanistic data are ambiguous (III).

Toxicological Significance - In an acute intraperitoneal lethality study, simultaneous injection of arsenic with cadmium appeared to antagonize the cadmium-induced testicular effects (hemorrhage) in rats, but did not affect testicular concentrations of cadmium (Diaz-Barriga et al. 1990). These results are supported by those of a sequential intraperitoneal lethality study, in which pretreatment with arsenic protected against testicular hemorrhagic necrosis in rats (Hochadel and Waalkes 1997). The relevance of these severe testicular effects in dying animals to a nonlethal intermediate or chronic exposure is uncertain. A rating of B for toxicological significance is appropriate.

Modifiers - A modifier for route is recommended because of uncertainties regarding the applicability of parenteral exposure. A modifier for duration is appropriate for application to intermediate or chronic exposure.

Table 43. Effect of **Chromium(VI)** on **Arsenic:** Dermal and Other Non-Renal Toxicities for Oral Exposure

BINWOE: >IIIC (+1 x 0.32 x 0.32 = +0.10) for Dermal Toxicity and other Non-Renal Toxicities (Neurological, Cardiovascular, Hematological, Carcinogenic)

Direction of Interaction - The direction of interaction can be inferred as greater than additive, based on mechanistic considerations: competition for glutathione and greater absorption of arsenic during co-exposure to chromium(VI).

Mechanistic Understanding - Concentrations of arsenic in skin of humans exposed to background levels of arsenic were higher than in other "live" tissues except blood (Liebscher and Smith 1968). Arsenic accumulated in the skin of animals given long-term exposure (Lingren et al. 1982). Arsenic reacts with the sulfhydryl groups of proteins, inactivates enzymes, and interferes with mitochondrial function. Relatively high-dose intermediate-duration toxicity to the skin is considered to be due to cytotoxic effects. Chronic, low-level exposure to arsenic is thought to stimulate keratinocyte secretion of growth factors. The resulting increase in cell division and DNA replication would afford greater opportunities for genetic damage. Reduction of arsenate to arsenite can be mediated by glutathione, and glutathione may be a co-factor for the methylation of arsenite (ATSDR 2000a). Chromium(VI) is thought to produce cellular damage during reduction to chromium(III); this process may generate oxygen radical species and involve glutathione (ATSDR 2000b). The absorption of arsenic from the gastrointestinal tract was higher following gavage administration of a mixture of chromium(VI) and arsenic than from the same dose of arsenic alone (Gonzalez et al. 1995). Thus, there are two potential points of interaction that could result in a greater-than-additive interaction, an enhancement of the absorption of arsenic by chromium(VI) and competition for glutathione. Thus, greater-than-additive joint action can be inferred, but a rating of III is appropriate because the mechanistic data are not in agreement with the limited toxicological data.

Toxicological Significance - Intraperitoneal injection of a mixture of chromium(VI) and arsenic appeared to result in less marked renal effects than either metal alone at the same dose as in the mixture (Mason and Edwards 1989), contrary to what would be expected from the mechanistic data. The relevance of the apparent antagonism for renal toxicity to potential interactions on dermal toxicity or other non-renal toxicities (neurological, cardiovascular, hematological, carcinogenic) is uncertain, and the use of a parenteral route adds to the uncertainty. Greater confidence is placed in the mechanistic data, from which greater-than-additive joint action can be inferred. The appropriate rating for toxicological significance is C.

Table 44. Effect of **Arsenic** on **Chromium(VI)**: Renal and Non-Renal Toxicities for Oral Exposure

BINWOE: <IIIBii (-1 x 0.32 x 0.71 x 0.79 = -0.16) for acute exposure, and
BINWOE: <IIIB2ii (-1 x 0.32 x 0.71 x 0.79 x 0.79 = -0.14) for intermediate or chronic exposure: Renal Toxicity

BINWOE: <IIICii (-1 x 0.32 x 0.32 x 0.79 = -0.08) for acute exposure, and
BINWOE: <IIIC2ii (-1 x 0.32 x 0.32 x 0.79 x 0.79 = -0.06) for intermediate or chronic exposure: Non-Renal Toxicities (Neurological, Hematological, Testicular)

Direction of Interaction - The direction of interaction is predicted to be less than additive, based on the antagonism of renal toxicity observed in an acute simultaneous intraperitoneal study in rats (Mason and Edwards 1989). The mechanistic data are ambiguous.

Mechanistic Understanding - An in vitro study in renal cortical slices showed an inhibition by arsenic of chromium(VI) uptake (Keith et al. 1995), but interpretation of these results is uncertain without additional information on the mechanism of uptake of these metals and their membrane-bound or intracellular location in the tissue slices. Both chromium(VI) and arsenic have oxidant properties (ATSDR 2000a, 2000b), which may indicate the potential for additive or greater-than-additive joint action. Thus, the mechanistic data are ambiguous. An appropriate rating is III.

Toxicological Significance - In an acute intraperitoneal study, simultaneous injection of chromium(VI) and arsenic appeared to antagonize the renal effects (increased relative kidney weight and increased serum creatinine) that resulted from the administration of each metal alone at the same dose as in the mixture (Mason and Edwards 1989). The results of this study are toxicologically relevant to chromium(VI) renal toxicity, but because they are not supported by other toxicological data, and the mechanistic data are ambiguous, an intermediate rating of B is chosen. The relevance of this determination to other toxicities of chromium(VI) involves additional uncertainties, reflected in the downgrading of the rating for toxicological significance to a C.

Modifiers - A modifier for route is recommended because of uncertainties regarding the applicability of parenteral exposure. A modifier for duration is appropriate for application to intermediate or chronic exposure.

Table 45. Effect of **Chromium(VI)** on **Arsenic**: Renal Toxicity for Oral Exposure

BINWOE: <IIIBii (-1 x 0.32 x 0.71 x 0.79) = -0.16) for acute exposure
BINWOE: <IIIB2ii (-1 x 0.32 x 0.71 x 0.79 x 0.79 = -0.14) for intermediate or chronic exposure

Direction of Interaction - The direction of interaction is predicted to be less than additive, based on the antagonism of renal toxicity observed in an acute simultaneous intraperitoneal study in rats (Mason and Edwards (1989). The mechanistic data are ambiguous.

Mechanistic Understanding - When the metals were administered simultaneously once by gavage, chromium (VI) increased the absorption of arsenic in rats, and also decreased the urinary and fecal excretion of arsenic. Results from intestinal perfusion experiments also indicated greater absorption of arsenic in the presence of chromium(VI) (Gonzales et al. 1995). These results would indicate chromium(VI) increased the body burden of arsenic and, thus, might be expected to potentiate arsenic toxicity. An in vitro study in renal cortical slices showed a slight inhibition by chromium(VI) of arsenic uptake (Keith et al. 1995), but interpretation of these results is uncertain without additional information on the mechanism of uptake of these metals and their membrane or intracellular location in the tissue slices. Both chromium(VI) and arsenic have oxidant properties (ATSDR 2000a, 2000b) that may indicate the potential for additive or greater-than-additive joint action. Thus, the mechanistic data are ambiguous. An appropriate rating is III.

Toxicological Significance - In an acute intraperitoneal study, simultaneous injection of chromium(VI) and arsenic appeared to antagonize the renal effects (increased relative kidney weight and increased serum creatinine) that resulted from the administration of each metal alone at the same dose as in the mixture (Mason and Edwards 1989). The results of this study are toxicologically relevant to arsenic renal toxicity, but because they are not supported by other toxicological data, and the mechanistic data are ambiguous, an intermediate rating of B is chosen.

Modifiers - A modifier for route is recommended because of uncertainties regarding the applicability of parenteral exposure. A modifier for duration is appropriate for application to intermediate or chronic exposure.