INTERACTION PROFILE FOR:
ARSENIC, CADMIUM, CHROMIUM, AND LEAD

U.S. Department of Health and Human Services
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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found. The Food Quality Protection Act (FQPA) of 1996 requires that factors to be considered in establishing, modifying, or revoking tolerances for pesticide chemical residues shall include the available information concerning the cumulative effects of substances that have a common mechanism of toxicity, and combined exposure levels to the substance and other related substances. The FQPA requires that the Administrator of the Environmental Protection Agency (EPA) consult with the Secretary of the Department of Health and Human Services (which includes ATSDR) in implementing some of the provisions of the act.

To carry out these legislative mandates, ATSDR's Division of Toxicology (DT) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists in collaboration with mixtures risk assessors and laboratory scientists have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

Literature searches for this Interaction Profile were conducted in January 2000. This final version of the document, released in 2004, includes changes based on additional literature searching and analysis of joint action for arsenic and chromium(VI) that were performed in 2002 for an ATSDR health consultation.

SUMMARY

Lead, arsenic, cadmium, and chromium constitute a very frequently occurring quaternary mixture at hazardous waste sites. This mixture was found in soil at 219 sites out of the 1,608 sites for which ATSDR has produced a Public Health Assessment, including waste storage, treatment or disposal, manufacturing and industrial, and government waste sites. The primary route of exposure for this mixture in soil is likely to be oral, and the duration of concern is intermediate and particularly chronic. The profile focuses on inorganic forms of these metals, consistent with the monitoring data, and on chromium(VI), the species of concern for chromium. Because no pertinent health effects data or physiologically based pharmacokinetic (PBPK) models were located for the quaternary mixture, exposure-based assessment of health hazards for this mixture depends on an evaluation of the health effects data for the individual metals and on the joint toxic action and mechanistic data for various combinations of these metals. This profile discusses and evaluates the evidence for joint toxic action among lead, arsenic, cadmium, and chromium(VI) and recommends how to incorporate concerns regarding possible interactions or additivity into public health assessments of sites where people may be exposed to mixtures of these chemicals.

An intermediate-duration dietary study of the trinary mixture lead, arsenic, and cadmium in rats indicates that results for the binary submixtures predicted the toxicity of the trinary mixture reasonably well, and that subthreshold doses of two metals can, when administered in combination, result in effects (Fowler and Mahaffey 1978; Mahaffey and Fowler 1977; Mahaffey et al. 1981). An intermediate-duration drinking water study of lead, cadmium, and chromium(VI+III) in diethylnitrosamine-initiated rats gave no evidence that the mixture had promoting activity (Benjamin et al. 1999).

Most of the information regarding joint toxic action for the metals in this mixture is for binary combinations of the metals. Data 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 because they employed simultaneous, intermediate or chronic oral exposure, and relevant endpoints of toxicity. Limitations in study design and reporting, and inconsistences in results across studies for the same target organ make it difficult to draw conclusions from these studies. The data for the other binary mixtures are less extensive, and tend to be less relevant in terms of sequence, duration, and route of exposure, as well as endpoints of toxicity. For these reasons, the weight-of-evidence (WOE) approach for the assessment of interactions was used to prepare binary weight-of-evidence determinations (BINWOE) for the binary mixtures (ATSDR 2001a, 2001b). The BINWOE determinations provide conclusions regarding the

expected direction of interaction, and the degree of confidence in these conclusions. BINWOE determinations need to take into account the potential endpoint-specificity of joint toxic action (ATSDR 2001b), particularly as the data for the lead-cadmium and lead-arsenic mixtures indicated that the direction of interaction may not be consistent across endpoints.

Each of the four metals affects a wide range of target organs and endpoints, and there are a number of target organs in common across two or more of the metals. These four metals do not, however, share the same critical effects (i.e., the most sensitive effect that is the basis for the MRL or other health criterion) for long-term oral exposure. For a mixture of this type, the recommended approach is to estimate endpoint-specific hazard indexes, using the target-organ toxicity dose (TTD) modification of the hazard index method (ATSDR 2001a). Uncertainties regarding the impact of interactions are taken into account through application of the qualitative WOE approach (ATSDR 2001a), including the BINWOEs developed for the binary mixtures.

Endpoints of concern for oral exposure to this mixture include the critical effects on which the oral minimal risk levels (MRLs) are based, other sensitive effects, and also endpoints in common that may become significant due to additivity or interactions. The critical effect for lead is neurological, particularly in infants and children. Although no MRLs have been derived for lead, the Centers for Disease Control (CDC 1991) has defined a level of concern for lead exposure in children in terms of a blood lead concentration (PbB), and ATSDR (1999b) suggests the use of media-specific slope facts and site-specific environmental monitoring data to predict media-specific contributions to PbB. The critical effect for arsenic is dermal (ATSDR 2000a) and for cadmium is renal (ATSDR 1999a); these effects are the bases for the chronic oral MRLs. The critical effect for chromium(VI) is uncertain; no oral MRLs have been derived, and other health effects guidelines are based on essentiality (because chromium(III) is essential) (ATSDR 2000b) and a free-standing no-observed-adverse-effect level (NOAEL) for chromium(VI) (IRIS 2001). Sensitive effects in common across two or more of these metals include neurological, renal, cardiovascular, and hematological effects. Although less sensitive, testicular effects also are an endpoint of concern because a synergistic interaction has been noted for lead and cadmium, and because chromium(VI) also affects the testes. TTDs and BINWOEs were developed for the endpoints of concern for the four metals using the methods recommended by ATSDR (2001a, 2001b).

The binary mixtures with the most extensive interaction databases are the lead-arsenic mixture and the lead-cadmium mixture. The predicted direction of interaction for the effects of these mixtures is not

consistent across endpoints. This observation is most striking for the effects of cadmium on the toxicity of lead. The predicted direction is greater than additive for the neurological effects (the critical effect) and testicular effects (a less sensitive effect), less than additive for renal and hematological effects, and additive for cardiovascular effects. Confidence in the BINWOE determinations ranges from relatively high for renal and testicular to low for neurological.

The observation of inconsistency in predicted direction of interaction underscores the uncertainty in extrapolating interactions from one endpoint to another. It also suggests the possibility that a less sensitive target organ may have the potential to impact a mixtures health assessment if it is affected synergistically. Concern would be heightened if several chemicals in the mixture affect that target organ, and if confidence in the interaction (as reflected by the BINWOE scores) is high.

The recommendations for assessing the potential hazard to public health of the joint toxic action of lead, arsenic, cadmium, and chromium(VI) is to use the hazard index and TTDs to estimate endpoint-specific hazard indexes for neurological, renal, cardiovascular, hematological, and testicular toxicity of the mixture. This approach is appropriate when hazard quotients of at least two of the components equal or exceed 0.1 (ATSDR 2001a). The hazard quotient for arsenic's dermal toxicity (critical effect for chronic oral MRL) and the cancer risk estimate for arsenic are estimated separately from the other mixture components, because dermal effects are a unique critical effect (oral exposure to the other components does not affect the skin) and because the other components are not carcinogenic by the oral route (ATSDR 2001a). The impact of interactions on the endpoint-specific hazard indexes, unique hazard quotient, and cancer risk, were predicted using the WOE approach (ATSDR 2001a, 2001b), and are summarized below.

Neurological: The predicted direction of joint toxic action for neurological effects, an endpoint common to all four components, is greater than additive for the effect of lead on arsenic (low-moderate confidence), arsenic on lead (moderate confidence), cadmium on lead (low confidence), and chromium(VI) on arsenic (low confidence), and less than additive for the effect of arsenic on chromium(VI). The remaining seven BINWOEs were indeterminate due to a lack of toxicological and mechanistic data. Thus, the potential health hazard may be somewhat greater than estimated by the endpoint-specific hazard index for neurological effects, particularly for waste sites with relatively high hazard quotients for lead and arsenic, and lower hazard quotients for the other components. Given the indeterminate ratings for the majority of the BINWOEs, confidence in this conclusion would be lower for

mixtures where cadmium and chromium(VI) account for a greater portion of the apparent neurological hazard.

Renal: The potential health hazard regarding renal effects is likely to be lower than the additive, endpoint-specific hazard index, because five of the BINWOEs were less than additive, two were additive, and five were indeterminate. Confidence in the less-than-additive and additive BINWOEs ranges from low-moderate to high-moderate. Uncertainty regarding the impact of interactions on this endpoint is less than for neurological toxicity, because more information was available and a greater number of BINWOEs could be determined.

Cardiovascular: The WOE will have little impact on the additive, endpoint-specific hazard index, because the two moderate-confidence BINWOEs for this endpoint (for the effects of cadmium and lead and vice versa) were additive, one low confidence BINWOE (for chromium(VI) on arsenic) was less than additive, six BINWOEs were indeterminate, and three were not applicable (for the effect of the other components on chromium(VI). For mixtures other than those predominated by lead and cadmium, uncertainty is high.

Hematological: The potential health hazard for hematological effects is likely to be lower than indicated by the endpoint-specific hazard index, because six of the BINWOEs were less than additive, one was greater than additive, one was additive, and four were indeterminate. Confidence in the less-than-additive and additive BINWOEs is primarily low-moderate, and confidence in the greater-than-additive BINWOE is low.

Testicular: The potential health hazard may be higher than the endpoint-specific hazard index for testicular effects for mixtures with relatively high hazard quotients for cadmium and lead, because BINWOEs for this pair were greater than additive, with relatively high confidence. The BINWOE scores for arsenic effects on cadmium and chromium(VI) testicular toxicity were less than additive, but the confidence was low and the impact on the hazard index will be low. For the other pairs, BINWOEs were indeterminate (five BINWOEs) or not applicable (three BINWOEs for the effect of the other components on arsenic).

Dermal: Interactions of the other mixture components on the dermal toxicity of arsenic are indeterminate for lead and cadmium, and greater than additive with low confidence for chromium(VI). Thus the

available data do not indicate a significant impact of interactions on the hazard quotient for the unique critical effect of arsenic, but uncertainty is high due to the lack of pertinent information.

Carcinogenic: Data regarding effects of the other mixture components on arsenic carcinogenicity were not available. Mechanistic considerations suggest that the effect of chromium(VI) on arsenic carcinogenicity may be greater than additive, but confidence in this assessment was low. The remaining BINWOEs are indeterminate and will have no impact on the cancer risk estimate for arsenic. Uncertainty regarding interactions is high due to the lack of pertinent information.

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PEER REVIEW

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All reviewers were selected in conformity with the conditions for peer review specified in Section 104(i)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ALA	delta-aminolevulinic acid
ALAD	delta-aminolevulinic acid dehydratase
ALAS	delta-aminolevulinic acid synthetase
As	arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
BINWOE	binary weight-of-evidence
BMC	benchmark concentration
Cd	cadmium
CdB	blood cadmium
CDC	Centers for Disease Control
CdMT	cadmium metallothionein
CdU	urinary cadmium
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
Cr	chromium
dL	deciliter
DMA	dimethylarsenite
DNA	deoxyribonucleic acid
DT	Division of Toxicology
EPA	Environmental Protection Agency
FQPA	Food Quality Protection Act
HOME	Home Observation for Measurement of the Environment
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
kg	kilogram
L	liter
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentrations
MCV	mean corpuscular volume
mg	milligram
mL	milliliter
MMA	monomethylarsenite
MRL	Minimal Risk Level
MT	metallothionein
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NRC	National Research Council
NTP	National Toxicology Program
Pb	lead
PbB	blood lead
PBPK	physiologically based pharmacokinetic
PBPK/PD	physiologically-based pharmacokinetic pharmacodynamic
ppb	parts per billion
ppm	parts per million
RfC	Reference Concentration
RfD	Reference Dose
RNA	ribonucleic acid
SCE	sister chromatid exchange
SGOT	serum glutamic-oxaloacetic transaminase
TTD	target-organ toxicity dose
U.S.	United States
WOE	weight-of-evidence
ZPP	zinc protoporphyrin

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
µg	microgram
µmole	micromole

1. Introduction

The primary purpose of this Interaction Profile for lead, arsenic, cadmium, and chromium is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture MRL, and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR DT's recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

The lead, arsenic, cadmium, and chromium mixture was chosen as the subject for this interaction profile because it is a very frequently occurring quarternary mixture at hazardous waste sites. This mixture was found in soil at 219 sites out of the 1,608 sites for which ATSDR has produced a Public Health Assessment. The principal activity at sites with this mixture in soil was waste storage, treatment, or disposal (30%), followed by manufacturing and industrial (23.5%), and government (12.2%), with various other types of site activities accounting for the remainder. The profile is restricted to inorganic forms of these metals, as per the monitoring data. In the case of chromium, although total chromium is often monitored at waste sites, the form of concern is chromium(VI). The primary route of concern for a mixture of these chemicals in soil is likely to be oral, and the duration intermediate to chronic. The term "metals" is used in this profile for brevity and convenience, and is intended to refer to lead, arsenic, cadmium, and chromium(VI) in inorganic compounds or as ions. Arsenic, a metalloid, is usually grouped with metals in terms of its toxicology.

Before evaluating the relevance of interactions data for these chemicals, some understanding of the endpoints of concern for oral exposure to this mixture is needed. The endpoints of concern include the critical effects that are the bases for minimal risk levels (MRLs) and other sensitive effects of these metals, and also endpoints in common that may become significant due to additivity or interactions. No MRLs have been derived for lead (Pb) (ATSDR 1999b). The effect of concern is neurological. ATSDR (1999b) suggests the use of media-specific slope factors and site-specific environmental monitoring data to predict media-specific contributions to blood lead (PbB). Chronic oral MRLs have been derived for arsenic, based on skin lesions in humans (ATSDR 2000a), and for cadmium (Cd), based on proteinuria (indicator of renal damage) in humans (ATSDR 1999a). No oral MRLs have been derived for chromium(VI) because of insufficient data to define no-observed-adverse-effect levels (NOAELs) for reproductive and developmental effects. Instead, the upper end of the range of the estimated rate and adequate daily dietary intake of 200 µg Cr/kg/day (NRC 1989) was adopted by ATSDR (2000b) as provisional guidance for oral exposure to chromium(VI) and chromium(III). In practice, health assessments may use the reference dose (RfD) for chromium(VI). The RfD is based on a "free-standing" NOAEL that is lower than lowest-observed-adverse-effect levels (LOAELs) for toxic effects, including reproductive and developmental effects, in other studies (IRIS 2001).

The bases for the MRLs (or health assessment approach in the case of lead), as well as other pertinent effects, are summarized in Table 1. No studies were located that investigated the effect of the quaternary mixture on these effects of concern. A few studies have investigated the effect of trinary mixtures of these metals on some of these endpoints, but the bulk of the available interactions information is for binary mixtures of these metals. Table 2 summarizes the availability of pertinent interactions data by endpoint for the binary mixtures. The table serves as an overview, and shows some striking data gaps: no studies of interactions relevant to the critical effect of arsenic (dermal lesions), and no studies on endpoints of concern for oral exposure for the lead-chromium(VI) pair. The lead-cadmium mixture has been studied the most extensively, including in epidemiological studies and in intermediate and chronic simultaneous oral exposure studies in animals.

A point of interest is there appears to be no good animal model for arsenic toxicity in humans. No other species has been found to develop the arsenic effect of greatest concern, cancer in the skin and other organs. Nor have the studied species of animals been found to develop the noncancer skin lesions seen in humans exposed to arsenic. The species most often used in these interactions studies, the rat, is significantly different from humans in terms of arsenic metabolism, distribution, and health effects.

Table 1. Potential Health Effects of Concern for Intermediate and Chronic Oral Exposure to the Mixture Lead, Arsenic, Cadmium, and Chromium(VI)[a]

Lead	Arsenic	Cadmium	Chromium (VI)
Neurological **Hematological** **Cardiovascular** Renal Testicular	***Dermal lesions*** **Cardiovascular** **Hematological** Renal **Neurological** **Cancer**	***Renal (proteinuria*)** Cardiovascular Hematological Hepatic Neurological Testicular	Hematological Hepatic Renal Neurological Testicular

[a]The basis for the MRL or health assessment approach is bolded and italicized; other sensitive effects are bolded; and less sensitive effects in common across two or more metals, or known to be affected synergistically by another metal in the mixture, are listed without bold or italics

Table 2. Availability of Pertinent Interactions Data for Pairs of Components

Endpoint	Lead-Arsenic	Lead-Cadmium	Lead-Chromium(VI)	Arsenic-Cadmium	Arsenic-Chromium(VI)	Cadmium-Chromium(VI)
Cardiovascular		X				
Hematological	X	X		X		
Hepatic	X	X		X		
Renal	X	X		X	X	X
Dermal (for arsenic)						
Immunological		X				
Reproductive (testicular)		X		X		
Neurological	X	X				
Cancer (for arsenic)	X					

X = Some data are available

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.

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

Lead, arsenic, cadmium, and chromium are frequently found together in the soil of hazardous waste sites. Although the monitoring data for hazardous waste do not usually distinguish between chromium(VI) and chromium(III), but rather are for total chromium, the form of concern is chromium(VI). The primary route of concern for a mixture of these chemicals in soil is likely to be oral, and the duration intermediate to chronic. Chronic exposure is of particular concern because of the cumulative nature of cadmium injury to the kidney, and the association of chronic oral exposure to arsenic with dermal lesions and cancer.

These metals probably constitute an incidental mixture at most waste sites where they co-occur. The components vary in concentration and in proportion to each other from one hazardous waste site to another, and one point of exposure to another. The ideal basis for the assessment of joint toxic action of this (or other) environmental mixtures would be data and models of joint toxic action for the toxicity and carcinogenicity of the complete mixture or validated PBPK/PD models that would support prediction of the effects of different doses and proportions of mixture components.

As discussed in Section 2.3, no adequate epidemiological or toxicological studies and no PBPK models are available for the quaternary 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). Results of an intermediate-duration dietary study of toxicity and interactions for lead, arsenic, and cadmium in rats (Fowler and Mahaffey 1978; Mahaffey and Fowler 1977; Mahaffey et al. 1981) indicated that effects of the trinary mixture generally reflected those for the binary mixtures, suggesting that components-based approaches that focus on interactions for the binary mixtures may be useful in predicting the toxicity of the mixture.

In addition, although mechanisms for hematological effects are different for lead and cadmium, subthreshold exposures to these metals in combination resulted in significant decreases in hemoglobin and hematocrit (Mahaffey and Fowler 1977; Mahaffey et al. 1981; Thawley et al. 1977) suggesting that a health assessment approach that deals with each metal separately may underestimate the potential for mixtures of these metals to cause effects. Epidemiological studies of children have indicated that lead

and arsenic, and lead and cadmium, may interact at environmental levels of exposure to produce adverse neurobehavioral consequences in children (Marlowe et al. 1985a; Moon et al. 1985).

Because suitable data, joint action models, and PBPK models are lacking for the complete mixture, the recommended approach for the exposure-based assessment of joint toxic action of this mixture is to use the hazard index method with the TTD modification and qualitative WOE method to assess the potential consequences of additive and interactive joint action of the components of the mixture. These methods are to be applied only under circumstances involving significant exposure to the mixture, i.e., only if hazard quotients for two or more of the metals equal or exceed 0.1 (Figure 2 of ATSDR 2001a). Hazard quotients are the ratios of exposure estimates to noncancer health guideline values, such as MRLs. If only one or if none of the metals have a hazard quotient that equals or exceeds 0.1, then no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. As discussed by ATSDR (1992, 2001a), the exposure-based assessment of potential health hazard is used in conjuction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

The TTD modification of the hazard index requires the estimation of endpoint-specific (target-organ-specific) hazard indexes for the endpoints of concern for a particular mixture. The endpoints of concern are neurological, dermal, renal, hematological, and cardiovascular. Although less sensitive than these endpoints, testicular effects also are potentially of concern because they are caused by lead, cadmium, and chromium(VI), and the joint action of lead and cadmium on this endpoint is synergistic. Therefore, these endpoints are candidates for TTD development for the components of this mixture. Because only arsenic causes dermal effects (basis for chronic oral MRL) after oral exposure, dermal TTDs were not developed for the other metals. The TTDs were derived as described in the Appendices to this document, using the methods recommended by ATSDR (2001a, 2001b). The derived values are listed in Table 46, which also lists the chronic oral MRLs or guidance values. BINWOEs have been developed for these endpoints also, as presented in Section 2.3, and summarized later in Section 3.

Table 46. MRLs and TTDs for Chronic Oral Exposure to Chemicals of Concern. See Appendices A, B, C, and D for Details of Derivations.

Endpoint	Chemical			
	Lead PbB µg/dL	Arsenic (mg/kg/day)	Cadmium (mg/kg/day)	Chromium(VI) (mg/kg/day)
Neurological	10[a]	$3x10^{-4}$	$2x10^{-4}$	$1x10^{-2}$
Dermal	NA	$3x10^{-4}$ (chronic MRL)	NA	NA
Renal	34	$9x10^{-2}$	$2x10^{-4}$ (chronic MRL)	$1x10^{-2}$
Cardiovascular	10	$3x10^{-4}$	$5x10^{-3}$	NA
Hematological	10	$6x10^{-4}$	$8x10^{-4}$	$3x10^{-3}$
Testicular	40	NA	$3x10^{-3}$	$5x10^{-3}$

[a]CDC PbB level of concern
NA = not applicable

The binary mixtures with the most extensive interaction databases are the lead-arsenic mixture and the lead-cadmium mixture. BINWOEs for relevant endpoints of concern for these mixtures are summarized in Tables 47 and 48. The predicted direction of interaction for the effects of these mixtures is not consistent across endpoints. This observation is most striking for the effects of cadmium on the toxicity of lead. The predicted direction is greater than additive for the neurological effects (the critical effect) and testicular effects (a less sensitive effect), less than additive for renal and hematological effects, and additive for cardiovascular effects.

Table 47. Summary of Endpoint-Specific BINWOEs for Lead and Arsenic		
	Lead on Arsenic	Arsenic on Lead
Endpoint	BINWOE Determinations	
Neurological	>IIIB (+0.23)	>IIB (+0.50)
Dermal	? (0)	NA
Renal	<IIIB (-0.23)	<IIIB (-0.23)
Cardiovascular	? (0)	? (0)
Hematological	<IIIB (-0.23)	<IIIB (-0.23)
Testicular	NA	? (0)

NA = not applicable

Table 48. Summary of Endpoint-Specific BINWOEs for Lead and Cadmium		
	Lead on Cadmium	Cadmium on Lead
Endpoint	BINWOE Determinations	
Neurological	? (0)	>IIIC (+0.1)
Renal	=IIAii (0)	<IIA (-0.71)
Cardiovascular	=IIIA (0)	=IIIA (0)
Hematological	=IIC (0)	<IIIB (-0.23)
Testicular	>IIA (+0.71)	>IIA (+0.71)

The observation of inconsistency in predicted direction of interaction underscores the uncertainty in extrapolating interactions from one endpoint to another. It also suggests the possibility that a less sensitive target organ may have the potential to impact a mixtures health assessment if it is affected synergistically. Concern would be heightened if several chemicals in the mixture affect that target organ, and if confidence in the interaction (as reflected by the BINWOE scores) is high.

BINWOE determinations for the critical effects of the mixture components—neurological (the critical effect of lead), dermal (the critical effect of arsenic), and renal (the critical effect of cadmium)—are summarized in Tables 49–51. Only five of the BINWOEs for neurological effects (Table 49) are non-zero scores: four of these are greater than additive. Confidence in the greater-than-additive

determinations is moderate (>IIB, +0.50) for the effect of arsenic on lead, low-moderate (>IIIB, +0.23) for the effect of lead on arsenic, and low (>IIIC, +0.10) for the effect of cadmium on lead and of chromium(VI) on arsenic. Confidence in the less-than-additive determination for the effect of arsenic on chromium(VI) is low (<IIIC2ii, -0.06). There are no data directly relevant to joint action on neurological endpoints for the other pairs of metals, and no clear mechanistic understanding. Therefore, the remaining BINWOEs are indeterminate with a score of 0.

Only one of the metals, arsenic, causes dermal effects following oral exposure. The BINWOEs for dermal effects (Table 50) are indeterminate (0) for the effect of lead or cadmium on arsenic toxicity, and greater than additive with low confidence (>IIIC, +0.10) for the effect of chromium(VI) on arsenic toxicity. BINWOEs are not applicable for effects on lead, cadmium, and chromium(VI) toxicity because these metals are not toxic to the skin by the oral route of exposure.

For renal toxicity (Table 51), BINWOEs are less than additive (with confidence ranging from low-moderate to high-moderate) for the effect of lead on arsenic (<IIIB, -0.23) for the effect of cadmium on lead (<IIA, -0.71), for the effects of arsenic on lead (<IIIB, -0.23) and chromium (<IIIB2iii, -0.14), and for the effect of chromium on arsenic (<IIIB2ii, -0.14). BINWOEs are additive (0) for the effects of lead on cadmium (=IIAii) and cadmium on arsenic (=IIB); confidence is moderate. Scores are indeterminate (0) for the remaining five BINWOEs.

Table 49. Matrix of BINWOE Determinations for Neurological Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Lead	Arsenic	Cadmium	Chromium(VI)
EFFECT OF	Lead		**>IIIB (+0.23)**	**? (0)**	? (0)
	Arsenic	**>IIB (+0.50)**		? (0)	**<IIIC2ii (-0.06)**
	Cadmium	**>IIIC (+0.10)**	? (0)		? (0)
	Chromium(VI)	? (0)	**>IIIC (=0.10)**	? (0)	

The BINWOE determinations shown in boldface type were explained in the tables in Section 2.3. As reviewed in Section 2.2, no pertinent interactions data were available for the remaining pairs of metals, and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings are appropriate for these remaining pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii different route of exposure (0.79).

Table 50. Matrix of BINWOE Determinations for Dermal Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Lead	Arsenic	Cadmium	Chromium(VI)
EFFECT OF	Lead		**? (0)**	NA	NA
	Arsenic	NA		NA	NA
	Cadmium	NA	**? (0)**		NA
	Chromium(VI)	NA	**>IIIC (+0.10)**	NA	

The BINWOE determinations shown in boldface type were explained in the tables in Section 2.3. As reviewed in Section 2.2, no pertinent interactions data were available for the remaining pairs of metals, and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings are appropriate for these remaining pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii different route of exposure (0.79).

Table 51. Matrix of BINWOE Determinations for Renal Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Lead	Arsenic	Cadmium	Chromium(VI)
EFFECT OF	Lead		**<IIIB (-0.23)**	**=IIAii (0)**	? (0)
	Arsenic	**<IIIB (-0.23)**		**? (0)**	**<IIIB2ii (-0.14)**
	Cadmium	**<IIA (-0.71)**	**=IIB (0)**		? (0)
	Chromium(VI)	? (0)	**<IIIB2ii (-0.14)**	? (0)	

The BINWOE determinations shown in boldface type were explained in the tables in Section 2.3. The remaining BINWOEs are marked NA = not applicable because oral exposure to this metal does not cause dermal effects.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
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).

The remaining endpoints of concern are not the critical effects for any of the metals, but are relatively sensitive effects of one or more of these metals, are effects in common across two or more of the metals, or are known to be affected synergistically by another metal in the mixture. These are cardiovascular, hematological, and reproductive (testicular) effects. BINWOEs have been developed for these effects as well, and are summarized in Tables 52–54.

The BINWOE determinations for cardiovascular toxicity (Table 52) are additive for effects of lead on cadmium and vice versa (=IIIA, 0), and greater than additive (>IIIC, +0.10) for the effect of chromium(VI) on arsenic. Six BINWOEs are indeterminate (0), and three are not applicable (for effects on chromium(VI), which is not known to be a cardiovascular toxicant). Thus, all but one of the BINWOE scores are zero, and that score is close to zero.

Six of the BINWOE determinations for hematological toxicity (Table 53) are less than additive, with low-moderate confidence (<IIIB, -0.23). One BINWOE is greater than additive with low confidence (IIIC, +0.10), one BINWOE is additive (=IIC, 0), and the remaining four, for pairs involving chromium(VI), are indeterminate (0).

For testicular toxicity (Table 54), the two BINWOEs for the lead-cadmium mixture are greater than additive, with moderately high confidence (>IIA, +0.71), the BINWOEs for the effects of arsenic on cadmium and on chromium(VI) are less than additive, with low confidence (<IIIB2ii, -0.14, and <IIC2ii, -0.06), BINWOEs for an effect on arsenic are not applicable because arsenic is not known to have testicular effects, and the remaining five BINWOEs are indeterminate (0).

Estimation of hazard quotients for lead is problematic because of the lack of an oral MRL or RfD. The use of media-specific slope factors and site-specific environmental monitoring data has been recommended by ATSDR to predict media-specific contributions to blood lead (ATSDR 1999b). The predicted contributions from the individual media are summed to yield a total predicted PbB level. The media-specific slope factors were derived from regression analysis of lead concentrations in water, soil, dust, diet, or air and PbBs for various populations. In order to estimate a hazard quotient, the predicted PbB can be divided by the PbB of 10 µg/dL, the level of concern (CDC 1991). The development of TTDs for lead is based on PbB as well.

Table 52. Matrix of BINWOE Determinations for Cardiovascular Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Lead	Arsenic	Cadmium	Chromium(VI)
EFFECT OF	Lead		**? (0)**	**=IIIA (0)**	NA
	Arsenic	**? (0)**		? (0)	NA
	Cadmium	**=IIIA (0)**	? (0)		NA
	Chromium(VI)	? (0)	**>IIIC (+0.10)**	? (0)	

The BINWOE determinations shown in boldface type were explained in the tables in Section 2.3. As reviewed in Section 2.2, no pertinent interactions data were available for the remaining pairs of metals, and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings are appropriate for these remaining pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii: different route of exposure (0.79).

Table 53. Matrix of BINWOE Determinations for Hematological Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Lead	Arsenic	Cadmium	Chromium(VI)
EFFECT OF	Lead		**<IIIB (-0.23)**	**=IIC (0)**	? (0)
	Arsenic	**<IIIB (-0.23)**		**<IIIB (-0.23)**	**<IIIC2ii (-0.06)**
	Cadmium	**<IIIB (-0.23)**	**<IIIB (-0.23)**		? (0)
	Chromium(VI)	? (0)	**>IIIC (+0.10)**	? (0)	

The BINWOE determinations shown in boldface type were explained in the tables in Section 2.3. As reviewed in Section 2.2, no pertinent interactions data were available for the remaining pairs of metals, and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings are appropriate for these remaining pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii: different route of exposure (0.79).

Table 54. Matrix of BINWOE Determinations for Testicular Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Lead	Arsenic	Cadmium	Chromium(VI)
EFFECT OF	Lead		NA	**>IIA (+0.71)**	? (0)
	Arsenic	? (0)		**<IIIB2ii (-0.14)**	**<IIIC2ii (-0.06)**
	Cadmium	**>IIA (+0.71)**	NA		? (0)
	Chromium(VI)	? (0)	NA	? (0)	

The BINWOE determinations shown in boldface type were explained in the tables in Section 2.3. Arsenic is not known to have testicular effects, so BINWOEs for the testicular toxicity of arsenic are marked NA = not applicable. As reviewed in Section 2.2, no pertinent interactions data were available for the remaining pairs of metals, and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings are appropriate for these remaining pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
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).

Oral MRLs have not been developed for chromium(VI), but health assessments often use the RfD (IRIS 2001). Alternatively, the upper end of the range of the estimated safe and adequate daily dietary intake of 200 µg Cr/kg/day (NRC 1989) has been adopted as provisional guidance for oral exposure to chromium(VI) and chromium(III) by ATSDR (2000b).

Proceeding with the TTD modification of the hazard index approach involves calculating endpoint-specific hazard indexes for each endpoint of concern, as described in ATSDR (2001a, Section 2.3.2 and Figure 2 with accompanying text). For example, a hazard index for neurological effects of this mixture is calculated as follows:

$$HI_{NEURO} = \frac{E_{Pb}}{CDC\ PbB_{Pb\ NEURO}} + \frac{E_{As}}{TTD_{As\ NEURO}} + \frac{E_{Cd}}{TTD_{Cd\ NEURO}} + \frac{E_{Cr(VI)}}{TTD_{Cr(VI)\ NEURO}}$$

where HI_{NEURO} is the hazard index for neurological toxicity, E_{Pb} is the exposure to lead (as predicted PbB in µg/dL), $CDC\ PbB_{NEURO}$ is the CDC PbB of concern (10 µg/dL) for the neurological toxicity of lead (ATSDR 1999b; CDC 1991), E_{As} is the exposure to arsenic (as the oral intake in the same units as the corresponding TTD, mg/kg/day), $TTD_{As\ NEURO}$ is the TTD for the neurological toxicity of arsenic, and so forth.

If one or more of the endpoint-specific hazard indexes exceed one, they provide preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of the components on that endpoint (ATSDR 2001a). The qualitative WOE method is then used to estimate the potential impact of interactions on the endpoint-specific hazard indexes (Figure 2, ATSDR 2001a), using the BINWOEs developed in this profile. As discussed in ATSDR (2001a), when the endpoint-specific hazard index is greater than unity and/or when the qualitative WOE indicates that joint toxic action may be greater than additive, further evaluation using methods described by ATSDR (1992) is needed.

Similarly, if the estimated cancer risk for arsenic equals or exceeds $1x10^{-4}$, this provides preliminary evidence of a health hazard (ATSDR 2001a). The qualitative WOE is then used to estimate the potential impact of interactions, but for arsenic carcinogenicity, the WOE does not significantly impact conclusions. Mechanistic considerations suggest that the effect of chromium(VI) on arsenic carcinogenicity may be greater than additive, but confidence in this assessment is low. The remaining BINWOEs for the effects of the mixture components on arsenic carcinogenicity are indeterminate.

Therefore, if the estimated cancer risk equals or exceeds $1x10^{-4}$, further evaluation using methods described by ATSDR (1992) is needed.

4. Conclusions

No pertinent health effects data or PBPK models were available for the mixture of lead, arsenic, cadmium, and chromium(VI). Endpoints of concern for this mixture include the critical effects of the individual components, and toxicity targets in common that may become significant due to additivity or interactions. These endpoints are neurological, dermal, renal, cardiovascular, hematological, testicular, and carcinogenic effects. The recommendations for assessing the potential hazard to public health of the joint toxic action of lead, arsenic, cadmium, and chromium(VI) is to use the hazard index and TTDs to estimate endpoint-specific hazard indexes for neurological, renal, cardiovascular, hematological, and testicular toxicity of the mixture. This approach is appropriate when hazard quotients of at least two of the components equal or exceed 0.1 (ATSDR 2001a). The qualitative WOE approach is then used to predict the impact of interactions on the endpoint-specific hazard index. The hazard quotient for arsenic's dermal toxicity (critical effect for chronic oral MRL) and the cancer risk estimate for arsenic are estimated separately from the other mixture components, because dermal effects are a unique critical effect (oral exposure to the other components does not affect the skin) and because the other components are not carcinogenic by the oral route (ATSDR 2001a). The impact of interactions on the endpoint-specific hazard indexes, unique hazard quotient, and cancer risk for the mixture are discussed below in terms of the WOE approach.

Neurological: The predicted direction of joint toxic action for neurological effects, an endpoint common to all four components, is greater than additive for the effect of lead on arsenic (+0.23), arsenic on lead (+0.50), cadmium on lead (+0.10), and chromium(VI) on arsenic (+0.10); less than additive for arsenic on chromium(VI) (-0.06); and indeterminate (0) for the remaining nine BINWOEs. The combined WOE score is +0.87, indicating that the potential health hazard may be somewhat greater than estimated by the endpoint-specific hazard index for neurological effects, particularly for waste sites with relatively high hazard quotients for lead and arsenic, and lower hazard quotients for the other components. Given the indeterminate ratings for the majority of the BINWOEs, confidence in this conclusion would be lower for mixtures where cadmium and chromium(VI) account for a greater portion of the apparent neurological hazard.

Renal: The potential health hazard regarding renal effects is likely to be lower than the additive, endpoint-specific hazard index, because five of the BINWOEs were less than additive, two were additive, and five were indeterminate. The combined WOE score is -1.45. Uncertainty regarding the impact of

interactions on this endpoint is less than for neurological toxicity, because more information was available and a greater number of BINWOEs could be determined.

Cardiovascular: The WOE will have little impact on the additive, endpoint-specific hazard index, because the two moderate confidence BINWOEs that could be determined for this endpoint (for the effects of cadmium and lead and vice versa) were additive, one low confidence BINWOE was greater than additive (+0.10), six BINWOEs were indeterminate, and three were not applicable for the effect on chromium(VI). Thus, the combine WOE score is +0.10. For mixtures other than those predominated by lead and cadmium, uncertainty is high.

Hematological: The potential health hazard for hematological effects is likely to be lower than indicated by the endpoint-specific hazard index, because six of the BINWOEs were less than additive, one was greater than additive, and four were indeterminate. The combined WOE score is -1.21.

Testicular: The potential health hazard may be higher than the endpoint-specific hazard index for testicular effects for mixtures with relatively high hazard quotients for cadmium and lead, because BINWOEs for this pair were greater than additive, with relatively high confidence (>IIA) and correspondingly high numerical scores (+0.71 for each). The BINWOE scores for arsenic effects on cadmium and chromium(VI) testicular toxicity were less than additive, but the confidence was low (IIIB2ii, -0.14, and <IIC2ii, -0.06) and the impact on the hazard index will be low. For the other pairs, BINWOEs were indeterminate (5 BINWOEs) or not applicable (3 BINWOEs for the effect on arsenic). The combined WOE score is +1.22.

Dermal: Interactions of the other mixture components on the dermal toxicity of arsenic are indeterminate (0) for lead and cadmium, and greater than additive with low confidence (+0.10) for chromium(VI). Thus, the available data do not indicate a significant impact of interactions, but uncertainty is high due to the lack of pertinent information.

Carcinogenic: Data regarding effects of the other mixture components on arsenic carcinogenicity were not available. Mechanistic considerations suggest that the effect of chromium(VI) on arsenic carcinogenicity may be greater than additive, but confidence in this assessment is low (+0.10). The remaining BINWOEs are indeterminate (0) and will have no impact on the cancer risk estimate for arsenic. Uncertainty regarding interactions is high due to the lack of pertinent information.

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Appendix A: Background Information for Lead

A.1 Toxicokinetics

Gastrointestinal absorption of soluble lead salts in adult humans can be high during fasting (40–50%), but is about 3–15% when ingested with food. On the basis of dietary balance studies, gastrointestinal absorption of lead in children appears to be higher and may account for 40–50% of the ingested dose. Studies in animals also provide evidence that gastrointestinal absorption of lead is much higher in younger organisms. Absorption is strongly affected by nutritional status, with higher absorption of lead in children who are iron deficient. Calcium deficiency also may increase lead absorption, based on studies in children. Coadministration of calcium with lead decreases lead absorption in adults, and in animal studies. Vitamin D administration has been shown to enhance lead absorption in animal studies. The distribution of lead appears similar across routes of exposure. Initially, lead is distributed to the blood plasma and soft tissues, but under steady state conditions 99% of the lead in blood is found in the erythrocyte, where much of it is bound to hemoglobin. Lead accumulates in blood, such that bone lead accounts for approximately 73% of the body burden in children, increasing to 94% in adults. Inorganic lead is not known to be metabolized, but lead ions are complexed by macromolecules. Unabsorbed lead is excreted in the feces; absorbed lead that is not retained is excreted through the urine and bile (ATSDR 1999b).

A.2 Health Effects

The effects of lead are similar across inhalation and oral routes of exposure. Lead has been shown to affect virtually every organ and system in the body in both humans and animals. The most sensitive effects of lead appear to be neurological (particularly in children), hematological, and cardiovascular. Epidemiological studies provide evidence for an association between prenatal and postnatal exposure to lead and adverse effects on neurodevelopment in infants and young children, and support the use of PbB as an index of toxicological effect. The neurological effects included impaired cognitive ability and IQ deficits in children. On the basis of several meta-analyses, it appears that a highly significant IQ decrement of 1–3 points is associated with a change in PbB from 10 to 20 μg/dL. In addition, associations between biomarkers of lead exposure and increased problem behavior in the classroom have been reported (ATSDR 1999b; Marlowe et al. 1985a). In adult humans, slowing of nerve conduction velocity occurs at PbBs of ≥30 μg/dL; peripheral nerve function appears to be affected in children at

similar PbBs. Oral studies in animals support the human evidence regarding neurobehavioral toxicity of lead to infants and children from prenatal and postnatal exposure. In animals, lead has been shown to alter a number of neurotransmitter systems including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid systems (ATSDR 1999b).

Lead interferes with the synthesis of heme, resulting in accumulation of ALA in tissues and elevated excretion of ALA in urine, elevation of zinc protoporphyrin in erythrocyte, reductions in blood hemoglobin, and in a hypochromic, normocytic anemia at higher levels of exposure. Many epidemiological studies have found increases in blood pressure to be associated with increases in PbB. The contribution of lead, as compared with other factors, is relatively small, and whether the observed associations represent causality is controversial. Animal data demonstrate that oral exposure to lead increases blood pressure. At higher levels of exposure in humans, lead produces cardiac lesions and electrocardiographic abnormalities. Chronic nephropathy in humans is associated with PbB levels of 40–>100 μg/dL. Oral exposure of animals to lead causes renal damage; histopathology is similar in humans and animals and includes intranuclear inclusion bodies, swollen mitochondria, and tubular damage. Adverse effects on the testes and sperm have been seen in occupationally exposed men with PbBs of 40–50 μg/dL, and the more recent literature suggest that PbB concentrations <40 μg/dL also may be associated with adverse effects on sperm counts and morphology (ATSDR 1999b).

A.3 Mechanisms of Action

Lead can affect virtually every organ or system in the body through mechanisms that involve fundamental biochemical processes. These mechanisms include the ability of lead to inhibit or mimic the action of calcium and to interact with proteins. In the interaction with proteins, lead binds with virtually every available functional group, including sulfhydryl, amine, phosphate, and carboxyl groups, with sulfhydryl having the highest affinity. In its binding with sulfhydryl groups, lead may interfere with the activity of zinc metalloenzymes, as zinc binds to a sulfhydryl group at the active site. Lead also binds to metallothionein, a sulfhydryl-rich protein, but does not appear to displace cadmium or zinc. Metallothionein is induced by cadmium, zinc, and arsenic, but apparently not by lead, although metallothionein sequesters lead in the cell. Another lead-binding protein is an acidic, carboxyl-rich protein found in the kidney and brain (ATSDR 1999b).

Lead interferes with heme synthesis by altering the activity of several mitochondrial and cytosolic enzymes. One of the most sensitive hematological effects is inhibition of the cytosolic enzyme ALAD, with no threshold apparent through the lowest PbB levels (≈3 µg/dL). Lead's inhibition of ALAD occurs through binding of lead to vicinal sulfhydryls at the active site of ALAD, where zinc is normally bound to a single sulfhydryl. Lead stimulates the mitochondrial enzyme ALAS, through feedback derepression, with a threshold in human leukocytes at a PbB of about 40 µg/dL. As a result of the inhibition of ALAD and stimulation of ALAS, ALA accumulates in blood, urine, and soft tissues. Lead inhibits the insertion of iron into protoporphyrin by the mitochondrial enzyme ferrochelatase, possibly through binding of lead to the sulfhydryl groups of the active site or indirectly through disruption of mitochondrial structure. Inhibition of ferrochelatase results in elevation of zinc protoporphyrin (ZPP) in erythrocytes; ZPP is a sensitive indicator of lead exposure, occurring in children at PbBs of about 25 µg/dL. Effects on heme synthesis are not restricted to the erythrocyte. A number of studies suggest that lead-impaired heme production itself may be a factor in lead's neurotoxicity (ATSDR 1999b).

Mechanisms by which lead might affect blood pressure include effects on several hormonal and neural regulatory systems, changes in vascular smooth muscle reactivity, cardiac muscle contractility, changes in cell membrane cation transport systems, and possible effects on vascular endothelial cells (ATSDR 1999b).

A.4 Health Guidelines

ATSDR (1999b) has not derived MRLs for lead. ATSDR (1999b) has suggested the use media-specific slope factors and site-specific environmental monitoring data to predict media-specific contributions to blood lead. The predicted contributions from the individual media are summed to yield a total predicted PbB level. The media-specific slope factors were derived from regression analysis of lead concentrations in water, soil, dust, diet, or air and PbBs for various populations.

The CDC determined in 1991 that blood lead levels of >10 µg/dL are to be considered elevated (ATSDR 1999b; CDC 1991).

EPA (IRIS 2001) has not developed a reference concentration (RfC) or RfD for lead. EPA stated that it would be inappropriate to develop an RfD for inorganic lead (and lead compounds) because some of the health effects occur at PbBs so low as to be essentially without a threshold. Instead, EPA defines lead

risk as the probability of exceeding a PbB of concern (i.e., 10 µg/dL) in children (EPA 1994a) or in fetuses (EPA 1996). This approach is supported by human epidemiological studies that have associated PbBs exceeding 10 µg/dL with impairment or delays in neurobehavioral development and other effects on children (e.g., blood enzymes). EPA estimates lead risk in children using the IEUBK model (EPA 1994b). This model translates estimates of site-specific exposure concentrations into estimates of the probability that children's blood leads will exceed a PbB of concern.

The National Toxicology Program (NTP 2001) has determined that lead acetate and lead phosphate can reasonably be anticipated to be human carcinogens, based on sufficient evidence of carcinogenicity in experimental animals. NTP (2001) considered lead chromate as one of the "Chromium Hexavalent Compounds." IARC (1987) has determined that the animal data are sufficient to classify lead and some lead compounds as possibly carcinogenic to humans (Group 2B). EPA (IRIS 2001) classified lead in Group B2—probable human carcinogen. EPA did not develop an oral slope factor for lead because of the many uncertainties, some of which may be unique to lead. An EPA inhalation unit risk also is not available for lead (IRIS 2001). ACGIH (1998) classified lead and certain inorganic lead compounds as A3 carcinogens—carcinogenic in animals at relatively high doses not considered relevant to worker exposure. Lead chromate, assessed on the basis of both lead and chromate, was classified by ACGIH (1998) as an A2 carcinogen—carcinogenic in animals at doses considered relevant to worker exposure, but with insufficient epidemiological data to confirm risk to humans.

A.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to lead were derived for endpoints affected by lead and one or more of the other chemicals in the lead, arsenic, cadmium, and chromium(VI) mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include neurological renal, cardiovascular, hematological, and testicular effects. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001a, Section 2.3.2). Because ATSDR's approach to the assessment of lead uses media-specific slope factors and site-specific contributions to PbB, the TTDs for lead are derived based on PbB as well (see rationale in Chapter 3 of this profile). The derivations are based on data provided in ATSDR (1999b), and particularly Sections 2.2.1 (Effects in Humans Based on Blood Lead (PbB) Levels), 2.5 (Relevance to Public Health), and 2.7 (Biomarkers of Exposure and Effect). The derivation methods used similar reasoning as for the CDC and EPA levels of concern (see neurological effects).

Neurological Effects

A large number of epidemiological studies and case reports indicate that exposure to lead causes neurological effects. Slowing of nerve conduction velocity is associated with PbBs of ≥30 μg/dL in children and adults. Of greater concern are the inverse linear relationships between IQ and other neurobehavioral measures in children at PbBs extending down through 10 μg/dL or possibly lower. Children appear to be more sensitive to the neurobehavioral toxicity of lead than are adults. Limited data suggest an association between decreased neurobehavioral performance and PbB in aging subjects at relatively low PbBs, indicating that the elderly may be another sensitive population. Although results of the epidemiological studies in children are not entirely consistent, several meta-analyses have indicated that a highly significant IQ decrement of 1–3 points is associated with a change in PbB from 10 to 20 μg/dL in children (IPCS 1995; Needleman and Gatsonis 1990; Pocock et al. 1994; Schwartz 1994). The CDC (1991) determined that blood lead levels of >10 μg/dL are to be considered elevated in children, based largely on concern for the effects of low-level lead exposure on the central nervous system. EPA defines lead risk as the probability of exceeding a PbB of concern (10 μg/dL) in children or fetuses (EPA 1994a, 1996). The CDC level of concern for lead of 10 μg/dL is adopted as the TTD_{NEURO}.

Renal Effects

Chronic nephropathy is associated with PbB levels of 40–>100 μg/dL in humans exposed to lead occupationally. There are some indications of renal damage in a study in children whose mean PbB was 34.2 μg/dL (increased N-acetyl-β-D-glucosaminidase activity in urine, a sensitive indicator) (Verberk et al. 1996). The value for children, supported by the occupational data, and rounded to 34 μg/dL, is taken as the TTD_{RENAL}.

Cardiovascular Effects

At higher levels of exposure, lead produces cardiac lesions and electrocardiographic abnormalities in humans. Many epidemiological studies have reported an association between increases in blood pressure and increases in PbB. The contribution of lead, as compared with other factors, is relatively small, and whether the associations indicate causality is controversial. Animal data demonstrate that oral exposure to lead increases blood pressure ATSDR (1999b). The correlation between PbB and blood pressure is

apparent at relatively low PbBs extending through 10 µg/dL (e.g., Schwartz 1995). Therefore, the CDC level of concern, 10 µg/dL, is adopted as the TTD_{CARDIO}.

Hematological Effects

Lead interferes with the synthesis of heme. The consequence at higher levels of exposure is a hypochromic, normocytic anemia. The most sensitive indicator of effect on heme synthesis is the inhibition of ALAD. ALAD activity is inversely correlated with PbB through the lowest levels of PbB in the general population. Even in the absence of detectable effects on hemoglobin levels, there is concern that effects on heme synthesis may have far-reach impacts, particularly on children (ATSDR 1999b). Accordingly, the CDC PbB of concern, 10 µg/dL (CDC 1991), is selected as the TTD_{HEMATO}.

Testicular Effects

Adverse effects of the testes and sperm have been reported in occupationally exposed men with PbBs of 40–50 µg/dL in some studies, but not in others, and are well-established at higher levels of exposure (PbBs ≥66 µg/dL) (ATSDR 1999b). The point of departure for increased risk of below normal sperm and total sperm count was 40 µg/dL (Alexander et al. 1996). This value is selected as the TTD_{TESTIC}.

Summary (TTDs for Lead)

TTD_{NEURO} = 10 µg/dL PbB = CDC level of concern
TTD_{RENAL} = 34 µg/dL PbB
TTD_{CARDIO} = 10 µg/dL PbB
TTD_{HEMATO} = 10 µg/dL PbB
TTD_{TESTIC} = 40 µg/dL PbB

Appendix B: Background Information for Arsenic

B.1 Toxicokinetics

Arsenic, as soluble arsenate or arsenite, is well-absorbed (≥80%) in both humans and animals exposed by the oral route. Judging from the oral toxicity data, arsenic trioxide also is well absorbed. Lower rates of absorption have been observed with insoluble or less soluble forms of arsenic, such as arsenic sulfide and lead arsenate. Absorption appears to occur by passive diffusion. Distribution occurs throughout the body (ATSDR 2000a). Concentrations 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 following long-term exposure (Lingren et al. 1982). Concentrations in hair and nails tend to be higher than in live tissues. The rat tends to sequester arsenic in erythrocytes. Arsenates (As(V)) and arsenites (As(III)) are interconverted in the body by reduction/oxidation reactions. Reduction of arsenate to arsenite can be mediated by glutathione. Arsenite is methylated to yield the less toxic forms monomethylarsenite (MMA) and dimethylarsenite (DMA). The liver is the major site for the methylation. The methylated forms are excreted primarily in the urine (ATSDR 2000a).

B.2 Health Effects

Chronic oral exposure to arsenic has resulted in serious damage to the vascular system in humans, including Blackfoot disease (a progressive loss of circulation in the fingers and toes that may lead to gangrene), Raynaud's disease, and cyanosis of fingers and toes. The intima of the blood vessels appeared to have thickened. Direct irritation of the gastrointestinal mucosa can occur. Arsenic has caused anemia in humans exposed by the oral route. Increased hemolysis and a toxic effect on the erythropoietic cells of bone marrow may be factors in the development of anemia. Leukopenia has been reported in humans. Hepatic effects seen in humans were thought to be secondary to portal tract fibrosis and portal hypertension, which may have originated from damage to the blood vessels. Signs of renal damage generally are not seen or are mild in humans exposed to arsenic by the oral route. Characteristic dermal lesions caused by long-term oral exposure of humans to arsenic include hyperkeratinization (particularly on the palms and soles), formation of hyperkeratinized corns or warts, and hyperpigmentation of the skin with associated spots of hypopigmentation. A fraction of the hyperkeratinized corns may progress to squamous cell carcinoma of the skin. Signs of peripheral and/or central neuropathy are commonly seen in humans exposed to arsenic orally, with high-dose exposure producing

central nervous system effects and low-dose exposure producing peripheral nervous system effects (ATSDR 2000a). The potential for arsenic to cause subtle neurological effects, such as neurobehavioral effects in children, has not been fully investigated. Studies of associations between hair arsenic concentrations (a biomarker of exposure) and neurobehavioral effects in children have observed an inverse association between hair arsenic and reading and spelling performance (Moon et al. 1985).

Effects on the skin, vascular system and neurological system appear to be relatively sensitive effects of ingested arsenic; dermal effects are the best documented sensitive effect, and the earliest observable sign of health effects from long-term exposure. The NOAEL and LOAEL for dermal effects in humans are 0.0008 and 0.014 mg/kg/day. Hematological effects may be somewhat less sensitive, and renal effects are less sensitive and less common. Epidemiological studies provide convincing evidence that ingestion of arsenic causes cancer of the skin in humans. The lesions include squamous cell carcinomas, which develop from some of the hyperkeratotic arts or corns, and multiple basal cell carcinomas, arising from cells not associated with hyperkeratinization. Evidence is mounting that ingested arsenic may increase the risks of internal cancers as well (ATSDR 2000a).

Some of the effects of arsenic seen in humans are supported by the animal data, but animals do not develop dermal lesions and cancer as a result of oral arsenic exposure. Changes in vascular reactivity have been reported in rats given repeated oral arsenic doses of 11 mg/kg/day (ATSDR 2000a). Hematological and hematopoietic effects, including decreased hematocrit and increased urinary excretion of porphyrins, have been observed in intermediate-duration dietary studies of arsenic in rats at doses of 2.5 mg/kg/day (Fowler and Mahaffey 1978; Mahaffey et al. 1981), and in chronic oral studies in dogs at 2.4 mg/kg/day (ATSDR 2000a). Intermediate oral studies in rats demonstrated alterations in renal mitochondria at 2.5 and 4.7 mg/kg/day (ATSDR 2000a; Mahaffey and Fowler 1977; Mahaffey et al. 1981). Mild proteinuria was observed rats following a single oral dose of 10 mg/kg/day (ATSDR 2000a). Oral administration of arsenic to mice at 8 mg/kg/day altered neurotransmitter concentrations in some areas of the brain (Mejia et al. 1997). Developmental effects have been seen following high oral doses of arsenic in animals, but these are not sensitive effects (ATSDR 2000a).

B.3 Mechanisms of Action

At relatively high oral exposure, methylation capacity may not be adequate to prevent cytotoxic levels of arsenic(III) from reaching tissues. Some of the effects of higher-dose oral exposure to arsenic are thought to be the result of direct cytotoxicity; these include gastrointestinal irritation, and dermal and neurological effects (ATSDR 2000a). Arsenic(III) reacts with the sulfhydryl groups of proteins, inactivates enzymes, and interferes with mitochondrial function by inhibiting succinic dehydrogenase activity and uncoupling oxidative phosphorylation. It has been proposed that arsenic may compete with phosphate during oxidative phosphorylation and may inhibit energy-linked reduction of nicotinamide adenine dinucleotide (NAD) (Goyer 1995). Chronic low-level exposure to arsenic is thought to stimulate keratinocyte secretion of growth factors the resulting increase in cell division and DNA replication affords greater opportunities for genetic damage. Arsenic induces metallothionein, a metal-binding protein. Only a small percentage of administered arsenic is bound to metallothionein, and the affinity of arsenic for metallothionein is much lower than that of cadmium or zinc (ATSDR 2000a). It has been suggested that metallothionein may protect against arsenic toxicity by acting as an antioxidant against oxidative injury produced by arsenic (ATSDR 2000a; NRC 1999).

B.4 Health Guidelines

ATSDR (2000a) did not derive inhalation MRLs or an intermediate oral MRL for arsenic due to lack of suitable studies.

ATSDR (2000a) derived a provisional acute oral MRL of 0.005 mg/kg/day for arsenic based on a LOAEL of 0.05 mg/kg/day for facial (periorbital) edema and gastrointestinal irritation in poisoning cases from arsenic-contaminated soy sauce in Japan (Mizuta et al. 1956). These effects were the initial effects, and in some patients, were followed by dermal lesions, neuropathy (hypesthesia in legs, abnormal patellar reflex), mild anemia, mild degenerative liver lesions and hepatic dysfunction, and abnormal electrocardiogram. An uncertainty factor of 10 was applied to account for the use of a LOAEL. The MRL is considered provisional because the gastrointestinal effects were serious and because serious neurological and cardiovascular effects also occurred at the same dose.

ATSDR (2000a) derived a chronic oral MRL of 0.0003 mg/kg/day for arsenic based on a NOAEL of 0.0008 mg/kg/day for dermal lesions in male and female farmers exposed to high levels of arsenic in well water in Taiwan. An uncertainty factor of 3 was applied to account for human variability.

EPA has not derived an RfC for arsenic (IRIS 2001).

EPA (IRIS 2001) derived a chronic RfD of 0.0003 mg/kg/day for arsenic based on a NOAEL of 0.0008 mg/kg/day for dermal lesions and possible vascular complications for farmers in Taiwan, which also was used as the basis for the ATSDR chronic oral MRL. An uncertainty factor of 3 was applied to account for the lack of reproductive data and to account for some uncertainty in which the NOAEL in the critical study accounts for all potentially sensitive individuals.

The National Toxicology Program (NTP 2001) has determined that inorganic arsenic compounds are known to be human carcinogens, based on sufficient evidence of carcinogenicity in humans. The International Agency for Research on Cancer (IARC 1987) concluded that there is sufficient evidence of a relationship between exposure to arsenic and human cancer, and classifies arsenic in Group 1. The American Conference of Governmental Industrial Hygienists (ACGIH) classifies arsenic (elemental and inorganic compound) as a confirmed human carcinogen; cancer category A1 (ACGIH 1998). EPA (IRIS 2001) has classified arsenic in Group A—Human carcinogen, based on increased lung cancer mortality in several human populations exposed primarily through inhalation, increased mortality from internal organ cancers (liver, kidney, lung, and bladder), and increased incidences of skin cancer in populations exposed to arsenic through drinking water. An oral slope factor of 1.5 per (mg/kg)/day was derived based on analysis of the skin cancer data from a Taiwanese population exposed through drinking water. An inhalation unit risk of 4.3×10^{-3} per $\mu g/m^3$ was derived based on age-specific mortality from lung cancer in male smelter workers.

B.5 Derivation of Target Organ Toxicity Dose (TTD) Values

TTDs for oral exposure to arsenic were derived for endpoints affected by arsenic and one or more of the other chemicals in the lead, arsenic, cadmium, and chromium mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include neurological, renal, cardiovascular, hematological, and testicular effects. Chronic oral TTDs for these endpoints are derived below, using the methods described by ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in

ATSDR (2000a), and in particular, the oral LSE table. Where the data are inadequate to derive a chronic oral TTD for a given endpoint, the chronic oral MRL is recommended as a conservative alternative that is protective of human health.

Neurological Effects

A large number of epidemiology studies and case reports indicate that ingestion of arsenic can cause injury to the nervous system. A symmetrical peripheral neuropathy has been observed in individuals exposed to 0.004–0.5 mg As/kg/day for an intermediate or chronic duration (ATSDR 2000a). The neuropathy is characterized by numbness in the hands and feet progressing to a painful pins and needles sensation and dying-back axonopathy with demyelination. Additionally, a significant association between decreased reading and spelling performance and hair arsenic levels was found in a group of elementary school children (Moon et al. 1985), suggesting that arsenic may also cause neurobehavioral effects. A TTD_{NEURO} can be derived using a study by Lianfang and Jianzhong (1994) of approximately 31,000 residents living in areas of China with high arsenic levels in the drinking water. This study identified NOAEL and LOAEL values of 0.003 and 0.004 mg As/kg/day, respectively, for an increased occurrence of numbness of the extremities. Dividing the NOAEL by an uncertainty factor of 10 for intrahuman variability results in a TTD_{NEURO} of 0.0003 mg As/kg/day.

Renal Effects

Although there have been some reports of kidney injury in humans ingesting arsenic, most studies did not report clinical signs of significant renal injury (ATSDR 2000a). When renal effects were observed they were often secondary to fluid imbalances or vascular injury. Several animal studies have reported renal effects following intermediate- or chronic-duration oral exposure. The effects include increased kidney weight, swollen mitochondria and increased numbers of dense autophagic lysosome-like bodies in the proximal tubules, increased pigmentation in the proximal tubules, and cysts (ATSDR 2000a). The ultrastructural changes in the proximal tubules were observed at 4.7 mg As/kg/day (Brown et al. 1976), which is the lowest identified LOAEL for renal effects in animal studies. However, the toxicological significance of this effect is not known. The next highest LOAEL is 20 mg As/kg/day identified in rats exposed to arsenic in the feed for 2 years (Byron et al. 1967). At 20 mg/kg/day and higher there was an increase in pigmentation in the proximal tubules and increased number of cysts in the renal cortex; no

effects were observed at 9 mg As/kg/day. The Byron et al. (1967) study was selected as the basis of the TTD_{RENAL}. The NOAEL of 9 mg As/kg/day was divided by an uncertainty factor of 100 (10 for interspecies differences and 10 for intrahuman variability) to derive a TTD_{RENAL} of 0.09 mg As/kg/day.

Cardiovascular Effects

The cardiovascular system is a very sensitive target of arsenic toxicity. A number of effects have been observed including heart damage (myocardial depolarization, hypertrophy of the ventricular wall, cardiac arrhythmias), vascular damage (Raynaud's disease, Blackfoot disease, arterial thickening), and hypertension (ATSDR 2000a). The series of studies by Tseng and associates (Tseng 1977; Tseng et al. 1968, 1995, 1996) provide suggestive evidence that Blackfoot disease and dermal hyperkeratosis and hyperpigmentation would occur at similar dose levels. Thus, the chronic-duration oral MRL of 0.0003 mg As/kg/day (based on the dermal effects reported by Tseng [1977] study) can also be used as the $TTD_{CARDIO.}$

Hematological Effects

Numerous studies have reported anemia in humans and animals ingesting arsenic (ATSDR 2000a). The available human studies reported significant increases in the occurrence of anemia at doses of 0.05 mg As/kg/day and higher (Franzblau and Lilis 1989; Wagner et al. 1979; Zaldivar and Guillier 1977). In a study of Utah residents with elevated levels of arsenic in the drinking water for at least 5 years, the incidence of anemia was not significantly higher than control populations (Southwick et al. 1981). This NOAEL of 0.006 mg As/kg/day and an uncertainty factor of 10 for intrahuman variability was used to derive a TTD_{HEMATO} of 0.0006 mg As/kg/day.

Testicular Effects

There is limited information on the potential reproductive toxicity of arsenic. In a 3-generation reproductive toxicity study, no alterations in reproductive success were observed at 1.2 mg As/kg/day (Schroeder and Mitchner 1971). Another study found an 8% decrease in testes weight in mice exposed to 0.0085 mg As/kg/day in drinking water for 32 days; no functional tests were conducted (Healy et al. 1998). Thus, the available data are inadequate to determine whether the testes are a target of concern for arsenic and a TTD_{TESTIC} was not derived.

Summary (TTDs for Arsenic)

TTD_{NEURO} = 0.0003 mg As/kg/day ($3x10^{-4}$ mg/kg/day)

TTD_{RENAL} = 0.09 mg As/kg/day ($9x10^{-2}$ mg/kg/day)

TTD_{CARDIO} = 0.0003 mg As/kg/day ($3x10^{-4}$ mg/kg/day)

TTD_{HEMATO} = 0.0006 mg As/kg/day ($6x10^{-4}$ mg/kg/day)

TTD_{TESTIC} = Not applicable

Appendix C: Background Information for Cadmium

C.1 Toxicokinetics

Ingested cadmium is poorly absorbed. Approximately 5% of the total cadmium ingested in food or water is absorbed. Cadmium absorption increases with iron or calcium deficiency. Absorption from the gut appears to take place in two phases—uptake from the lumen into the mucosa, and transfer from the mucosa into the circulation. Cadmium is distributed throughout the body, but the major portion is found in the liver and kidney. The majority of absorbed cadmium is retained in the tissues. Half-times for cadmium in the human kidney have been estimated at 6–38 years, and in human liver at 4–19 years. Cadmium concentrations in the kidney are near zero at birth, but rise with age to a peak (generally around 40–50 μg Cd/g wet weight) between ages 50 and 60, after which renal concentrations plateau or decline. Hepatic concentrations of cadmium also are near zero at birth, increasing to values of 1–2 μg/g wet weight by age 20–25, and increase only slightly thereafter. Thus, renal concentrations far exceed hepatic concentrations following prolonged exposure. Cadmium does not undergo metabolic conversion, but the cadmium ion can readily bind to anionic groups, especially sulfhydryl groups, in proteins and other molecules. Cadmium is bound to the protein metallothionein in the liver, which releases the metallothionein-cadmium complex, rather than free cadmium, into the bloodstream. Metallothionein is a low-molecular-weight, sulfhydryl-rich protein that normally binds zinc. Metallothionein-bound cadmium is readily filtered by the renal glomerulus and reabsorbed from the glomerular filtrate by the proximal tubule cells, at which point the "exogenous" metallothionein is catabolized in tubular lysosomes, releasing free cadmium. The free cadmium stimulates the synthesis of metallothionein in the tubular cells, is then bound to the tubular metallothionein, and remains in the cells. Most of the ingested cadmium is excreted unabsorbed in the feces. Most of the absorbed cadmium is retained; some excretion of cadmium occurs through the urine, and urinary excretion increases with renal damage (ATSDR 1999a).

C.2 Health Effects

Cadmium is considered a cumulative toxicant. The human exposure scenarios of greatest concern are long-term oral exposures. Cadmium accumulates in the kidney over a period of approximately 50 years; renal damage appears to be a consequence of this accumulation, such that the ability of the kidney to sequester cadmium through synthesis of metallothionein may be overwhelmed. Renal effects have been

seen in humans and animals by both inhalation and oral exposure, and are the most sensitive effects of chronic oral exposure, occurring at intakes as low as 0.0078 mg/kg/day. Effects of cadmium other than renal damage are not considered by ATSDR (1999a) to be sensitive effects. Nevertheless, some effects that are seen at moderately low levels of oral exposure are cardiovascular, hematological, neurological, and testicular effects. Cardiovascular effects (hypertension) have been reported in humans and animals in some studies and not in others. ATSDR (1999a) has concluded that the magnitude of any effect of cadmium on blood pressure is small compared with other determinants of hypertension, and that cardiovascular effects are not a sensitive endpoint for cadmium. Oral exposure to cadmium can cause anemia in humans and animals, but is not considered by ATSDR (1999a) to be likely to result from low level exposure. Hepatic effects occur with higher oral doses of cadmium, usually for acute or intermediate durations. A few studies have reported associations between environmental cadmium exposure (using hair cadmium as a biomarker) and neurobehavior effects including verbal IQ in children and disruptive behavior in young adults. Neurological effects have been seen in animals exposed to cadmium orally, and include changes in behavior, including a decrease in motor activity, alterations in neurotransmitter levels, histopathological changes in the brain, and peripheral neuropathy. These effects occurred in animals at doses as low as 1.4 mg/kg/day. Testicular effects have been seen from oral dose ranges of 5–14 mg/kg/day in animal studies. Although inhalation exposure to cadmium appears to be carcinogenic, oral exposure does not (ATSDR 1999a).

C.3 Mechanisms of Action

Cadmium is a cumulative renal toxicant. Cadmium accumulates in the kidney over the lifetime; toxicity is thought to result when a critical concentration of cadmium is reached in the kidney. Much of the cadmium in the kidney and in other tissues is bound to metallothionein, which is thought to sequester cadmium, preventing damage to cellular constituents, but which also retains cadmium in the cell. Metallothionein is thought to function in the storage of the essential metals zinc and copper, and to serve as an antioxidant. Details regarding the mechanism of cadmium renal toxicity are uncertain; renal damage is hypothesized to occur when an excessive concentration of free cadmium occurs intracellularly in the kidney, perhaps due to an insufficient rate of renal metallothionein synthesis to bind the intrarenal cadmium. The free cadmium may bind to other intracellular ligands, including metalloenzymes, and may destabilize proximal tubule cell membranes (ATSDR 1999a; IRIS 2001). Whether the accumulation of the CdMT complex devotes disproportionate cellular resources to sequestration of cadmium and may

contribute to toxicity through lack of metallothionein for other needs does not appear to have been considered as a possible mechanism.

Although intracellular renal metallothionein protects against the toxicity of cadmium, when released from the liver or administered by injection, CdMT is directly and indirectly toxic to the kidney. The CdMT that reaches the kidney through the circulation is filtered by the glomerulus, is directly toxic to the brush border membrane of the proximal convoluted tubules (Cherian 1985; Suzuki and Cherian 1987), and, following reabsorption by the proximal convoluted tubules, is indirectly toxic through degradation of the metallothionein and 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).

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 multi-organ 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 carry 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) metallothionein 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 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 metallothionein-inducers 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.

Cadmium is known to alter neurotransmitter levels in the brain, and may inhibit calcium entry into neurons (ATSDR 1999a; Nation et al. 1989). Testicular effects of cadmium may be due to cadmium interference with zinc-protein complexes that control DNA transcription, subsequently leading to apoptosis (ATSDR 1999a).

C.4 Health Guidelines

ATSDR (1999a) did not derive inhalation MRLs or acute or intermediate oral MRLs for cadmium due to lack of suitable studies.

ATSDR (1999a) derived a chronic oral MRL of 0.0002 mg/kg/day for cadmium based on a NOAEL for β_2-microglobulinuria (an indicator of renal damage) of 0.0021 mg/kg/day in humans, corresponding to a total lifetime cadmium intake of 2,000 mg. An uncertainty factor of 10 was applied to the NOAEL to account for human variability.

EPA has not derived an RfC for cadmium (IRIS 2001).

EPA derived chronic RfDs of 0.0005 mg/g/day for water and 0.001 mg/kg/day for food for cadmium, based on NOAELs of 0.005 mg/kg/day for water and 0.01 mg/kg/day for food (IRIS 2001). The NOAELs were estimated with a toxicokinetic model from a human NOAEL of 200 μg Cd/g wet renal cortex for proteinuria (an indicator of renal damage). The different values for food and water reflect EPA opinion regarding bioavailability from these media.

The National Toxicology Program (NTP 2001) has classified cadmium and cadmium compounds as known to be human carcinogens, based on sufficient evidence of carcinogenicity from studies in humans. IARC (1993) concluded that cadmium and cadmium compounds are carcinogenic to humans (Group 1). EPA (IRIS 2001) classified cadmium in Group B1—probable human carcinogen, and derived an inhalation unit risk of 1.8×10^{-3} per $\mu g/m^3$ for cadmium based on lung, trachea, and bronchus cancer mortality in male workers in a cadmium smelter. EPA (IRIS 2001) noted that seven oral studies of

cadmium salts in rats and mice have given no evidence of carcinogenicity, and that studies of ingestion in humans are inadequate to assess carcinogenicity.

C.5 Derivation of Target Organ Toxicity Dose (TTD) Values

TTDs for oral exposure to cadmium were derived for endpoints affected by cadmium and one or more of the other chemicals in the lead, arsenic, cadmium, and chromium mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include neurological, renal, cardiovascular, hematological, and testicular effects. Chronic oral TTDs for these endpoints are derived below, using the methods described by ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (1999a), and in particular, the oral LSE table. Where the data are inadequate to derive a chronic oral TTD for a given endpoint, the chronic oral MRL for cadmium is recommended as a conservative alternative that is protective of human health.

Neurological Effects

Neurological effects consisting of decreased motor activity, weakness and muscle atrophy, aggressive behavior, increased passive avoidance, and alterations in brain dopamine, 5-hydroxytryptamine, succinic dehydrogenase, and monoamine oxidase levels have been observed in rats exposed to 3.1–24 mg Cd/kg/day for an intermediate duration (ATSDR 1999a). In mice, necrosis of the choroid plexus epithelial cells have been observed following intermediate duration exposure to 1.4 mg Cd/kg/day as cadmium chloride in drinking water, but not after exposure to 0.2 mg Cd/kg/day (Valois and Webster 1989). Chronic exposure to 3.6 mg Cd/kg/day as cadmium chloride in drinking water resulted in peripheral neuropathy in rats (Sato et al. 1978). The lowest LOAEL for neurological effects was identified in the intermediate duration mouse study; this study was selected as the basis of the TTD_{NEURO}. A TTD_{NEURO} of 0.0002 mg Cd/kg/day was calculated by dividing the NOAEL of 0.2 mg Cd/kg/day identified in the Valois and Webster (1989) study by an uncertainty factor of 1,000 (10 for use of an intermediate-duration study, 10 for interspecies differences and 10 for intrahuman variability)

Renal Effects

Numerous human and animal studies indicate that the kidney is the main target of cadmium toxicity (ATSDR 1999a). The kidney damage is characterized as decreased reabsorption of filtered low

molecular weight proteins and mild tubular lesions progressing to necrosis. The chronic oral MRL for cadmium of 0.0002 mg Cd/kg/day is based on renal effects.

Cardiovascular Effects

A number of human and animal studies have found a relationship between ingestion of cadmium and increased blood pressure, but other studies have not found any significant association (ATSDR 1999a). ATSDR (1999a) concluded that the evidence for cardiovascular toxicity resulting from oral exposure to cadmium is suggestive of a slight effect and that the magnitude of any effect of cadmium on blood pressure is small compared with other determinants of hypertension. Increases in blood pressure have been observed in animals exposed to doses of 0.0081–1.6 mg Cd/kg/day and 0.01–1.71 mg Cd/kg/day following intermediate or chronic exposure, respectively. The Perry et al. (1989) and Kopp et al. (1982) studies identified the lowest LOAELs for hypertension following intermediate- and chronic-duration exposure, respectively; however, these studies were not selected as the basis of the TTD_{CARDIO} because of the uncertainty regarding the relevance to human exposures of the very low metal diet and environment used in the animal studies. (See "Animal Studies—Oral Exposure" in Section 2.2.4 for further discussion of the studies by this group of investigators.) Thus, the Akahori et al. (1994) chronic monkey study was selected as the basis of the TTD_{CARDIO}. This study identified NOAEL and LOAEL values of 0.53 and 1.71 mg Cd/kg/day, respectively, for increases in blood pressure in Rhesus monkeys exposed to cadmium chloride in the diet for 9 years; blood pressure effects were only observed during the first 1.5 years. Dividing this NOAEL by an uncertainty factor of 100 (10 for interspecies differences and 10 for intrahuman variability) results in a TTD_{CARDIO} of 0.005 mg Cd/kg/day.

Hematological Effects

Oral cadmium exposure reduces gastrointestinal uptake of iron, which can result in anemia if dietary intake of iron is low. In animal studies, administration of additional iron prevents the anemia. Anemia has been observed in some human oral studies and in a number of animal oral studies of cadmium. Following intermediate-duration exposure, anemia has been observed in rats, mice, and rabbits exposed to doses of 0.8 mg Cd/kg/day and higher (ATSDR 1999a). In chronic-duration studies, anemia was observed in monkeys exposed to 4.0 mg Cd/kg/day. Although a human study (Shiwen et al. 1990) identified a NOAEL of 0.0078 mg Cd/kg/day for anemia in individuals exposed to cadmium for at least 25 years, this study was not selected as the basis of a TTD_{HEMATO} because both the control and exposed

populations had very high incidences of anemia (65–73%), which are much higher than in the U.S. population. Additionally, the monkey study was not selected as the basis of the TTD because an intermediate-duration study identified a lower LOAEL. The TTD_{HEMATO} is based on the LOAEL of 0.8 mg Cd/kg/day identified in rats exposed to cadmium chloride in drinking water for 4 weeks (Ogoshi et al. 1989); a NOAEL was not identified in this study. The NOAEL is divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for interspecies differences, and 10 for intrahuman variability) resulting in a TTD_{HEMATO} of 0.0008 mg Cd/kg/day. Because the hematological effects are secondary to decreased iron intake rather than a direct effect of cadmium on the hematological system, it is not likely that the effect is duration-related, thus, an uncertainty factor was not used to account for the use of an intermediate-duration study.

Testicular Effects

Testicular effects have been observed in animals exposed to cadmium for acute or intermediate durations; the testicular effects included necrosis and atrophy of seminferous tubule epithelium, increased testes weight, and decreased sperm count and motility (ATSDR 1999a). Chronic oral studies have not tested the reproductive toxicity of cadmium. The oral studies suggest that the testicular effects occur at doses of 5.8 mg Cd/kg/day and higher. NOAEL and LOAEL values of 2.9 and 5.8 mg Cd/kg/day, respectively, for increased relative testes weight were identified in a study in which rats were exposed to cadmium chloride in the drinking water for 14 weeks (Pleasants et al. 1992). TTD_{TESTIC} of 0.003 mg Cd/kg/day is based on this NOAEL and an uncertainty factor of 1,000 (10 for use of an intermediate-duration study, 10 for interspecies differences, and 10 for intrahuman variability).

Summary (TTDs for Cadmium)

TTD_{NEURO} = 0.0002 mg Cd/kg/day ($2x10^{-4}$ mg/kg/day)
$MRL_{(RENAL)}$ = 0.0002 mg Cd/kg/day ($2x10^{-4}$ mg/kg/day)
TTD_{CARDIO} = 0.005 mg Cd/kg/day ($5x10^{-3}$ mg/kg/day)
TTD_{HEMATO} = 0.0008 mg Cd/kg/day ($8x10^{-4}$ mg/kg/day)
TTD_{TESTIC} = 0.003 mg Cd/kg/day ($3x10^{-3}$ mg/kg/day)

Appendix D: Background Information for Chromium(VI)

D.1 Toxicokinetics

The absorption of chromium(VI) through the gastrointestinal tract after oral exposure of humans is about 2–10% for potassium chromate. The chromate anion can enter cells by facilitated diffusion through nonspecific anion channels, similarly to phosphate and sulfate anions. Absorption efficiency appears to increase with increasing dose. Once in the blood, chromium is distributed to all organs of the body; preferential distribution to any particular organ does not appear to occur. Chromium(VI) does not appear to accumulate in the body. Chromium(VI) is unstable in body fluids and tissues, including the gastric juice, and is reduced to chromium(V), chromium(IV), and ultimately to chromium(III) by many substances, including ascorbate and glutathione. Absorbed chromium is excreted primarily in the urine; the half-time for excretion of chromium following administration of potassium chromate in drinking water was estimated at 35–40 hours in humans. Minor pathways of excretion are through the hair and nails. Much of the chromium from ingested chromium(VI) passes through the body without being absorbed and is excreted in the feces (ATSDR 2000b).

D.2 Health Effects

Accidental or intentional ingestion of very high doses of chromium(VI) compounds has resulted in severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects. Respiratory and cardiovascular effects are not generally seen at nonlethal doses. Gastrointestinal effects are associated with irritating effects on the mucosa at high concentrations of chromium(VI). Hematological effects (reduced MCV and MCH) have been seen in rats and mice fed chromium(VI) in the diet for intermediate durations, with a LOAEL of approximately 8 mg/kg/day in rats and 32 mg Cr(VI)/kg/day in mice. Renal effects included accumulation of lipids and inhibition of membrane enzymes in rats given chromium(VI) at 13.5 mg/kg/day by gavage, and proteinuria in rats given chromium(VI) at 98 mg/kg/day from drinking water. An exacerbation of chromium contact dermatitis has been seen in chromium(VI)-sensitized individuals given an oral dose of chromium(VI). In a single study in rats, chromium(VI) produced increased proliferative responses to T- and B-lymphocytes to mitogens, effects consistent with sensitization. Decreased motor activity and balance were seen in rats given 98 mg/kg/day of chromium (VI) in drinking water for 28 days. Testicular effects occurred in rats given 40 mg/kg/day for 90 days, and altered male sexual behavior has been noted at a similar dose.

Chromium(VI) administration to rats and mice during gestation by the oral route was developmentally toxic at ≥51 mg/kg/day. Although chromium(VI) is a human carcinogen by the inhalation route of exposure, there is no evidence that it is carcinogenic by the oral route (ATSDR 2000b).

D.3 Mechanisms of Action

As previously mentioned, chromium(VI) enters the cells through membrane channels that also admit sulfate and phosphate. Once in the cell chromium(VI) is reduced to chromium(III), with chromium(V) and (IV) as intermediates. The reactions commonly involve intracellular species such as ascorbate, glutathione, or amino acids. Chromium(VI), (V), and (IV) have been shown to be involved in oxidative cycling, generating oxygen radical species. The formation of these radicals may be responsible for many of the deleterious effects of chromium on cells, which can be blocked by radical scavengers. This mechanism appears to have been explored in terms of their potential impact on the induction of carcinogenic responses, and the data appear to have been obtained primarily in vitro. *In vivo* studies, however, reported that the antioxidant ascorbate protected against the lethality of dermally administered chromium(VI) and the nephrotoxicity of subcutaneously injected chromium(VI), through reduction of chromium(VI) to chromium(III) (ATSDR 2000b). Once formed from reduction of chromium(VI) within the cell, chromium(III) is thought to complex with intracellular macromolecules (Goyer 1995). It may bind to proteins through a variety of functional groups (de Meester and Hodgson 1977; de Meester et al. 1977).

D.4 Health Guidelines

ATSDR (2000b) derived an inhalation MRL of 0.000005 mg Cr(VI)/m^3 (as chromic acid [chromium trioxide mist] and other dissolved chromium(VI) aerosols and mists) for intermediate-duration exposure. The MRL was based on a LOAEL of 0.002 mg Cr(VI)/m^3 for nasal lesions in workers. To derive the MRL, the LOAEL was adjusted for continuous exposure (0.0005 mg Cr(VI)/m^3) and divided by an uncertainty factor of 100 (10 for human variability and 10 for extrapolating from a LOAEL). ATSDR also derived an MRL of 0.001 mg Cr(VI)/m^3 for intermediate exposure to particulate chromium(VI) compounds, based on a benchmark concentration (BMC) of 0.016 mg/m^3 for increased levels of lactate dehydrogenase in bronchoalveolar lavage fluid in rats. The BMC was converted to a BMC_{ADJ} and divided by an uncertainty factor of 30 (3 for pharmacodynamic differences not addressed by the dose conversion and 10 for human variability).

ATSDR (2000b) did not derive oral MRLs for chromium(VI) (or chromium(III)) because of insufficient to conflicting data on reproductive and developmental effects. Instead, the upper end of the range of the estimated safe and adequate daily dietary intake of 200 µg Cr/kg/day (NRC 1989) was adopted as provisional guidance for oral exposure to chromium(VI) and chromium(III).

The NRC (1989) derived its estimated safe and adequate daily dietary intakes for (trivalent) chromium of 50–200 µg/day for adults, based on data regarding chromium intake from typical Western diets, the beneficial effect of chromium supplementation in the United States on subjects with impaired glucose tolerance, and the low toxicity of trivalent chromium. The NRC further stated that because humans cannot oxidize the nontoxic trivalent food chromium to the potentially carcinogenic hexavalent chromate compounds, the carcinogenicity of certain chromates is not relevant to the nutritional role of the trivalent form.

EPA (IRIS 2001) derived a chronic inhalation RfC of 0.008 µg Cr(VI)/m^3 for chromic acid mists and dissolved chromium(VI) aerosols, based on a LOAEL for nasal septum atrophy in workers exposed to 0.002 mg Cr(VI)/m^3. An uncertainty factor of 90 (3 for extrapolation from subchronic to chronic, 3 for extrapolation from a LOAEL to NOAEL, and 10 for human variation) was applied to a $LOAEL_{ADJ}$.

EPA (IRIS 2001) also derived a chronic inhalation RfC of 0.0001 mg Cr(VI)/m^3 for chromium(VI) particulates, based on a benchmark concentration of 0.016 mg Cr(VI)/m^3 derived from data for lactate dehydrogenase activity in bronchoalveolar lavage fluid in rats.

EPA (IRIS 2001) derived a chronic oral reference dose (RfD) of 0.003 mg Cr(VI)/kg/day for soluble salts of chromium(VI) (e.g., potassium chromate, sodium chromate, potassium dichromate, and sodium dichromate), based on a NOAEL of 2.5 mg Cr(VI)/kg/day for systemic effects in rats exposed to potassium chromate in the drinking water for 1 year.

NTP (2001) lists certain chromium(VI) compounds as substances that are known to be human carcinogens, based on sufficient evidence of carcinogenicity in humans. This classification is based on sufficient evidence for calcium chromate, chromium trioxide, lead chromate, strontium chromate, and zinc chromate. IARC (1990) classifies chromium(VI) in Group 1, carcinogenic to humans, based on sufficient evidence in humans for the carcinogenicity of chromium(VI) compounds as encountered in the chromate production, chromate pigment production, and chromium plating industries; sufficient evidence

in experimental animals for the carcinogenicity of calcium chromate, zinc chromates, strontium chromate, and lead chromates; limited evidence in experimental animals for the carcinogenicity of chromium trioxide and sodium dichromate; and data that support the concept that chromium(VI) ions generated at critical sites in the target cells are responsible for the carcinogenic action observed. EPA has classified chromium(VI) in Group A, a known human carcinogen by the inhalation route of exposure. For the oral route, chromium(VI) is classified as Group D, not classified as to human carcinogenicity (IRIS 2001).

D.5 Derivation of Target Organ Toxicity Dose (TTD) Values

TTDs for oral exposure to chromium(VI) were derived for endpoints affected by chromium(VI) and one or more of the other chemicals in the lead, arsenic, cadmium, and chromium mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include neurological, renal, cardiovascular, hematological, and testicular effects. Chronic oral TTDs for these endpoints are derived below, using the methods described by ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2000b), and in particular, the oral LSE table. Where the data are inadequate to derive a chronic oral TTD for a given endpoint, the RfD for chromium(VI) is recommended as a conservative alternative that is protective of human health.

Neurological Effects

There is limited information on the neurotoxicity of chromium(VI). Dizziness, headache, and weakness were reported by workers exposed to high concentrations of chromium(VI) oxide (chromium trioxide) (Lieberman 1941). In rats, decreased motor activity and ponderal balance were observed following a 28-day exposure to 100 mg Cr(VI)/kg/day as sodium chromate in drinking water (Diaz-Mayans et al. 1986); no effects were observed at 10 mg Cr(VI)/kg/day. A decrease in motor activity was observed in rats following intraperitoneal administration of sodium chromate (Diaz-Mayans et al. 1986). The NOAEL identified in the Diaz-Mayans et al. (1986) drinking water study is a suitable basis for a TTD. Application of an uncertainty factor of 1,000 (10 for extrapolation from rats to humans, 10 for intrahuman variability, and 10 to extrapolate from an intermediate-duration study) to the NOAEL results in a TTD_{NEURO} of 0.01 mg Cr(VI)/kg/day.

Renal Effects

Severe renal impairment, renal failure, and necrosis of the renal tubules have been reported in cases of fatal or near fatal ingestion of chromium(VI) and impaired renal function has been reported in workers exposed to airborne chromium(VI) (ATSDR 2000b). Renal effects have also been reported in experimental animal studies. An accumulation of lipids and inhibition of membrane enzymes were observed in rats administered via gavage 13.5 mg Cr(VI)/kg/day as potassium chromate for 20 days (Kumar and Rana 1982, 1984) and oliguria and proteinuria were observed in rats receiving 100 mg Cr(VI)/kg/day as sodium chromate in drinking water for 28 days (Diaz-Mayans et al. 1986). In a series of studies conducted by NTP, no histological alterations were observed in rats or mice exposed to doses as high as 9.8 or 48 mg Cr(VI)/kg/day, respectively, as potassium dichromate for 9 weeks, (NTP 1996a, 1996b); however, no tests of renal function were performed. The available human and animal data provide strong evidence that the kidney is a target of chromium toxicity. A TTD_{RENAL} of 0.01 mg Cr(VI)/kg/day was derived using the NOAEL of 10 mg Cr(VI)/kg/day identified in the Diaz-Mayans et al. (1986) study and an uncertainty factor of 1,000 (10 for extrapolation from rats to humans, 10 for intrahuman variability, and 10 to extrapolate from an intermediate-duration study). The Kumar and Rana (1982, 1984) studies were not selected as the basis of the TTD because the potassium chromate was administered via gavage and there is some human evidence which suggests a higher absorption rate following bolus administration versus three divided dose administration (Kerger et al. 1997).

Cardiovascular Effects

Cardiovascular effects (e.g., cardiopulmonary arrest, hypoxic changes in myocardium, and progressive drop in cardiac output, heart rate and blood pressure) have been observed in humans following lethal ingestion of chromium(VI) (ATSDR 2000b). However, cardiovascular effects have not been observed in humans or animals exposed to nonfatal doses, suggesting that cardiovascular toxicity is not a target of concern. Thus, a TTD_{CARDIO} was not derived.

Hematological Effects

A series of intermediate-duration studies conducted by NTP (1996a, 1996b, 1997) have consistently shown slight dose-related decreases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in rats and mice exposed to potassium dichromate in the diet for at least 9 weeks.

The lowest identified LOAEL for these alterations, 7.8 mg Cr(VI)/kg/day, was found in F_1 female mice (NTP 1997); this study did not identify a NOAEL. This LOAEL and an uncertainty factor of 3,000 (3 for use of a minimal LOAEL, 10 for extrapolation from intermediate to chronic duration, 10 for interspecies extrapolation, and 10 for intrahuman variability) were used to derive a TTD_{HEMATO} of 0.003 mg Cr(VI)/kg/day.

Testicular Effects

Several animal studies have found reproductive effects in males orally exposed to 14–42 mg Cr(VI)/kg/day. These effects included decreases in testes, seminal vesicle, and preputial gland weights, decreased sperm counts, morphological sperm alterations, and alterations in sexual behavior (ATSDR 2000b). However, no histological or organ weight alterations were observed in rats and mice exposed to 9.8 or 32.2 mg Cr(VI)/kg/day as potassium dichromate in feed (NTP 1996a, 1996b) and no adverse effects were observed in a multigeneration reproductive study in which mice were exposed to 36.7 mg Cr(VI)/kg/day (NTP 1997). Although there are conflicting results, the available data suggest that chromium(VI) can adversely affect the male reproductive system. The lowest identified reliable LOAEL is 14 mg Cr(VI)/kg/day for decreased seminal vesicle and preputial gland weights in mice exposed to potassium dichromate in drinking water for 12 weeks (Elbetieha and Al-Hamood 1997). Application of a 3,000 uncertainty factor (3 for use of a minimal LOAEL, 10 for extrapolation from intermediate to chronic duration, 10 for interspecies extrapolation, and 10 for intrahuman variability) to this LOAEL yields a TTD_{TESTIC} of 0.005 mg Cr(VI)/kg/day. The Elbetieha and Al-Hamood (1997) study reported an increase in testes weight at 6 mg Cr(VI)/kg/day; however, this LOAEL was not selected for TTD derivation because this is the only study which found an increase in testes weight.

Summary (TTDs for Chromium(VI))

TTD_{NEURO} = 0.01 mg Cr(VI)/kg/day ($1x10^{-2}$ mg/kg/day)
TTD_{RENAL} = 0.01 mg Cr(VI)/kg/day ($1x10^{-2}$ mg/kg/day)
TTD_{CARDIO} = Not applicable
TTD_{HEMATO} = 0.003 mg Cr(VI)/kg/day ($3x10^{-3}$ mg/kg/day)
TTD_{TESTIC} = 0.005 mg Cr(VI)/kg/day ($5x10^{-3}$ mg/kg/day)