

**DRAFT**  
**INTERACTION PROFILE FOR:**  
**LEAD, MANGANESE, ZINC, AND COPPER**

**U.S. Department of Health and Human Services**  
**Public Health Service**  
**Agency for Toxic Substances and Disease Registry**

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Public Comment Period Ends September 2, 2002



## 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.

The public comment period ends on September 2, 2002. Comments should be sent to:

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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. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

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.



## SUMMARY

Lead, manganese, zinc, and copper were chosen as the subject mixture for this interaction profile based on an analysis of the most frequently occurring binary mixtures in completed exposure pathways at hazardous waste sites. These metals are commonly found in soil. The primary route of exposure for this mixture is likely to be oral and the durations of concern are intermediate and particularly chronic. The term “metals” is used in this profile for brevity and convenience, and is intended to refer to lead, manganese, zinc, and copper in inorganic compounds or as ions. 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, manganese, zinc, and copper, 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.

Although occupational and environmental exposure studies of the trinary mixture of lead, zinc, and copper are available, they are not adequate as the basis for conclusions regarding the toxicity of this mixture due to inconsistencies in results across studies by a single group of investigators and deficiencies in study design. In general, the effects in workers coexposed to lead, zinc, and copper were characteristic of lead toxicity. Whether the coexposure to zinc and copper provided partial protection against these effects cannot be determined from the data. In animals exposed environmentally to lead, zinc, and copper (and cadmium), high bone burdens of lead and low tissue levels of copper were seen, in comparison with unexposed animals. The decreased copper in the tissues may reflect zinc inhibition of copper absorption, but again, no clear conclusions can be drawn from this study.

Most of the data regarding joint toxic action of the components of this mixture are for binary combinations of these metals. Data for the lead-zinc mixture are extensive, and for the lead-manganese, lead-copper, and zinc-copper mixtures are adequate to support some conclusions regarding the mode of joint toxic action. Many of these studies are reasonably relevant because they employed intermediate oral exposure, and investigated endpoints relevant to the critical and sensitive effects of the mixture components. Data for the manganese-zinc and manganese-copper mixtures, however, are inadequate. Based on these data, and on mechanistic data for the individual components, the predicted directions of

interaction for the binary mixtures were primarily less than additive or additive, with exception of the effects of manganese on the toxicity of lead, which were predicted to be greater than additive. Further discussion of the predicted interactions is presented later in this section.

Because no adequate studies or PBPK models for the whole mixture are available, a components-based approach to the exposure-based assessment of the potential hazard to the public is recommended in this profile, consistent with ATSDR (2001a) guidance. The effects of concern for the mixture include the critical effects of the individual components, and effects in common that may become significant due to additivity or interactions. The critical effects of two of the mixture components, lead and manganese, are neurological. The critical effects of zinc are hematological, which is also a sensitive effect of lead. The recommended approach is to estimate endpoint-specific hazard indexes for the neurotoxicity of lead and manganese and for the hematotoxicity of lead and zinc in order to screen for noncancer health hazards from potential additivity. The qualitative weight-of-evidence (WOE) is applied to assess the potential impact of interactions of the mixture components on the neurological and hematological hazard. The critical effect of copper is hepatic. The recommended approach is to use the hazard quotient for copper, with application of the qualitative WOE to assess the potential impact of the other metals on the hepatic toxicity of copper. If an endpoint-specific hazard index or if the hazard quotient for copper is greater than unity, and/or if the qualitative WOE indicates that joint toxic action may be greater than additive, further evaluation is needed (ATSDR 2001a), using biomedical judgment and community-specific health outcome data, and taking into account community health concerns (ATSDR 1992).

Estimation of endpoint-specific hazard indexes and application of the WOE are appropriate when hazard quotients for two or more of the mixture components equal or exceed 0.1 (ATSDR 2001a). If only one or if none of the mixture components has a hazard quotient that equals or exceeds 0.1, further assessment of the joint toxic action is not needed because additivity and/or interactions are unlikely to result in significant health hazard. Appropriate health guidance values for use in estimating hazard quotients and hazard indexes are summarized (Minimal Risk Levels [MRLs]) or derived (target-organ toxicity doses [TTDs]) in this profile.

The WOE method was used to prepare binary weight-of-evidence determinations (BINWOEs) for the binary mixtures (ATSDR 2001a, 2001b). The BINWOEs are predictions of the plausible direction of interactions, when they occur, and the degree of confidence in the prediction (indicated by a numerical score). The BINWOEs are used qualitatively to estimate the impact of interactions on the endpoint-

specific hazard indexes for neurological and for hematological effects, and on the unique hazard quotient for hepatic effects of copper. The BINWOE scores, when summed to give a combined WOE score, provide an overall indication of direction of interaction and confidence.

*Neurological:* The predicted direction of joint toxic action for neurological effects, an endpoint common to two components, is greater than additive for the effect of manganese on lead, less than additive for the effects of zinc and copper on lead, additive (no effect) for the effect of lead on manganese, and indeterminate for the effects of zinc and copper on manganese. The WOE score indicates that the potential health hazard may be less than estimated by the endpoint-specific hazard index for neurological effects, particularly for waste sites with relatively high hazard quotients for lead, copper, and zinc, and a lower hazard quotient for manganese. The indeterminate ratings for two of the BINWOEs (zinc and copper on manganese) are a source of uncertainty in assessments where manganese accounts for a great portion of the apparent neurological hazard.

*Hematological:* The potential health hazard for hematological effects is likely to be lower than indicated by the endpoint-specific hazard index for mixtures where lead, zinc, and copper predominate, because three of the BINWOEs for combinations of these metals were less than additive with moderate to high confidence, and the remaining one was additive. The BINWOE for manganese on lead was greater than additive with low-moderate confidence, for lead on manganese was additive, and for manganese on zinc was indeterminate. The WOE score indicates that the potential health hazard may be less than estimated by the endpoint-specific hazard index for hematological effects. The indeterminate BINWOE for manganese on zinc is a source of uncertainty.

*Hepatic:* The predicted effects of the other mixture components on the hepatic toxicity of copper are less than additive for zinc with high-moderate confidence, additive for lead, and indeterminate for manganese. Thus, the available data indicate the potential health hazard for hepatic effects may be less than predicted by the hazard quotient for mixtures where zinc and copper predominate. There is uncertainty with regard to the potential effect of manganese due to the lack of pertinent information.



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## LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists	mg	milligram
ALA	delta-aminolevulinic acid	Mn	manganese
ALAD	delta-aminolevulinic acid dehydratase	MRL	Minimal Risk Level
ALAS	delta-aminolevulinic acid synthetase	NIH	National Institutes of Health
ATPase	adenosine triphosphatase	NMDA	N-methyl-D-aspartate
ATSDR	Agency for Toxic Substances and Disease Registry	NOAEL	no-observed-adverse-effect level
		NRC	National Research Council
		NTP	National Toxicology Program
BINWOE	binary weight-of-evidence		
BMD	benchmark dose	Pb	lead
BMDL <sub>10</sub>	benchmark dose, increased risk of 10%	PbB	blood lead concentration
		PBPK	physiologically based pharmacokinetic
CDC	Centers for Disease Control	PBPK/PD	physiologically-based pharmacokinetic pharmacodynamic
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act	ppm	parts per million
CI	confidence interval	RDA	Recommended Dietary Allowance
Cu	copper	RfC	reference concentration
		RfD	reference dose
dL	deciliter	RNA	ribonucleic acid
DNA	deoxyribonucleic acid		
DT	Division of Toxicology	SGOT	serum glutamic-oxaloacetic transaminase
EDTA	calcium disodium ethylenediamine		
EPA	Environmental Protection Agency	TLV	threshold limit value
ESADDI	estimated safe and adequate daily dietary intake	TTD	target-organ toxicity dose
		µg	microgram
FDA	Food and Drug Administration	UL	tolerable upper intake level
FEP	free erythrocyte protoporphyrin	µmole	micromole
FQPA	Food Quality Protection Act	U.S.	United States
GABA	gamma-aminobutyric acid	WOE	weight-of-evidence
GOT	glutamic-oxaloacetic transaminase		
		Zn	Zinc
HDL	high density lipoprotein	ZPP	zinc protoporphyrin
IARC	International Agency for Research on Cancer	>	greater than
		≥	greater than or equal to
IEUBK	Integrated Exposure Uptake Biokinetic	=	equal to
		≈	approximately equal to
i.p.	intraperitoneal	<	less than
IRIS	Integrated Risk Information System	≤	less than or equal to
kg	kilogram		
L	liter		
LOAEL	lowest-observed-adverse-effect level		



## 1. Introduction

The primary purpose of this Interaction Profile for lead, manganese, zinc, and copper 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, manganese, zinc, and copper mixture was chosen as the subject for this interaction profile based on an analysis of the most frequently occurring binary mixtures in completed exposure pathways at hazardous waste sites. These metals are commonly found in soil. The primary route of exposure for this mixture is likely to be oral and the durations of concern are intermediate and particularly chronic. The term “metals” is used in this profile for brevity and convenience, and is intended to refer to lead, manganese, zinc, and copper in inorganic compounds or as ions.

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 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 1999). 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) of 10  $\mu\text{g}/\text{dL}$ , and ATSDR (1999) suggests the use of media-specific slope factors and site-specific environmental monitoring data to predict media-specific contributions to PbB. The critical effect for manganese is neurological. ATSDR (2000) has not derived oral MRLs for manganese (Mn) because no clear threshold level for neurological effects could be determined from the acute and intermediate duration data, and because no firm conclusions were considered possible regarding a critical effect level of chronic intake versus essential dietary levels of manganese. ATSDR (2000) derived a provisional guidance value for total dietary intake of 0.07 mg Mn/kg/day, based on the upper end of the estimated safe and adequate daily dietary intake (ESADDI) range (5 mg/day, divided by 70 kg, the weight of an average adult), to be used in ATSDR human health assessments. ATSDR (1994) derived an intermediate oral MRL of 0.3 mg/kg/day for zinc (Zn) based on hematological effects (decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase activity) in humans. The hematological effects may be related to disruption of the copper balance. The intermediate oral MRL was adopted as the chronic oral MRL for zinc due to the lack of adequate chronic studies. ATSDR did not derive oral MRLs for copper (Cu) because of the lack of human data, lack of no-observed-adverse-effects level (NOAEL) values in the animal studies, development of tolerance in rats, and because of the essentiality of copper. Nor did ATSDR (1990) suggest a suitable guidance value in this relatively early toxicological profile. If an approach analogous to that for manganese were taken, i.e., to adopt the upper end of the National Research Council (NRC 1989) ESADDI range for copper (3 mg/day for adults, divided by 70 kg, the weight of an adult) as a guidance value for total dietary intake for copper, the resulting value would be 0.04 mg/kg/day. More recently, a Recommended Dietary Allowance (RDA) has been estimated at 0.9 mg/day ( $\approx 0.013$  mg/kg/day) (Institute of Medicine 2001: prepublication document, final version not published as of December 2001). The critical effect for overexposure to copper was considered to be liver damage, and a tolerable upper intake level (UL) of 10 mg/day ( $\approx 0.14$  mg/kg/day) of copper was established for adults ( $\geq 19$  years old). This value, once available in the final published document, may be appropriate as a provisional guidance value.

The bases for the MRLs or guidance value (or health assessment approach in the case of lead), as well as other sensitive effects, are summarized in Table 1. No pertinent studies of the toxicity or interactions of the quaternary mixture were located. Studies of one trinary mixture (lead, zinc, and copper) were located but limitations in study design and relevance of the studied endpoints limit the conclusions that can be drawn. The bulk of the available interactions information is for binary mixtures of these metals. Table 2 summarizes the availability of pertinent data on joint toxic action data by endpoint for the binary

mixtures. The table serves as an overview, and shows some data gaps: no relevant studies of joint toxic action for manganese and zinc or for manganese and copper. The lead-zinc mixture has been studied the most extensively.

**Table 1. Potential Health Effects of Concern for Intermediate and Chronic Oral Exposure to the Mixture Lead, Manganese, Zinc, and Copper<sup>a</sup>**

Lead	Manganese	Zinc	Copper
<b>Neurological</b> Hematological Cardiovascular	<b>Neurological</b>	<b>Hematological</b>	<b>Hepatic</b> Gastrointestinal <sup>b</sup>

<sup>a</sup>The basis for the MRL or health assessment approach is bolded; other sensitive effects are listed in regular typeface.

<sup>b</sup>Particularly for acute exposure to copper in drinking water

**Table 2. Availability of Pertinent Joint Toxic Action Data for Pairs of Components**

Endpoint	Lead-Manganese	Lead-Zinc	Lead-Copper	Manganese-Zinc	Manganese-Copper	Zinc-Copper
Cardiovascular						
Hematological	X	X	X			
Hepatic			X			X
Renal		X				
Immunological						X
Reproductive (testicular)		X				
Neurological	X	X	X			

X = Some data are available

Potential neurological effects are a particular concern for this mixture. Lead produces neurobehavioral effects in young children (ATSDR 1999), and manganese has the potential to do so, because infants absorb or retain a greater proportion of oral manganese than do adults, and the manganese in breast milk may be more bioavailable than manganese in foods consumed by other age groups (ATSDR 2000). For this reason, the order of discussion of the toxicities of the individual metals and of the binary mixtures starts with lead and manganese.



## **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 gastrointestinal absorption of lead and sensitivity to its effects are affected by the adequacy of essential metals, such as calcium, zinc, iron, and selenium, and also other nutrients in the diet. This interdependence seems to be true for zinc and copper, and may be for manganese as well. 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. Studies focusing on animals in deficiency states for the essential elements that are the topic of this profile, or for other elements, are not considered relevant to the interactions of excess manganese, zinc, or copper, and are not reviewed in detail in this profile.

### **2.1 Mixture of Concern**

No studies were located regarding the toxicity of the complete mixture.

No PBPK models were found for mixtures of lead, manganese, zinc, and copper.

## 2.2 Component Mixtures

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

Studies of the interactions or toxicity of one trinary mixture were located and are reviewed in the following subsection. 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.

At the end of each binary section, the *in vivo* data are summarized by exposure route, duration, and endpoint in tables. These summary tables are designed to give an overview of the pattern of interactions across different exposure conditions, endpoints, and studies. Consistent with the guidance for the preparation of these documents, two sets of tables are presented for each binary mixture—one for the effect of chemical A on the toxicity of B, and the other for the effect of B on the toxicity of A. Because the database for the effect of zinc on the toxicity of lead is large, the information for the influence of zinc on the tissue concentrations of lead is presented in a separate table. The rest of tables include the tissue concentration data 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, Zinc, and Copper**

Studies of neurological effects have been conducted in foundry workers exposed to lead, zinc, and copper.

A series of studies on Japanese workers exposed to lead, zinc, and copper at a gun metal foundry concluded the zinc and copper may antagonize some of the neurological effects of lead in occupationally exposed adults. Indirect evidence of potential antagonism by zinc and copper (Araki et al. 1993a; Murata et al. 1993) or by zinc (Araki et al. 1993b) of lead inhibition of peripheral nerve conduction velocity was found in some of these studies, but not in others (Araki et al. 1992; Murata and Araki 1991). Other information from this series of studies includes indirect evidence of potential antagonism by zinc and copper of lead inhibition of central nerve conduction (Araki et al. 1992), and potential antagonism by zinc of lead inhibition of autonomic nervous function (Murata and Araki 1991). Mean PbB values for the groups of foundry workers were  $\approx 40 \mu\text{g/dL}$ . The numbers of exposed workers and matched controls were very small (14-22/group); comparisons with controls were used to determine endpoints affected by lead. The indirect evidence of protection by the two essential metals against lead neurotoxicity was obtained by separate correlation analysis of the measures of nervous system function with indicators of lead, zinc, or copper absorption (blood, plasma, and erythrocyte concentrations) and also in one study (Murata et al. 1993) with indicators of lead toxicity (urinary delta-aminolevulinic acid [ALA] and coproporphyrin). No analyses of interactions were performed. The endpoints studied were relatively insensitive endpoints, or endpoints of uncertain sensitivity. In addition, the investigators noted

considerable variability among the results of these studies and also a previous series of studies they had performed in the 1980s. Thus, these findings should be viewed with caution.

Another study of foundry workers exposed to lead at concentrations above the threshold limit value (TLV), and zinc and copper at concentrations below the TLV, mentioned an inverse relationship between PbB and serum zinc concentrations, but did not include detailed information (Antonowicz et al. 1990). Blood lead levels were higher in exposed workers than in controls. The study reported effects of the mixed exposure on the workers' erythrocytes, including inhibition of delta-aminolevulinic acid dehydratase (ALAD) and increased free erythrocyte protoporphyrin (FEP) in comparison with values in unexposed controls; these effects are characteristic of lead. A stimulatory effect on glycolytic enzymes also was reported. Thus, characteristic hematological effects of lead were seen in workers coexposed to low levels of zinc and copper. Whether or not zinc and copper afforded some protection against these effects cannot be determined from this study.

A 14-day intraperitoneal injection study of lead, zinc, and copper in rats reported that lead and copper, administered individually, changed the cholesterol, phospholipid, and glycoprotein composition of erythrocyte membranes, whereas zinc alone did not (Jehan and Motlag 1995). Administration of all three metals together at the same doses as given individually resulted in lesser effects than for lead or copper alone or no effect on these endpoints. Thus, the mixture appeared to be less "toxic" than either lead or copper alone at the same doses as in the mixture.

Lead, zinc, and copper (as well as cadmium) are often found at elevated concentrations in the environment near zinc smelters. A survey of wildlife in the vicinity of a zinc smelter site reported higher concentrations of Pb in bone than seen in animals from a relatively uncontaminated area (Storm et al. 1994). Thus, for this particular site, zinc and copper exposure were not completely protective against an increased body burden of lead when exposure to elevated levels of all three metals (and cadmium) occurred. Whether these essential elements were partially protective against lead accumulation cannot be determined from this study. Levels of copper in tissues of animals tended to be lower than in animals from a relatively uncontaminated area. It is possible that decreased copper in the tissues may have been related to zinc inhibition of copper absorption, but no clear conclusions can be drawn from these data.

### 2.2.2 Lead and Manganese

Data in humans are limited to a few epidemiology studies which reported a positive correlation between blood lead and blood manganese in various populations including children in urban environments in the U.S. and England (Delves et al. 1973; Joselow et al. 1978) and children and workers in various environments in the Netherlands (Zielhuis et al. 1978). This finding may simply reflect that environmental and/or occupational exposure to one metal is likely to be accompanied by exposure to other metals as well. Another study, of adults in Canada residing near a former manganese alloy plant, where exposures to manganese were elevated, found no correlation between lead and manganese blood levels (Baldwin et al. 1999).

In an oral/intraperitoneal study in adult rats, manganese (490 mg Mn/kg/day from manganese chloride in the drinking water) and/or daily intraperitoneal injections of lead (5, 8, or 12 mg Pb/kg, as lead acetate) were administered for 14 days (Chandra et al. 1981). Neurobehavioral endpoints were assessed on days 7 and 14. Slight but not significant increases in spontaneous motor activity were seen with manganese or low-dose lead. Mid- and high-dose lead increased spontaneous activity, whereas co-treatment with the metals resulted in decreased (lead dose-related) spontaneous motor activity relative to controls. In a test of learning ability that measured conditioned avoidance responses, lead plus manganese impaired learning to a greater extent than did lead alone; manganese slightly decreased learning at both time periods. The impairment of learning ability appeared to be additive, in comparison with each metal tested separately at the same dose as in the mixture. In a learning test that measured escape failure, the response to the mixture was similar to that for lead alone (increased escape failure); manganese alone had no significant effect on this response. Manganese alone increased aggressive behavior on day 14, but not on day 7. Lead alone (8 and 12 mg/kg) increased aggressive behavior on both test days more than did manganese alone; the mixture (at 8 and 12 mg/kg lead) induced a further increase in aggressive behavior on day 7, but on day 14 aggressive behavior was comparable to that seen with manganese alone. The most significant neurochemical effect of the manganese-lead combination was a decrease in norepinephrine in the brain relative to increases produced by either metal alone. The concentration of manganese in the brain was not affected by coadministration of lead, relative to manganese alone. The concentration of lead in the brain was increased approximately 3-fold by the coadministration of manganese as compared with the same dose of lead alone.

In a study of combined pre- and postnatal treatment, daily intraperitoneal injections of lead (5 mg/kg at 10 am), manganese (6 mg/kg at 5 pm), or both were administered to rats during gestation and/or lactation (Chandra et al. 1983). The combination of metals induced a significant and greater decrease in body weight and brain weight in the offspring at age 21 days than did lead alone; manganese alone had no significant effect. The brain to body weight ratio in the mixture group, however, was higher than in the other groups. Thus, the effect of the mixture appeared to be on general growth rather than selectively on the brain. Whether these findings are evidence of potentiation cannot be determined because only a single dose of each metal was administered singly, and the same dose of each administered in combination. The greater effects in the combination group could be due to the greater total dose of metals rather than to an interaction. As in the previous study by these investigators, coadministration of lead had no effect on the concentration of manganese in the brain, but coadministration of manganese increased the concentration of lead in the brain approximately 3-fold.

In a study in rabbits given a single intravenous injection of 5  $\mu$ mole/kg of lead (1 mg/kg) plus 5  $\mu$ mole/kg of manganese (0.28 mg/kg), the recovery of blood ALAD activity (which was inhibited by lead) was slightly delayed over a 123-day period compared with a group treated with lead (1 mg/kg) alone (Chiba and Kikuchi 1984b). Manganese alone did not affect ALAD. Of 29 clinical chemistry indices of liver and kidney function, only three,  $\beta$ -lipoprotein, triglycerides, and aldolase activity, were significantly increased ( $\approx$ 10-fold above vehicle controls) by treatment with the metal combination relative to no significant changes seen with either metal alone, but data for the single chemicals were not presented, so the only conclusion that can be drawn is that subthreshold doses of each of these metals, given as a mixture, produced an effect. The toxicological significance of this finding is uncertain as these reported effects are not known to be effects of exposure to these metals by natural routes. The estimated half-life of lead in blood was prolonged by manganese, which could have accounted for the slower ALAD recovery in the group treated with the mixture. In a related study, manganese did not restore ALAD activity when added *in vitro* as manganese chloride to blood from lead-exposed subjects, or from rabbits and mice after parenteral administration of lead as the acetate (Chiba and Kikuchi 1984a).

In a sequential intraperitoneal study, pretreatment of mice with 5 mg Mn/kg (as manganese chloride) completely protected against the 70% mortality caused by a challenge intraperitoneal dose of 60 mg Pb/kg (as the nitrate) administered 1 day later (Yoshikawa and Ohta 1982). Pretreatment with lead (10 mg/kg) did not protect against the mortality from a subsequent (2 days later) dose of manganese (50 mg/kg), which was 70% without and 90% with lead pretreatment. The increase was not considered

toxicologically significant by the investigators. Because of the endpoint studied, as well as the study design, these studies are considered inappropriate as the basis for conclusions regarding joint toxic action relevant to environmental contamination.

In a study of effects of these metals on each other's tissue distribution, simultaneous administration of lead acetate in the drinking water (5 ppm lead,  $\approx 2$  mg Pb/kg/day) and manganese chloride intraperitoneally (1 or 4 mg Mn/kg/day) for 30 days to weanling rats resulted in increased accumulation of lead in the five of the seven regions of the brain analyzed, as compared with lead alone at the same dose (Shukla and Chandra 1987). These regions were the cerebellum, cerebral cortex, corpus striatum, hippocampus, and midbrain. The high dose of manganese increased lead concentrations in the kidneys relative to administration of lead alone at the same dose. However, lead concentrations in blood and liver were lower after coadministration of both manganese doses, and no significant effect of manganese on testicular lead levels was seen. Lead significantly increased the concentration of manganese in only two of the seven brain regions (corpus striatum and midbrain) and only at the low dose of manganese, as compared with manganese alone at the same dose. Lead decreased the concentration of manganese in the kidneys and increased the concentration of manganese in the liver, again only at the low dose of manganese. Lead had no impact on testicular manganese concentrations at either dose of manganese relative to manganese alone at the same dose.

The mechanism by which manganese increases brain lead is unknown, but in an additional studies *in vitro*, the same group of investigators (Kalia et al. 1984) demonstrated that coadministration of the metals alters the affinity of binding proteins in the brain, which could account for increased retention of lead. The affinity of binding proteins for manganese in the brain was not altered by lead. Increased affinity of binding proteins in the liver was seen for both metals *in vitro*, but increased accumulation of these metals in liver was not seen in the *in vivo* coadministration study (Shukla and Chandra 1987), perhaps due to excretion in the bile or release into the circulation.

Table 3 provides an overview of the interaction data regarding the effects of lead on the toxicity and tissue concentrations of manganese and Table 4 summarizes the effects of manganese 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 Manganese by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (7-14 days)	Neurological (conditioned avoidance)		5-12 i.p. + 490 <sup>a</sup> (r) <sup>b</sup>		additive	Chandra et al. 1981
Acute (7-14 days)	Neurological (brain norepinephrine, motor activity)		5-12 i.p. + 490 (r)		indeterminate: significant decreases for the mixture, slight increases for each metal alone at same dose as in mixture	Chandra et al. 1981
Acute (14 days)	Brain Mn levels		5-12 i.p. + 490 (r)		additive	Chandra et al. 1981
Intraperitoneal injection (mg/kg/day)						
Intermediate (gestation+lactation)	Brain Mn levels		5 + 6 (r)		additive	Chandra et al. 1983
Intermediate	Brain Mn levels	2 oral + 1 (r) (in two of seven regions of brain)	2 oral + 4 (r)		>additive at lower Mn	Shukla and Chandra 1987

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

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal injection (mg/kg/day)						
Intermediate	Liver Mn levels	2 oral + 1 (r)	2 oral + 4 (r)		>additive at lower Mn	Shukla and Chandra 1987
Intermediate	Kidney Mn levels		2 oral + 4 (r)	2 oral + 1 (r)	<additive at lower Mn	Shukla and Chandra 1987
Intermediate	Testis Mn levels		2 oral + 1-4 (r)		additive	Shukla and Chandra 1987

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat

**Table 4. Summary of Available Data on the Influence of Manganese 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	Blood and Hepatic Pb levels			1-4 i.p. + 2 <sup>a</sup> (r) <sup>b</sup>	<additive	Shukla and Chandra 1987
Intermediate	Renal Pb levels	1-4 i.p. + 2 (r)			>additive	Shukla and Chandra 1987
Intermediate	Brain Pb levels	1-4 i.p. + 2 (r) (in 5 of 7 brain regions)			>additive	Shukla and Chandra 1987
Intraperitoneal injection (mg/kg/day)						
Acute (7-14 days)	Neurological (conditioned avoidance)		490 oral + 5-12 (r) <sup>b</sup>		additive	Chandra et al. 1981
Acute (7-14 days)	Neurological (brain norepinephrine, motor activity)		490 oral + 5-12 (r)		indeterminate: significant decreases for the mixture, slight increases for each metal alone at same dose as in mixture	Chandra et al. 1981
Acute (14 days)	Brain Pb levels	490 oral + 5-12 (r)			>additive	Chandra et al. 1981
Intermediate (gestation+lactation)	Developmental (growth of offspring at age 21 days)		6 + 5 (r)		indeterminate: greater effects in mixture group than in Pb group could be due to greater total metal dose	Chandra et al. 1983

**Table 4. Summary of Available Data on the Influence of Manganese on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure (*continued*)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal injection (mg/kg/day)						
Intermediate (gestation+lactation)	Brain Pb levels	6 + 5 (r)			>additive	Chandra et al. 1983
Intravenous injection (mg/kg)						
Acute (once)	Blood ALAD	0.28 + 1 (b)			>additive	Chiba and Kikuchi 1984b
Acute (once)	Blood Pb half life	0.28 + 1 (b)			>additive	Chiba and Kikuchi 1984b

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat; b = rabbits

### 2.2.3 Lead and Zinc

The database for this pair is large, and includes studies of workers, who probably were exposed primarily by inhalation, and of environmentally exposed populations, who may have been exposed primarily by the oral route. In addition, studies of oral coexposure to these metals in animals are available. Many of the studies focused on indicators of absorption or hematopoietic effects.

#### Human Studies-Inhalation Exposure

A few studies of foundry workers exposed to lead, zinc, and copper, reviewed in Section 2.2.1, suggest that zinc may antagonize some (but not other) aspects of lead neurotoxicity in adults (Araki et al. 1992, 1993a, 1993b; Murata and Araki 1991; Murata et al. 1993). The results were inconsistent from study to study, were derived from correlation analysis of each metal separately with the indices of neurotoxicity with no analysis for interactions, and concerned relatively insensitive endpoints for lead (peripheral nerve conduction velocity) or endpoints of uncertain sensitivity.

In a single blind study, workers from a lead smelter were given an oral zinc gluconate supplement providing 60 mg Zn/day (0.9 mg Zn/kg/day) or a placebo, 5 days/week for 8 weeks, during which they continued to work at their regular jobs at the smelter (Lauwerys et al. 1983). Groups were matched for age, years, and intensity of exposure, job distribution, and national origin. Zinc supplementation did not affect PbB values; mean values in both groups were  $\approx 50 \mu\text{g/dL}$  throughout the study. In addition, zinc supplementation had no effect on urinary excretion of ALA and  $\beta_2$ -microglobulin (indicators of hematological and renal toxicity, respectively). Other studies of the effect of zinc supplements on PbB in adults exposed to lead orally (Sohler et al. 1977) or occupationally (Papaioannou et al. 1978) lacked concurrent controls. Although results of these studies were suggestive of a decrease in PbB with zinc (and vitamin C) supplementation, similar but smaller decreases in PbB and an increase in urine lead were seen in lead-exposed workers after 8 weeks of placebo treatment in a study of vitamin C supplementation (which had no effect on PbB or urine lead relative to controls) (Lauwerys et al. 1983). Therefore, it is possible that the small decreases in blood lead values seen in the zinc-vitamin C studies were due to normal fluctuations in PbB.

Oral administration of high zinc doses for 5 days significantly increased erythrocyte ALAD activity in a single worker  $\approx 44$  days after termination of chelation therapy of occupational lead poisoning

(PbB=160 µg/dL) (Thomasino et al. 1977). ALAD activity actually decreased during calcium disodium ethylenediamine (EDTA) therapy, probably related to the removal of zinc as well as lead from the body. The increase in ALAD with zinc supplementation was modest, variability in ALAD from day to day was high, and the study involved only a single worker.

### **Human Studies-Oral Exposure**

Studies of the general population have investigated the potential relationship between zinc intake and blood lead or correlations between blood levels of the two metals. In a study of men and women with adequate dietary zinc (and copper and iron) intakes and a lead intake from the diet of 0.0036 mg/kg/day, ingestion of a zinc sulfate supplement that provided 15 mg Zn/day ( $\approx 0.21$  mg Zn/kg/day) did not significantly affect PbB or urinary or fecal excretion of lead, or lead balance (Kies and Ip 1990). Total zinc intake from the diet plus supplement was 0.40 mg/kg/day. Mean PbB values were 4.5 µg/dL during two 7-day periods without supplemental zinc and 4.28 µg/dL during two 7-day periods with supplemental zinc. These results suggest that supplemental zinc at the level of the RDA does not affect the bioavailability of lead in humans already receiving adequate zinc.

A study in men with no occupational exposure to these metals found no correlations between blood lead (which ranged from 3.3-18.4 µg/dL) and blood zinc levels or between seminal plasma lead and zinc levels (Xu et al. 1994). Dietary intakes of zinc were probably adequate as these men came from a population that had a high consumption of seafood, which is a good source of zinc.

A study in children investigated potential correlations between blood lead and dietary intakes of zinc or calcium (Johnson and Tenuta 1979). Blood lead values ranged from 12-67 µg/dL. All the children were ingesting less than the RDA for zinc. Although mean zinc intakes did not differ among low, medium, and high PbB groups, the percentage of children who consumed less than 55% of the RDA for zinc was 41% in the high PbB group, compared with 19% in the moderate, and 27% in the low PbB groups. On the other hand, the study showed a clear inverse correlation between dietary calcium intake and PbB. The children in the moderate and high PbB groups were more likely to have pica for paint chips, which contained up to 37% lead. Thus, although this study is widely cited as evidence of a protective effect of zinc against lead, no clear conclusions can be drawn from these data. In addition, the study provides no information about the effects of zinc intakes above the RDA. Another study, also widely cited, but published only as an abstract (Markowitz and Rosen 1981), reported that serum zinc levels in children

with elevated blood leads (40-71  $\mu\text{g}/\text{dL}$ ) were  $\approx 20\%$  lower than in control children. The mean serum zinc values for both groups were considered to be within the normal range (Mahaffey 1985).

In children treated with EDTA chelation therapy for lead poisoning, a positive correlation was found between urinary ALA and chelatable lead (Chisolm 1981). EDTA mobilizes lead and also the essential elements zinc, iron, and manganese, from soft tissue and bone; the resulting chelates are excreted in the urine and can serve as a measure of the mobile stores of these metals. Although no association between urinary ALA and chelatable zinc was found, an increase in urinary ALA above the normal range was significantly associated with a decrease in the chelatable zinc/lead ratio to 18.45 or less (Chisolm 1981). This association occurred in the range of blood leads from 45 to 60  $\mu\text{g}/\text{dL}$ . The results suggest a potential protection against ALAD inhibition by higher zinc/lead ratios.

Blood lead levels are known to correlate inversely with ALAD activity (Appendix A). In 143 patients of unspecified age and exposure, PbBs ranged from 4 to 115  $\mu\text{g}/\text{dL}$ , and correlated significantly and inversely with ALAD activity (Mauras and Allain 1979). The percent activation of ALAD that could be obtained by the *in vitro* addition of zinc to their blood was directly correlated with blood lead. These results suggest a reactivation of lead-inhibited ALAD by zinc. A number of studies have demonstrated zinc protection against lead inhibition or reactivation of lead-inhibited ALAD activity when both lead and zinc were added to human blood *in vitro* (e.g., Abdulla et al. 1979; Border et al. 1976; Davis and Avran 1978; Mauras and Allain 1979; Thomasino et al. 1977; Tomukuni 1979).

A study of trace element concentrations in drinking water and cognitive function among 1,016 elderly residents in rural China found no significant interaction between lead and zinc on cognitive function (assessed with the Community Screening Interview for Dementia) (Emsley et al. 2000). Many of the subjects had lived in the same village for their entire lives, and their food and beverages were almost entirely grown and prepared locally. After adjustment for the other elements and possible interactions, the only single element which was associated with cognitive function was calcium. Thus, neither lead nor zinc nor the combination was associated either positively or negatively with cognitive function in the elderly.

Concentrations of lead and zinc in breast milk from 30 mothers were not correlated (Kies and Umoren 1989). These mothers were exposed through their normal diets, so this is not a study of interactions between excess zinc and lead. Additional detail regarding adequacy of zinc in the diet or levels of

dietary lead were not provided. An inverse correlation between lead and copper was reported in this study and is discussed in Section 2.2.4.

### **Animal Studies-Oral exposure**

In a study of the potential effects of zinc on the absorption and toxicity of lead, groups of rats were fed inadequate (8 ppm,  $\approx 0.6$  mg/kg/day), adequate (35 ppm,  $\approx 2.7$  mg/kg/day), and excess (200 ppm,  $\approx 15.6$  mg/kg/day) zinc as the carbonate in the diet for 3-7 weeks, and/or low (50 ppm,  $\approx 3.9$  mg/kg/day) and high (200 ppm,  $\approx 15.6$  mg/kg/day) lead as the acetate (Cerklewski and Forbes 1976). The inadequate zinc dose was insufficient to maintain normal ALAD values in the rats, although body weight gain was not affected. Excess zinc alone had no effect on urinary ALA excretion, but caused an  $\approx 45\%$  reduction in the excretion of ALA due to high lead, as compared with adequate zinc plus high lead. Excess zinc also protected against the increase in free erythrocyte porphyrin (actually zinc protoporphyrin [Cerklewski and Forbes 1977]) caused by high lead, and protected against inhibition of renal ALAD caused by both low and high lead. Lead did not affect hemoglobin levels. Based on analysis of excreta during week 3, an inverse relationship between dietary zinc and absorption of high lead was seen across all three dietary doses of zinc, including excess zinc versus adequate zinc. Excess zinc decreased blood, kidney, and liver lead concentrations due to high lead and tibia lead concentrations due to low and high lead, by about 50%, but did not affect urinary lead excretion, as compared with adequate zinc. Note that significant differences due to excess versus adequate zinc for the low lead group (3.9 mg/kg/day) were limited to protection against renal ALAD inhibition. No significant differences on blood or tissue lead were seen with excess versus adequate zinc in the groups receiving low lead ( $\approx 3.9$  mg/kg/day). Lead did not affect zinc absorption and retention or testes zinc concentrations.

Similar results regarding tissue lead were reported for low lead exposures in another study in rats (Bebe and Panemangalore 1996). Rats were given zinc in the diet at negligible, 12 ppm (adequate), and 60 ppm (negligible,  $\approx 2$ , and 10 mg Zn/kg/day) as the sulfate and/or lead at 20 mg/L in the drinking water ( $\approx 2.4$  mg Pb/kg/day) as the acetate for 4 weeks. The negligible zinc groups had a 35% decrease in food intake and an 80% decrease in body weight gain. No significant differences across zinc dietary levels were seen in the concentrations of lead in the erythrocytes, liver, and kidney. Erythrocyte lead is roughly equivalent to blood lead because under steady state conditions 99% of the lead in blood is found in the erythrocyte (Appendix A). Lead did not affect the concentrations of zinc in plasma, liver, or kidney at any dietary level of zinc. Zinc produces its hematological effects through interference with copper

homeostasis, producing a *de facto* copper deficiency, when exposure occurs long enough to deplete copper stores. The most sensitive indicator is reduced activity of erythrocyte Cu/Zn superoxide dismutase. Effects on this endpoint were not studied in any of the joint action studies on lead and zinc, but Bebe and Panemangalore (1996) did investigate the effect of lead and zinc on copper concentrations in plasma, liver, and kidney. High zinc, as compared with adequate zinc, significantly decrease the concentrations of copper in plasma and liver (but not kidney); lead plus high zinc was not different from high zinc alone. Lead (as compared with no lead) did not affect tissue copper in the adequate and high zinc groups.

Hematological parameters were investigated in another study comparing the impact of excess versus marginal zinc intakes on lead toxicity (El-Gazzar et al. 1978). While maintained on a very low zinc diet (content not reported), weanling rats were exposed to zinc in their drinking water at 5 ppm ( $\approx 0.7$  mg/kg/day, “marginal zinc”) or 50 ppm ( $\approx 7.0$  mg/kg/day, “excess zinc”), with or without 100 ppm lead ( $\approx 14$  mg/kg/day). Both metals were administered as the acetates. The minimum adequate zinc intake is  $\approx 2$  mg/kg/day for weanling rats and  $\approx 1$  mg/kg/day for adult rats (Appendix C), indicating that the intake of 0.7 mg Zn/kg/day in this study was less than the minimal requirement. Growth in the group of rats that consumed marginal zinc was initially slower than in the group that consumed excess zinc, but according to the authors, subsequently caught up. Thus, data from the study confirm that the intake of zinc in the marginal zinc group was not adequate for growing rats. Excess zinc decreased urinary ALA excretion in lead-treated groups as compared with marginal zinc, but did not significantly ameliorate the depression of erythrocyte ALAD in lead-treated rats. Tissue levels of lead were decreased in tibia, spleen, and liver, but were not affected in brain and kidney, of excess zinc plus lead rats as compared with marginal zinc plus lead rats. Correlation analysis showed significant inverse correlations between plasma zinc and erythrocyte lead and between erythrocyte lead and ALAD, and significant direct correlations between plasma zinc and ALAD, between erythrocyte lead and zinc protoporphyrin, and between erythrocyte lead and urinary ALA excretion. Lead decreased plasma zinc in both marginal and excess zinc groups and decreased liver and bone zinc only in the marginal zinc group. The investigators stated that lead did not affect brain or kidney zinc, but did not show the data. Results from this study regarding the joint action of excess zinc and lead are included in the summary tables at the end of this section.

A 6-week gavage study compared the effect of lead alone (10 mg/kg on 6 days/week, 8.6 mg/kg/day) with lead plus supplemental zinc (25 mg/kg on 6 days/week, 21 mg/kg/day) on hematopoietic endpoints and

tissue levels of lead in rats receiving adequate dietary zinc (Flora et al. 1989b). Supplemental zinc protected against the inhibition of erythrocyte ALAD and accumulation of zinc protoporphyrin, but did not significantly affect the increased urinary excretion of ALA due to lead. Concentrations of lead were decreased by supplemental zinc in blood, liver, and kidney, but were not affected in brain. The study did not include a group treated with supplemental zinc alone. Similar results were reported on ALAD and liver and kidney lead levels in rats given lead at a dose of 10 mg/kg/day and supplemental zinc at 2 mg/kg/day (on top of adequate dietary zinc) for 21 days in an earlier study by the same group of investigators (Flora et al. 1982). In the 1982 study, lead slightly but significantly decreased blood hemoglobin; supplemental zinc did not restore hemoglobin to normal in lead-treated rats. Hematocrit was not affected by lead or lead plus zinc. These endpoints were not investigated in the 1989 study.

In rats administered lead in drinking water as 2.63 Mmole/L of lead acetate (545 mg Pb/L,  $\approx$ 87 mg/kg/day) with or without gavage administration of supplemental zinc at 1 mg/kg/day, the decrease in erythrocyte ALAD activity was partially prevented by zinc, as was the increase in urinary ALA (Flora et al. 1991). Blood hemoglobin was significantly reduced in the lead group versus controls. In the lead plus zinc group, hemoglobin was intermediate in value between lead alone and controls and did not differ significantly from either. As in the 1989 study by this same group of investigators, supplemental zinc decreased lead in blood, liver, and kidney, but did not affect brain lead.

Additional intermediate-duration oral (drinking water or diet) studies comparing the effect of excess zinc with inadequate zinc intakes on lead toxicity in animals have reported protective effects of the excess zinc. Intakes of zinc were known to be deficient because of the sustained low growth rates of the young rats on the low zinc diets, and in comparison with known zinc requirements. For example, quicker recovery of erythrocyte ALAD activity in lead-treated rats was seen with high zinc as compared with deficient zinc intakes. This study used a very low zinc artificial diet, with high and deficient zinc in drinking water for 7 weeks before, 2 weeks during, and 5 weeks after exposure to drinking water with high lead. Lead caused virtually complete inhibition of ALAD at 2 weeks in both deficient and high zinc groups (Finelli et al. 1975).

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 zinc (and cadmium) (Thawley et al. 1977). In this study, young male rats were fed lead carbonate (5,000 ppm Pb,  $\approx$ 430 mg Pb/kg/day) and zinc carbonate (6,300 ppm Zn,  $\approx$ 542 mg Zn/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 zinc 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 of this study were an exposure duration 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 calcium and vitamin D indicates slight decreases in blood hemoglobin from exposure to each metal alone and a more obviously decreased blood hemoglobin from exposure to the mixture, consistent with additivity, but none of the values appeared statistically significantly different. 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 alone group and the lead plus zinc group to support meaningful conclusions; the standard deviations were nearly as large as the means.

Weanling pigs fed 1,000 ppm lead ( $\approx 44$  mg/kg/day) as lead acetate with or without 4,000 ppm zinc ( $\approx 176$  mg/kg/day) as zinc oxide in diets containing low calcium for up to 13 weeks developed behavioral changes including extreme nervousness, aggressiveness after 1 week on the diets, followed by incoordination, lameness, and reduction of weight gain and food intake at 2 weeks (Hsu et al. 1975). Effects were more severe in the lead plus zinc group than in the lead alone; the zinc alone group had lower weight gain than the low calcium controls but no behavioral signs. The calcium level in the diet was inadequate as judged by a significantly lower weight gain of the control pigs on the low calcium diet as compared with high calcium controls, and by comparison with NRC (1998) estimates of minimum requirements for pigs of this weight (7.5 kg). In weanling pigs given a high calcium diet and the same levels of lead and/or zinc, no behavioral signs were seen in any group and only a slight effect on body weight gain was seen in the lead plus zinc group (Hsu et al. 1975). In the low calcium lead-treated groups blood lead levels were unaffected, and kidney levels were increased by coadministration of zinc. In the high calcium lead-treated groups, blood lead was decreased by zinc, but tissue lead (kidney, liver, and bone) was increased by zinc. In the low calcium zinc-treated groups, blood and kidney zinc levels were decreased by coadministration of lead in the low calcium group, but were unaffected in the high calcium group. In the high-calcium zinc-treated groups, higher levels of zinc were noted in liver, but not in other tissues. Lead and calcium interactions are well established (see Appendix A).

The study by Hsu et al. (1975) is cited in other reviews and documents as showing an enhancement of lead toxicity by zinc, but this enhancement was evident only when dietary calcium was inadequate. Other studies conducted under calcium-deficient conditions have reported no change in blood lead but some protection of excess zinc against some indicators of lead hematopoietic effects (urinary ALA) and possibly blood and red blood cell hemoglobin, which were decreased by each metal given separately, with no additional decrease from the two together at the same doses as when given separately in rats (Thawley et al. 1977, 1978). The applicability of these results to populations not grossly deficient in calcium intakes is questionable.

Few studies were located regarding the joint action of zinc and lead on neurological endpoints in animals, and the quality of the available studies is not as good as the studies on hematological endpoints. One study reported that zinc *deficiency* or oral lead exposure in rats impaired their choice behavior in a maze, but the zinc deficiency plus lead did not interact (Bushnell and Levin 1983); the effect of excess zinc was not studied.

Oral coexposure to zinc prevented a lead-induced decrease in the *in vitro* contractility of smooth muscle in response to a cholinergic agonist (Vassilev et al. 1994). In this study, rats received 100 mg/kg/day of lead and/or 100 mg/kg/day of zinc (as lead acetate and zinc sulfate in drinking water) for 30 days; the *in vitro* assay was conducted 24 hours after termination of exposure.

A study in rabbits investigated the effects of oral exposure to lead and zinc on peripheral nerve conduction and on tissue distribution of these metals (Hietanen et al. 1982). The rabbits were given lead (2,000 ppm) and/or supplemental zinc (5,000 ppm) as the acetates in their drinking water for 2 or 4 weeks (3 rabbits/treatment/duration). The daily intake of the metals from the drinking water was 0 (controls), 170 mg Pb/kg/day, 194 mg Zn/kg/day, and 196 mg Pb/kg/day plus 213 mg Zn/kg/day, based on body weights and metal intake data reported in the study, and correcting an obvious typographic error in daily intake for the mixture group. Decreases in nerve conduction velocity of  $\approx 75\%$  (to  $\approx 10$  meters/second) were seen in the sciatic nerve of the lead group and the mixture group; zinc did not appear to protect against this effect of lead. This decrease is far greater than usually encountered in the literature. Some differences in tissue lead and zinc and in blood lead were discussed by the investigators, but numbers of animals per group and duration were small, statistical analyses were limited to comparisons of each treatment group with the control group (when three animals were tested), and variability in these values within a group was high. An additional concern regarding this study is that some of the assays for

the lead plus zinc group do not include all three rabbits in the group, but no explanation was given for this discrepancy.

An additional study, included because it is one of the very few studies to give any information of the potential effect of lead on the toxicity of zinc, reported a protective effect of zinc against lead neurotoxicity, but no effect of lead against zinc toxicity, in horses (Willoughby et al. 1972). Young horses (3-4 weeks old) were fed increasing doses of zinc ( $\approx 25$ -187 mg/kg/day) and/or lead ( $\approx 2.5$ -105 mg/kg/day) for up to 38 weeks until moribund or dead. The neurological effects seen in the lead group (pharyngeal and laryngeal paralysis which occurred after PbBs reached 60  $\mu\text{g/dL}$ ) were not seen in the lead plus zinc group. The anemia; joint swelling, pain, and lameness; and depressed growth seen in the zinc group also occurred in the lead plus zinc group. Thus, zinc protected against lead toxicity, but lead did not protect against zinc toxicity when both were given orally at highly toxic doses. Limitations of this study include the small number of animals tested, relevance of results obtained with doses that ultimately resulted in moribundity or death, uncertainty regarding the relevance of findings in horses to human health (because of the differences in digestive tracts, and in characteristic effects: for example, very high exposure to lead did not cause anemia in the horses, and the effects of zinc on the joints of the horses were not encountered in the literature on humans and laboratory animals).

A study of the effects of supplemental zinc on the testicular toxicity of lead in the rat reported that zinc was protective (Batra et al. 1998). Histopathological examination of the testes of young adult male rats that had been gavaged with lead at 50 mg/kg/day for 3 months revealed damage to the seminiferous tubules, which was much less pronounced in animals that had also been supplemented with zinc at 1 mg/kg/day by gavage. Testicular ALAD was significantly decreased by lead alone and comparable to controls in the lead plus zinc group. Concentrations of lead in the blood, bone, liver, kidney, spleen, and testis were significantly reduced (by 30-50%) in the zinc plus lead group versus the lead alone group.

A study of the effects of zinc on the developmental toxicity of lead compared 12 ppm basal zinc (the dietary level thought to be the minimum needed to support normal reproduction,  $\approx 1.6$  mg/kg/day) with 120 ppm excess zinc ( $\approx 15.6$  mg/kg/day) with or without 500 ppm lead ( $\approx 65$  mg/kg/day) throughout gestation and lactation day 16 (Cerklewski 1979). In the zinc alone groups, body weight gain of the dams and pup weight at day 16 of lactation were the same in the basal and excess zinc groups. Although lead did not affect food intake, lead decreased the growth of both the dams and the pups in the basal zinc group. Excess zinc protected against this effect. Excess zinc appeared to decrease the lead levels in milk

and in pup tibias, and the accumulation of blood porphyrin in pups, but the values were not significantly different from the basal zinc plus lead group. Excess zinc was protective against inhibition of hepatic ALAD in the pups, and decreased the concentration of lead in liver and blood of the dams.

The following sequential oral study was performed in an effort to determine whether the interactive effects of lead and zinc occur during or after absorption through the gastrointestinal tract. It provides some useful information, but is thought to be less relevant, and is not included in the summary tables. This study, in which 200 ppm lead was fed in a 12 ppm basal zinc diet for 3 weeks, followed by no lead plus basal or 200 ppm zinc for 2 weeks, reported that high zinc had little effect on the recovery of indices of lead toxicity (liver ALAD, urinary ALA, soft tissue lead levels), which occurred at similar rates in the basal zinc versus excess zinc groups (Cerklewski 1979). Loss of lead from the erythrocytes, however, was enhanced by the high zinc diet. These findings were interpreted to mean that the interaction between zinc and lead primarily involves absorption.

### **Animal Studies-Injection**

The following sequential injection study is even less relevant to determining the mode of joint action, but includes all the pairs of metals studied in this interaction profile. In this sequential intraperitoneal study, pretreatment of mice with zinc (4 mg Zn/kg as the sulfate) completely protected against the 70% mortality resulting from a challenge dose of lead (60 mg Pb/kg as the nitrate) administered 1 day later (Yoshikawa and Ohta 1982). Pretreatment with lead (10 mg/kg) decreased the lethality of a subsequent (2 days later) dose of zinc (12 mg/kg) from 90 to 30%. Because of the endpoint studied, as well as the study design, this study is considered inappropriate as the basis for conclusions regarding joint toxic action relevant to environmental contamination.

### **Potential Mechanisms of Interaction**

Lead alters heme synthesis by stimulating mitochondrial delta-aminolevulinic acid synthetase (ALAS), directly inhibiting cytosolic 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 1999). ALAD is present in most cells, including neurons. Zinc is contained at the catalytic center of the enzyme and can be readily removed or replaced by lead (Simons 1997). The inhibition occurs through the binding of lead to vicinal sulfhydryls at the

active site of ALAD. Inhibition of ALAD results in accumulation of ALA in blood and soft tissues, including brain. ALA is structurally similar to gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. ALA appears to act as a GABA agonist at the presynaptic GABA receptors, causing negative-feedback inhibition of GABA release. In addition, ALA undergoes autooxidation, generating free radicals that may contribute to toxicity, and ALA promotes oxyhemoglobin oxidation. At relatively high levels of lead exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Decreases in tissue heme pools can have deleterious effects throughout the body, not only because heme is a constituent of hemoglobin, but also because heme is a prosthetic group of cytochrome P450 and the cytochromes of cellular energetics (ATSDR 1999; EPA 1986).

Zinc also influences heme synthesis, by interfering with copper homeostasis. The principal mechanism appears to involve zinc induction of metallothionein in the mucosa of the gastrointestinal tract. Copper has a higher affinity than zinc for binding to metallothionein, and displaces zinc. This binding of copper reduces its absorption into the blood and increases its fecal excretion as the cells of the intestinal mucosa containing copper-metallothionein are exfoliated (ATSDR 1994). Copper is an essential part of several enzymes including ceruloplasmin, which oxidizes ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is critically important to provide iron for hemoglobin synthesis (Friberg et al. 1986).

Potential mechanisms of interaction between lead and zinc, other than on ALAD, include an inhibition of lead gastrointestinal absorption by zinc, which has been demonstrated directly in rats (Cerklewski and Forbes 1976). Indirect evidence includes the observation that supplemental zinc decreased the concentrations of lead in blood and in a variety of tissues, although not in brain, in lead-exposed rats, as reviewed in the previous section. The protective effect of supplemental zinc was not seen at low lead or low zinc plus low lead exposures, but occurred primarily at higher lead exposures.

Zinc released from vesicles in presynaptic terminals of certain glutaminergic neurons is thought to modulate postsynaptic N-methyl-D-aspartate (NMDA) receptors for glutamate (Sandstead et al. 2000). Lead and zinc inhibit receptor binding to the NMDA receptor channel, but the inhibition is non-competitive, indicating that lead does not bind to the zinc allosteric site (Lasley and Gilbert 1999). Thus, these cations have a combined inhibitory effect on this endpoint, but this is a relatively insensitive neuronal endpoint for lead (Lasley and Gilbert 1999). Lead has been shown to interfere with the

deoxyribonucleic acid (DNA) binding properties of zinc-finger regions of transcription factors (Zawia et al. 2000), but implications for interactions regarding lead target organs have not yet been examined.

Another possible mechanism for protective effects of zinc on lead-induced toxicity is zinc induction of a metallothionein that sequesters lead. Goering and Fowler (1987b) showed that liver and kidney cytosol from rats pretreated with zinc contained a Zn-metallothionein, which chelated lead, thus decreasing the pool of free lead ions. Similarly, Liu et al. (1991) reported that pretreatment of cultured hepatocytes with zinc induces metallothionein and significantly protects against the cytotoxicity of lead. Additional studies with other metals (silver and mercury) indicated that the reduction in cytotoxicity was attributable to binding of the metals to metallothionein. Church et al. (1993a, 1993b) have found a metallothionein-like protein in erythrocytes, which bound Pb during *in vitro* incubations. This protein was present in higher concentrations in workers exposed to inorganic lead than in unexposed subjects. Studies of one worker who had an exceptionally high blood lead (180  $\mu\text{g}/\text{dL}$ ) but no symptoms of lead poisoning revealed that lead was bound to the metallothionein-like protein isolated from his erythrocytes. Another heavily exposed worker (blood lead 160  $\mu\text{g}/\text{dL}$ ) who had severe symptoms of lead poisoning had relatively low concentrations of the erythrocyte metallothionein-like protein and less lead bound to this protein. These observations suggest that this protein protects against some aspects of lead toxicity. Metallothioneins have been identified in the brain as well as other tissues (Sandstead et al. 2000). In addition, an acidic, soluble lead-binding protein in brain, kidney, and erythrocytes that normally binds zinc is postulated to attenuate lead toxicity to ALAD through lead binding and zinc donation (Fowler 1998).

## Summary

Table 5 provides an overview of the interaction data regarding the effects of lead on the toxicity and tissue concentrations of zinc. Because of the more voluminous database on the effects of zinc on the toxicity and tissue concentrations of lead, this information is presented in two tables. Table 6 summarizes the effects of zinc on the toxicity of lead and Table 7 summarizes the effects of zinc on 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 5. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Zinc by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Intermediate	Hematological (anemia)		103 + 187 <sup>a</sup> (s) <sup>b</sup>		additive	Willoughby et al. 1972
Intermediate	Zn absorption		15.6 + 15.6 (r)		additive	Cerklewski and Forbes 1976
Intermediate	Plasma Zn levels		2.4 + 2 (r) 2.4 + 10 (r)	14 + 7 (r)	<additive at higher lead and lead/zinc	Bebe and Panemangalore 1996 El-Gazzar et al. 1978
Intermediate	Erythrocyte Zn levels		14 + 7 (r)		additive	El-Gazzar et al. 1978
Intermediate	Bone Zn levels		14 + 7 (r)		additive	El-Gazzar et al. 1978
Intermediate	Hepatic Zn levels		2.4 + 2 (r) 2.4 + 10 (r) 14 + 7 (r)		additive	Bebe and Panemangalore 1996 El-Gazzar et al. 1978
Intermediate	Renal Zn levels		2.4 + 2 (r) 2.4 + 10 (r) 14 + 7 (r)		additive	Bebe and Panemangalore 1996 El-Gazzar et al. 1978
Intermediate	Brain Zn levels		14 + 7 (r)		additive	El-Gazzar et al. 1978
Intermediate	Testes Zn levels		15.6 + 15.6 (r)		additive	Cerklewski and Forbes 1976

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat, s = horse

**Table 6. Summary of Available Data on the Influence of Zinc on Toxicity/Carcinogenicity of Lead by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation (occupational) exposure (Zn mg/kg/day + PbB µg/DL)						
Intermediate	Hematopoietic (urinary ALA)		0.9 oral + 50 <sup>a</sup> (ha) <sup>b</sup>		additive	Lauwerys et al. 1983
Intermediate	Renal (urinary β <sub>2</sub> -microglobulin)		0.9 oral + 50 (ha)		additive	Lauwerys et al. 1983
Oral exposure (mg/kg/day)						
Intermediate	Hematopoietic (blood or erythrocyte ALAD)		7 + 14 (r)	2 + 10 (r) 21 + 8.6 (r) 1 + 87 (r)	<additive in studies with adequate basal Zn	Flora et al. 1982 El-Gazzar et al. 1978 Flora et al. 1989b Flora et al. 1991
Intermediate	Hematopoietic (zinc proto-porphyrin)		15.6 + 3.9 (r)	1 + 87 (r) 15.6 + 15.6 (r) 21 + 8.6 (r)	<additive at higher lead dose	Flora et al. 1991 Cerklewski and Forbes 1976 Flora et al. 1989b
Intermediate	Hematopoietic (urinary ALA)		15.6 + 3.9 (r) 21 + 8.6 (r)	exposure biomarkers: chelatable Zn/Pb (hc) 7 + 14 (r) 15.6 + 15.6 (r) 1 + 87 (r)	<additive at higher lead doses	Chisholm 1981 El-Gazzar et al. 1978 Cerklewski and Forbes 1976 Flora et al. 1989b Flora et al. 1991

**Table 6. Summary of Available Data on the Influence of Zinc 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	Renal (ALAD)			15.6 + 3.9 (r) 15.6 + 15.6 (r)	<additive	Cerklewski and Forbes 1976
Intermediate	Neurological (cholinergic stimulation of smooth muscle)			100 + 100 (r)	<additive	Vassilev et al. 1994
Intermediate	Neurological (pharyngeal and laryngeal paralysis)			187 + 105 (s)	<additive	Willoughby et al. 1972
Intermediate	Neurological (nerve conduction velocity)		213 + 196 (b)		additive	Hietanen et al. 1982
Intermediate	Testicular (histopathology and ALAD)			1 + 50 (r)	<additive	Batra et al. 1998
Intermediate	Developmental (pup growth, hepatic ALAD)			15.6 + 65 (r)	<additive	Cerklewski 1979

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat, b = rabbit, ha = human (adult), hc = human (child), s = horse

**Table 7. Summary of Available Data on the Influence of Zinc on Tissue Concentrations of Lead by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation (occupational) exposure (Zn mg/kg/day + PbB µg/dL)						
Intermediate	Blood lead		0.9 oral +50 <sup>a</sup> (ha) <sup>b</sup>		additive	Lauwerys et al. 1983
Oral exposure (mg/kg/day)						
Acute (7 days)	Blood Pb levels, Pb balance		0.21 + 0.0036 (ha) (PbB = 4.5 µg/dL)		additive	Kies and Ip 1990
Intermediate	Pb absorption		15.6 + 3.9 (r)	15.6 + 15.6 (r)	<additive at high lead dose	Cerklewshi and Forbes 1976
Intermediate	Blood Pb levels		10 + 2.4 (r)  15.6 + 3.9 (r)	1 + 50 (r) 1 + 87 (r)  15.6 + 15.6 (r)  15.6 + 65 (r) 21 + 8.6 (r)	<additive at higher lead doses	Batra et al. 1998 Flora et al. 1991 Bebe and Panemangalore 1996 Cerklewshi and Forbes 1976 Cerklewski 1979 Flora et al. 1989b
Intermediate	Bone Pb levels			1 + 50 (r) 7 + 14 (r) 15.6 + 3.9 (r) 15.6 + 15.6 (r)	<additive	Batra et al. 1998 El-Gazzar et al. 1978 Cerklewski and Forbes 1976

**Table 7. Summary of Available Data on the Influence of Zinc 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	Hepatic Pb levels		10 + 2.4 (r) 15.6 + 3.9 (r)	1 + 50 (r) 1 + 87 (r) 2 + 10 (r) 7 + 14 (r)  15.6 + 15.6 (r)  15.6 + 65 (r) 21 + 8.6 (r)	<additive at higher lead doses	Batra et al. 1998 Flora et al. 1991 Flora et al. 1982 El-Gazzar et al. 1978 Bebe and Panemangalore 1996 Cerklewski and Forbes 1976 Cerklewski 1979 Flora et al. 1989b
Intermediate	Renal Pb levels		7 + 14 (r) 10 + 2.4 (r) 15.6 + 3.9 (r)	1 + 50 (r) 1 + 87 (r) 2 + 10 (r)  15.6 + 15.6 (r)  21 + 8.6 (r)	<additive in studies with higher lead doses and/or adequate basal dietary zinc	Batra et al. 1998 Flora et al. 1991 Flora et al. 1982 El-Gazzar et al. 1978 Bebe and Panemangalore 1996 Cerklewski and Forbes 1976 Flora et al. 1989b
Intermediate	Spleen Pb levels			1 + 50 (r) 7 + 14 (r)	<additive	Batra et al. 1998 El-Gazzar et al. 1978
Intermediate	Testis Pb levels			1 + 50 (r)	<additive	Batra et al. 1998
Intermediate	Brain lead levels		1 + 87 (r) 7 + 14 (r) 21 + 8.6 (r)		additive	Flora et al. 1991 El-Gazzar et al. 1978 Flora et al. 1989b

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's tissue concentrations.

<sup>b</sup> Species code: r = rat, ha = human (adult)

## 2.2.4 Lead and Copper

### Human Studies

A few studies of foundry workers exposed to lead, zinc, and copper, reviewed in Section 2.2.1, provide indirect evidence that copper may antagonize some (but not other) aspects of lead neurotoxicity in adults (Araki et al. 1992, 1993a; Murata and Araki 1991; Murata et al. 1993). The results were derived from correlation analysis of indices of absorption of each metal with neurotoxicity and no analysis for interactions, were inconsistent from study to study (e.g., positive correlation between copper and nerve conduction velocity in one study, negative in another), and concerned relatively insensitive endpoints for lead (peripheral nerve conduction velocity) or endpoints of uncertain sensitivity.

A population-based case-control study in men and women  $\geq 50$  years of age investigated the potential role of occupational exposure to metals (including lead and copper) as risk factors for Parkinson's disease (Gorell et al. 1997, 1999). In cases with  $>20$  years of exposure, the adjusted odds ratio for copper was 2.4 (95% confidence interval [CI]=1.06, 5.89), for lead was 2.05 (borderline significance, 95% CI=0.97, 4.31), and for combined exposure to lead and copper was 5.24 (95% CI=1.40, 17.21). The number of cases for 20 years of exposure to copper was 10, to lead was 13, and to lead and copper appears to have been 7 (Table 5 of Gorell et al. 1999). As a point of interest, greater than 20 years of exposure to manganese also was a risk factor for Parkinson's, but there were only three cases, and therefore no analyses of combined exposure involving manganese were conducted. These results indicate that lead and excess copper may act together to increase the risk of Parkinson's disease, but do not delineate the type of joint action. Note that neither metal has previously been established as an etiological agent for this disease.

In a study of men and women with adequate dietary copper (and zinc and iron) intakes and a lead intake from the diet of 0.0036 mg/kg/day, ingestion of a copper supplement that provided 5 mg Cu/day ( $\approx 0.071$  mg Cu/kg/day as amino acid chelate) significantly decreased PbB, and increased fecal but not urinary excretion of lead (Kies and Ip 1990). Mean lead balance was -0.022 with supplemental copper versus +0.024 without supplements, primarily due to the higher fecal lead excretion during copper supplementation. Dietary intakes of copper averaged 1.12 mg/day (0.016 mg/kg/day). The dietary intake appears adequate. By way of comparison, the ESADDI range was determined to be 1.5-3.0 mg/day (NRC 1989) and, more recently, an RDA has been estimated at 0.9 mg/day (Institute of Medicine 2001:

prepublication document, final version not published as of December 2001). Total copper intake from the diet plus the supplement was 0.087 mg/kg/day. Mean PbB values were 4.50  $\mu\text{g}/\text{dL}$  during two 7-day periods without supplemental copper and 4.11  $\mu\text{g}/\text{dL}$  during two 7-day periods with supplemental zinc. These results suggest that supplemental copper may decrease the absorption and/or increase the biliary/fecal excretion of lead in humans already receiving adequate copper, creating a negative lead balance, thus having a protective effect.

Concentrations of lead and copper in breast milk from 30 mothers were significantly and inversely correlated (Kies and Umoren 1989). These mothers were exposed through their normal diets, so this study does not provide information regarding interactions between excess copper and lead. Additional detail regarding adequacy of copper in the diet or levels of dietary lead were not provided.

### **Animal Studies**

In rats treated with 10 mg/kg/day of lead and receiving adequate dietary copper, ALAD activity was significantly higher and hepatic and renal lead levels were significantly lower in the group that also received supplemental copper at 2 mg/kg/day (Flora et al. 1982). Hepatic and renal levels of copper were not affected by lead. The metals were administered by gavage as lead acetate and copper sulfate for 21 days. Lead slightly, but significantly, decreased blood hemoglobin; supplemental copper did not restore hemoglobin to normal in lead-treated rats. Hematocrit was not affected by lead or lead plus copper.

In a 4-week feeding study in rats, neither excess copper (20 ppm,  $\approx 1.8$  mg/kg/day) as the chloride, nor lead (200 ppm,  $\approx 18.2$  mg/kg/day) as the acetate, nor the mixture at the same dose as tested separately, affected serum ceruloplasmin as compared with adequate copper (5 ppm,  $\approx 0.5$  mg/kg/day) with or without lead (Cerklewski and Forbes 1977). Blood hemoglobin levels also were the same in all these groups. Hepatic copper increased in rats given excess copper as compared with adequate copper; lead had no effect on the hepatic levels of copper in rats given excess copper. Concentrations of lead in blood, bone, liver, and kidney in lead-treated rats were not affected by excess copper as compared with adequate copper. Comparisons between adequate and deficient copper groups in this study, with and without lead exposure, are discussed subsequently in this section.

A 30-day study of exposure of rats to 100 ppm lead ( $\approx 14$  mg/kg/day) as the acetate in drinking water and/or 100 ppm copper ( $\approx 8.6$  mg/kg/day) as the sulfate in the diet investigated hematological endpoints, neurotransmitter levels, and tissue concentration of the metals (Flora et al. 1989a). The basal diet contained adequate copper. Erythrocyte ALAD was inhibited and urinary ALA and zinc protoporphyrin were increased by lead alone, but not by copper alone, as compared with controls on the normal diet. Coexposure to excess copper significantly attenuated these responses to lead. Lead decreased brain concentrations of dopamine, copper alone did not, and the effect of the mixture was similar to that of lead alone. Copper alone increased brain concentrations of norepinephrine, lead did not, and the mixture effect was similar to copper alone. Neither metal nor the mixture affected brain concentrations of serotonin. Copper decreased the concentrations of lead in blood, kidney, and liver, but not in brain, as compared with lead alone. Lead decreased the concentrations of copper in kidney, increased the concentrations in brain, and had no effect on the concentration in liver, as compared with copper alone.

An oral/intraperitoneal study in rats also investigated effects of lead and copper on neurotransmitters in rat brains (Malhotra et al. 1982). Rats were administered lead in drinking water at 100 ppm providing 4.5 mg/rat/day ( $\approx 20$  mg/kg/day) as the acetate, and/or copper intraperitoneally at 2 mg/rat/day ( $\approx 10$  mg/kg/day) as the chloride for 21 days. No information regarding the basal diet or the general condition of the animals (e.g., body weight gain) was reported. Other studies by this laboratory on lead and manganese have used regular pelleted diets (Chandra et al. 1981, 1983; Shukla and Chandra 1987). Lead did not affect dopamine, copper increased dopamine, and the mixture greatly decreased dopamine concentrations in the brain (Malhotra et al. 1982). Lead alone and copper alone increased norepinephrine, but the mixture decreased norepinephrine concentrations in the brain. Neither metal alone affected brain serotonin concentration, but the mixture significantly decreased it. Lead decreased the concentration of copper in the brain; copper did not affect the concentration of lead in the brain.

In a sequential oral study, rats were exposed to 0 or 500 ppm lead in drinking water for 6 weeks, followed by 0 or 12 ppm copper for 2 weeks (Miniuk et al. 1989). The animals were fed standard laboratory diets and thus had an adequate basal copper intake. Hemoglobin and hematocrit were depressed by lead, but not by copper, and the group given lead plus subsequent copper had hemoglobin levels that were elevated not only above lead alone, but also slightly above controls. Hematocrits in the mixture group were slightly but not significantly elevated above the lead alone values. Blood ALAD activity was depressed by lead and this depression was not alleviated by subsequent exposure to excess copper. Serum copper and ceruloplasmin concentrations were decreased by lead alone and were not

different from controls in the lead plus copper group. Although the sequential protocol is considered inappropriate as the basis for conclusions regarding simultaneous environmental exposure, the results do indicate that the hematological effects of lead in animals receiving adequate copper can be alleviated by subsequent exposure to excess copper, and that some of the lead effects may be mediated through an interference with copper.

In a sequential intraperitoneal study, pretreatment of mice with copper (2 mg Cu/kg as the sulfate) decreased the mortality resulting from a challenge dose of lead (60 mg Pb/kg as the nitrate) administered 1 day later from 70 to 30% (Yoshikawa and Ohta 1982). Pretreatment with lead (10 mg/kg) decreased the mortality from a subsequent (2 days later) dose of copper (4 mg/kg) from 100 to 10%. Because of the endpoint studied, as well as the study design, these studies are considered inappropriate as the basis for conclusions regarding joint toxic action relevant to environmental contamination.

The following studies do not provide information regarding potential interactions of lead and excess copper, but rather focus on inadequate versus adequate copper. They are included because they are widely cited in the literature. In rats receiving high lead through the diet and inadequate to subadequate copper in the diet, erythrocyte lead was inversely proportional to dietary copper (Klauder et al. 1972). Lead appeared to interfere with copper status, as indicated by a decrease in plasma copper and ceruloplasmin in lead-treated groups, as compared with no-lead groups (Klauder et al. 1972). Inadequate dietary copper and high drinking water lead provided to rats for 12 weeks resulted in decreased hematocrit and hemoglobin as compared with inadequate copper and no lead (Klauder and Petering 1975, 1977). In rats given adequate copper, however, there was no significant change in these endpoints with high lead versus no lead (Klauder and Petering 1975, 1977). A 4-week feeding study comparing the toxicity of lead in the presence of adequate or inadequate copper found a greater increase in urinary excretion of ALA in lead-treated rats with adequate copper, as compared with those with deficient copper (Cerklewski and Forbes 1977). Blood hemoglobin and serum ceruloplasmin were lower in copper-deficient than copper-sufficient rats, but were unaffected by lead. Concentrations of copper in liver were increased by lead in deficient and adequate copper groups. No significant changes in concentrations of lead in blood, bone, liver, and kidney were seen between deficient and adequate copper groups that received lead. Thus, results of the studies by Klauder and coworkers are not entirely consistent with those of the study by Cerklewski and Forbes (1977), and as stated above, are not directly relevant to interactions between excess copper and lead.

## Potential Mechanisms of Interaction

Lead and copper appear to act on different components related to hematopoietic 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. ALA is toxic to the nervous system (ATSDR 1999). Copper is essential for heme synthesis because of its role, as part of the metalloenzyme ceruloplasmin, in oxidizing ferrous iron to the ferric form. Only ferric iron is bound to transferrin and transported to the bone marrow, so this transformation is critically important to provide iron for hemoglobin synthesis (Friberg et al. 1986). Copper at excessive levels, however, inhibits enzymes such as glucose-6-phosphatase and glutathione reductase by binding to enzyme sulfhydryl groups, thus interfering with their protection of cells from free radical damage. Inhibition of glucose-6-phosphatase leads to hemolysis at acutely toxic doses of copper (Barceloux 1999).

Whether lead interferes with copper status in animals receiving adequate or deficient oral intakes of copper is unclear. In a study in rats receiving high lead through the diet and inadequate to subadequate copper in the diet, lead appeared to interfere with copper status, as indicated by a decrease in plasma copper and ceruloplasmin in lead-treated groups, as compared with no-lead groups (Klauder et al. 1972). In another dietary study, however, serum ceruloplasmin was lower in copper-deficient than copper-sufficient rats, but was unaffected by lead (Cerklewski and Forbes 1977).

Copper metalloenzymes include dopamine  $\beta$ -hydroxylase, tyrosinase (tyrosine hydroxylase), and ascorbic acid oxidase (ATSDR 1990; Institute of Medicine 2001). Dopamine  $\beta$ -hydroxylase transforms dopamine to norepinephrine, tyrosinase metabolizes tyrosine to dopa (precursor of dopamine), and monoamine oxidase is important in the metabolic degradation of serotonin, epinephrine, norepinephrine, and dopamine (Hardman and Limbird 1996). Thus, it is conceivable that both deficient and excess copper could affect neurotransmitter levels, but clear evidence that copper is a neurotoxicant is lacking. Lead also has been shown to affect neurotransmitter levels (ATSDR 1999).

## Summary

Table 8 provides an overview of the interaction data regarding the effects of lead on the toxicity and tissue concentrations of copper and Table 9 summarizes the effects of copper 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 8. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Copper by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation (occupational) Exposure						
Chronic	Neurological (Parkinson's Disease)		>20 years exposure, levels not quantitated (ha)		indeterminate; odds ratio significantly elevated for Pb + Cu exposure, for Cu alone, and marginally for Pb alone	Gorell et al. 1997, 1999
Oral exposure (mg/kg/day)						
Intermediate	Neurological (brain norepinephrine levels)		14 + 8.6 <sup>a</sup> (r) <sup>b</sup>		additive	Flora et al. 1989a
Intermediate	Serum ceruloplasmin, a Cu metalloenzyme		18.2 + 1.8 (r)		additive: neither Pb nor Cu nor the mixture affected this endpoint relative to normal Cu	Cerklewski and Forbes 1977
Intermediate	Hepatic Cu levels		10 + 2 (r) 14 + 8.6 (r) 18.2 + 1.8 (r)		additive	Flora et al. 1982 Flora et al. 1989a Cerklewski and Forbes 1977
Intermediate	Renal Cu levels		10 + 2 (r)	14 + 8.6 (r)	<additive at higher copper dose	Flora et al. 1982 Flora et al. 1989a
Intermediate	Brain Cu levels	14 + 8.6 (r)			>additive	Flora et al. 1989a

**Table 8. Summary of Available Data on the Influence of Lead on Toxicity/Carcinogenicity of Copper by Simultaneous Exposure (*continued*)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal injection (mg/kg/day)						
Intermediate	Neurological (brain norepinephrine levels)		20 oral + 10 (r)		indeterminate: increase from each metal alone; decrease from the mixture at same doses as given alone	Malhotra et al. 1982
Intermediate	Neurological (brain dopamine levels)		20 oral + 10 (r)		indeterminate: no effect from lead alone, increase from copper alone, marked decrease from the mixture at same doses as given alone	Malhotra et al. 1982
Intermediate	Neurological (brain serotonin levels)		20 oral + 10 (r)		indeterminate: no effect from either metal alone but decrease from the mixture at same doses as given alone	Malhotra et al. 1982
Intermediate	Brain Cu levels			20 oral + 10 (r)	<additive	Malhotra et al. 1982

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat, ha = human (adult)

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

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation (occupational) exposure						
Chronic	Neurological (Parkinson's Disease)		>20 years exposure, levels not quantitated (ha)		indeterminate; odds ratio significantly elevated for Pb + Cu exposure, for Cu alone, and marginally for Pb alone	Gorell et al. 1977, 1999
Oral exposure (mg/kg/day)						
Acute (7 days)	Blood Pb, Pb balance			0.071 + 0.0036 <sup>a</sup> (ha) <sup>b</sup>	<additive	Kies and Ip 1990
Intermediate	Hematological (hemoglobin)		2 + 10 (r)		additive	Flora et al. 1982
Intermediate	Hematological (erythrocyte ALAD)			8.6 + 14 (r)	<additive	Flora et al. 1989a
Intermediate	Hematopoietic (zinc protoporphyrin)			8.6 + 14 (r)	<additive	Flora et al. 1989a
Intermediate	Hematopoietic (urinary ALA)			8.6 + 14 (r)	<additive	Flora et al. 1989a
Intermediate	Neurological (brain dopamine levels)		8.6 + 14 (r)		additive	Flora et al. 1989a
Intermediate	Neurological (brain norepinephrine levels)		10 i.p. + 20 (r)		indeterminate: increase from each metal alone; decrease from the mixture at same doses as given alone	Malhotra et al. 1982

**Table 9. Summary of Available Data on the Influence of Copper 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	Neurological (brain serotonin levels)		10 i.p. + 20 (r)		indeterminate: no effect from either metal alone but decrease from the mixture at same doses as given alone	Malhotra et al. 1982
Intermediate	Blood Pb levels		1.8 + 18.2 (r)	8.6 + 14 (r)	<additive at higher Cu and Cu/Pb	Cerklewski and Forbes 1977 Flora et al. 1989a
Intermediate	Bone Pb levels		1.8 + 18.2 (r)		additive	Cerklewski and Forbes 1977
Intermediate	Hepatic Pb levels		1.8 + 18.2 (r)	2 + 10 (r) 8.6 + 14 (r)	<additive at higher Cu and Cu/Pb	Cerklewski and Forbes 1977 Flora et al. 1982 Flora et al. 1989a
Intermediate	Renal Pb levels		1.8 + 18.2 (r)	2 + 10 (r) 8.6 + 14 (r)	<additive at higher Cu and Cu/Pb	Cerklewski and Forbes 1977 Flora et al. 1982 Flora et al. 1989a
Intermediate	Brain Pb levels		8.6 + 14 (r) 10 i.p. + 20 (r)		additive	Flora et al. 1989a Malhotra et al. 1982

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat, ha = human (adult)

### **2.2.5 Manganese and Zinc**

Manganese, zinc, and copper (as well as cadmium) are common industrial pollutants, and were therefore studied to see if there was any correlation among concentrations of pairs of these metals in urine of 1,464 women who lived in eight nonpolluted regions of Japan (Watanabe et al. 1991). Values were adjusted to a specific gravity of urine of 1.016. For manganese and zinc, no correlation was found.

In a sequential intraperitoneal study, pretreatment of mice with zinc (4 mg Zn/kg as the sulfate) did not affect the 70% mortality resulting from a challenge dose of manganese (50 mg Mn/kg as the chloride) administered 1 day later (Yoshikawa and Ohta 1982). Pretreatment with manganese (5 mg/kg) decreased the mortality from a subsequent (1 day later) dose of zinc (12 mg/kg) from 90 to 40%. Because of the endpoint studied, as well as the study design, these studies are considered inappropriate as the basis for conclusions regarding joint toxic action relevant to environmental contamination.

Information regarding the mechanisms of action of these two metals, summarized in the appendices to this profile, also does not clearly indicate what the mode of joint toxic action might be for these metals.

The data for manganese and zinc reviewed above are not adequate for supporting predictions regarding the joint toxic action of intermediate or chronic exposure to manganese and zinc, and do not warrant inclusion in summary tables.

### **2.2.6 Manganese and Copper**

Few data are available regarding the interactions of this pair of chemicals.

Manganese, zinc, and copper (as well as cadmium) are common industrial pollutants, and were therefore studied to see if there was any correlation among concentrations of pairs of these metals in urine of 1,464 women who lived in eight nonpolluted regions of Japan (Watanabe et al. 1991). Values were adjusted to a specific gravity of urine of 1.016. For manganese and copper, the correlation coefficient was positive, very small, and although statistically significant, was considered toxicologically insignificant.

A cross-sectional epidemiological study among men exposed to manganese in a manganese oxide and manganese salt producing plant revealed significantly elevated ceruloplasmin, copper, and ferritin in the serum of the exposed group versus the control group (Roels et al. 1987). Potential occupational exposure to copper was not mentioned, and apparently was not a concern. Roels et al. (1987) stated that the above changes suggest a potential metabolic interaction among manganese, copper, and iron. The information is not sufficient to suggest a mode of joint action and may not be relevant to interactions with excess copper. Ceruloplasmin is a copper-containing enzyme that oxidizes ferrous to ferric iron, and also may oxidize Mn(II) to Mn(III). Mn(II) binds to transferrin, as does ferric iron (ATSDR 2000; Friberg et al. 1986).

In a sequential intraperitoneal study, pretreatment of mice with copper (2 mg Cu/kg as the sulfate) did not significantly affect the 70% mortality resulting from a challenge dose of manganese (50 mg Mn/kg as the chloride) administered 1 day later (Yoshikawa and Ohta 1982). Pretreatment with manganese (5 mg/kg) decreased the mortality from a subsequent (1 day later) dose of copper (4 mg/kg) from 100 to 50%. Because of the endpoint studied, as well as the study design, these studies are considered inappropriate as the basis for conclusions regarding joint toxic action relevant to environmental contamination.

Additional mechanistic information from assays of rat brain homogenates indicated that manganese inhibited copper-induced lipid peroxidation *in vitro* (Sziraki et al. 1999). On the other hand, manganese and copper ions accelerated the oxidation of dopamine to a toxic quinone metabolite, which bound covalently with sulfhydryl groups of proteins, including to serotonin binding proteins, in calf brain soluble extracts (Velez-Pardo et al. 1995). The investigators suggested that this mechanism could contribute to the degeneration of dopaminergic neurons.

The data for manganese and copper reviewed above are not adequate for supporting predictions regarding the joint toxic action of intermediate or chronic exposure to manganese and zinc, and do not warrant inclusion in summary tables.

### **2.2.7 Zinc and Copper**

Manganese, zinc, and copper (as well as cadmium) are common industrial pollutants, and were therefore studied to see if there was any correlation among concentrations of pairs of these metals in urine of 1,464 women who lived in eight nonpolluted regions of Japan (Watanabe et al. 1991). Values were

adjusted to a specific gravity of urine of 1.016. For zinc and copper, a significant positive correlation was found.

Concentrations of zinc and copper in breast milk from 30 mothers were not correlated (Kies and Umoren 1989). These mothers were exposed through their normal diets, so this is not a study of interactions between excess zinc and lead. Additional detail regarding adequacy of zinc or copper in the diet were not provided. An inverse correlation between lead and copper was reported in this study and is discussed in Section 2.2.4.

Oral administration of zinc is an effective maintenance therapy for Wilson's disease, a genetic disease involving impaired biliary excretion of copper, excessive retention of copper in the liver, hepatic and renal lesions, hemolytic anemia, and neurological signs (ATSDR 1990). Zinc therapy of patients with Wilson's disease inhibits the absorption of copper through the induction of metallothionein, which sequesters copper in the intestinal mucosa. When the mucosal cells are exfoliated, the copper is excreted in the feces (Anderson et al. 1998; Brewer 2000). A similar hereditary disease occurs in Bedlington terriers; this disease also responds favorably to oral zinc therapy (Brewer et al. 1992). The success of these therapies indicates that high zinc can inhibit the absorption of normal levels of copper, but does not provide information regarding interactions between oral exposure to high zinc and high copper.

Most of the available studies on zinc and copper have explored the induction of a copper deficiency or altered copper status by the oral administration of excess zinc to humans (e.g., Prasad et al. 1978; Yadrick et al. 1989) or animals (e.g., Grant-Frost and Underwood 1958; Johnson and Flagg 1986) receiving adequate or inadequate intakes of copper through the diet. This type of information forms the basis for the oral MRL for zinc derived by ATSDR (1994) and will not be reviewed further here.

Few studies of potential interactions between excess zinc and excess copper have been performed. A study of the effect of supplemental oral copper on the toxicity of high oral zinc in rats reported that copper protected against the depression in hemoglobin caused by zinc (Smith and Larson 1946). In this study, rats were fed an adequate basal diet with excess zinc (7,000 ppm in the diet,  $\approx 897$  mg/kg/day) as zinc carbonate, with or without supplemental oral copper (0.2 mg/day,  $\approx 2.3$  mg/kg/day) as copper sulfate, for 6 weeks.

In a study of the effect of high oral copper on the toxicity of high oral zinc, rats were fed control diets (presumed adequate in mineral content), or 7,500 ppm zinc ( $\approx 900$  mg/kg/day) as the carbonate, with or without 200 ppm copper (24 mg/kg/day) as the sulfate for 5 weeks (Magee and Matrone 1960). Hemoglobin levels were markedly depressed by zinc alone; copper attenuated this response. Copper did not decrease the concentration of zinc in the liver.

A study of the effects of zinc on copper toxicity was conducted in calves fed milk replacer containing excess copper (1,000 ppm dry weight;  $\approx 15$  mg/kg/day) with and without excess zinc (1,000 ppm dry weight;  $\approx 15$  mg/kg/day) from day 3 to day 45 of age. Copper alone resulted in death of three of the seven calves and in lack of weight gain (Jenkins and Hidioglou 1989). Zinc prevented death and permitted some weight gain when fed with copper. Signs of jaundice (yellow coloration of the mucous membranes of the eye) and of hemolytic anemia (brownish-red urine) were seen in the calves that died from exposure to copper. Milder signs of jaundice were seen in surviving calves in the copper group, and in two of the seven calves receiving zinc plus copper. Necropsy revealed severe generalized jaundice in the calves that died from copper exposure, and mild generalized jaundice in the survivors in this group and in the calves of the zinc plus copper group. In the copper alone group, plasma glutamic-oxaloacetic transaminase (GOT) values were 50-fold higher than control values in the calves that died and 2.5-fold higher in the calves that survived than in control calves receiving an adequate basal diet. Hematocrits were significantly decreased in the copper alone group. Coexposure to zinc partially protected against the elevation in plasma GOT and the decrease in hematocrit. In addition, zinc administered with copper slightly but significantly decreased the concentration of copper in the liver, and significantly increased the excretion of copper in the feces, as compared with copper alone.

A study of the effect of intraperitoneally injected zinc (1.14 mg/kg/week = 0.16 mg/kg/day) and/or 100 or 200 ppm copper in the drinking water (26 or 52 mg/kg/day) in mice for 8 weeks investigated immunological endpoints (Pocino et al. 1990). Both metals were given as the sulfates; the diets were standard laboratory diets. Copper alone at either dose greatly depressed the lymphocytic proliferative response to mitogens and the antibody response to sheep red blood cells, as compared with controls. Zinc restored the immune competence in copper-treated rats as measured by these assays.

A developmental toxicity study in rats that investigated deficient through excess dietary levels of zinc and copper reported few effects in the range of adequate to excess dietary levels of these metals (Reinstein et al. 1984). Excess copper (100 ppm,  $\approx 6$  mg/kg/day) and/or excess zinc (1,000 ppm,

≈60 mg/kg/day) fed on days 0-21 of gestation did not affect reproductive and developmental endpoints (maternal weight, rats with resorptions, number of implantations, live fetuses, fetal weight, and malformed fetuses [none in these groups]). Tissue concentrations of copper and zinc were measured in maternal plasma, liver, kidney, and intestine and in whole fetuses, and fetal liver and brain (zinc only). Significant effects on tissue levels were limited to the following: excess zinc plus excess copper increased maternal renal levels of copper and decreased fetal hepatic levels of copper, as compared with excess copper and adequate zinc; and excess zinc plus excess copper decreased fetal hepatic levels of zinc, as compared with excess zinc and adequate copper.

Coadministration of single high doses of zinc (80 or 240 mg/kg) and copper (40 mg/kg) by gavage to rats decreased the concentration of copper in the liver as compared with the same dose of copper alone (Ogiso et al. 1974). The impact of zinc on liver copper was dose-related. In the same study, a sequential exposure of rats to 10,000 ppm zinc (as the carbonate) in the diet for 5 days followed by a single gavage dose of 40 mg/kg of copper (compound not specified) also resulted in a greatly decreased concentration of copper in the liver, as compared with the same dose of copper alone. The sequential data are considered less relevant to environmental exposure, but are consistent with the simultaneous exposure data.

A study of potential interactive effects of copper and zinc on absorption used a vascularly perfused rat intestine system to assay absorption of copper and zinc into the vascular perfusate from a solution of 6 ppm copper and 30 ppm zinc in the intestinal lumen (Ostreicher and Cousins 1985). The rats were pretreated for 7 days with inadequate, adequate, or high dietary copper and zinc using a 3x3 factorial design. It was thought that the pretreatment would allow for induction of metallothionein. The rats were then fasted for 16 hours before the assay was conducted. Dietary levels of copper were 1, 6, and 36 ppm (0.08, 0.5, and 2.9 mg/kg/day) and of zinc were 5, 30, and 180 ppm (0.4, 2.4, and 14.4 mg/kg/day). No significant differences in absorption of either metal were seen across the groups. The relevance of the assay to absorption in the intact organism is uncertain.

In a study of interactive effects on body burden, rats were injected subcutaneously on every other day with 5 mg/kg of zinc (2.5 mg/kg/day) as the chloride, with or without 2 mg/kg copper (1 mg/kg/day) as the chloride for 2 weeks (Chmielnicka et al. 1988). Coadministration of copper with zinc increased concentrations of zinc in blood, liver, kidney, intestines, and increased body burden of zinc. No details regarding animal condition or basal levels of minerals in the diet were provided, and this method of

administration bypasses homeostatic mechanisms and possible points of interaction during gastrointestinal absorption.

In a sequential intraperitoneal study, pretreatment of mice with copper (2 mg Cu/kg as the sulfate) decreased the mortality resulting from a challenge dose of zinc (12 mg Zn/kg as the sulfate) administered 1 day later from 90 to 70% (Yoshikawa and Ohta 1982). Pretreatment with zinc (4 mg/kg) decreased the mortality from a subsequent (1 day later) dose of copper (4 mg/kg) from 100 to 70%. The investigators did not consider these decreases toxicologically significant. Because of the endpoint studied, as well as the study design, these studies are considered inappropriate as the basis for conclusions regarding joint toxic action relevant to environmental contamination.

Table 10 provides an overview of the interaction data regarding the effects of zinc on the toxicity and tissue concentrations of copper and Table 11 summarizes the effects of copper on the toxicity and tissue concentrations of zinc. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

**Table 10. Summary of Available Data on the Influence of Zinc on Toxicity/Carcinogenicity of Copper by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (once)	Liver Cu levels			80-240 + 40 <sup>a</sup> (r) <sup>b</sup>	<additive	Ogiso et al. 1974
Acute (7 days)	Absorption (vascularly perfused rat intestine after <i>in vivo</i> exposure)		14.4 + 2.9 (r)		additive	Ostreicher and Cousins 1985
Intermediate	Immunological			0.16 i.p. + 26-52 (m)	<additive	Pocino et al. 1990
Intermediate	Death			15 + 15 (q)	<additive	Jenkins and Hidioglou 1989
Intermediate	Hematological (hematocrit, clinical signs of hemolysis, jaundice)			15 + 15 (q)	<additive	Jenkins and Hidioglou 1989
Intermediate	Hepatic (plasma GOT; jaundice)			15 + 15 (q)	<additive	Jenkins and Hidioglou 1989
Intermediate	Body weight gain			15 + 15 (q)	<additive	Jenkins and Hidioglou 1989
Intermediate	Plasma, intestine Cu levels		60 + 6 (r)		additive	Reinstein et al. 1984

**Table 10. Summary of Available Data on the Influence of Zinc on Toxicity/Carcinogenicity of Copper by Simultaneous Exposure (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intermediate	Liver Cu levels		60 + 6 (r)	15 + 15 (q)	<additive at higher Cu	Jenkins and Hidioglou 1989 Reinstein et al. 1984
Intermediate	Kidney Cu levels	60 + 6 (r)			>additive	Reinstein et al. 1984
Intermediate	Developmental (fetal liver Cu levels)			60 + 6 (r)	<additive	Reinstein et al. 1984
Intermediate	Fecal Cu excretion			15 + 15 (q)	<additive because increased fecal Cu excretion is protective	Jenkins and Hidioglou 1989

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat, m = mouse, q = cow

**Table 11. Summary of Available Data on the Influence of Copper on Toxicity/Carcinogenicity of Zinc by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral exposure (mg/kg/day)						
Acute (7 days)	Absorption (vascularly perfused rat intestine after <i>in vivo</i> exposure)		2.9 <sup>a</sup> + 14.4 (r) <sup>b</sup>		additive	Ostreicher and Cousins 1985
Intermediate	Hematological (hemoglobin)			2.3 + 897 (r) 24 + 900 (r)	<additive	Smith and Larson 1946 Magee and Matrone 1960
Intermediate	Plasma, intestine kidney Zn levels		6 + 60 (r)		additive	Reinstein et al. 1984
Intermediate	Liver Zn levels		6 + 60 (r) 24 + 900 (r)		additive	Reinstein et al. 1984 Magee and Matrone 1960
Intermediate	Developmental (fetal liver Zn levels)			6 + 60 (r)	<additive	Reinstein et al. 1984
Intermediate	Developmental (fetal brain and whole fetal Zn levels)		6 + 60 (r)			Reinstein et al. 1984

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

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Subcutaneous injection (mg/kg/day)						
Acute (2 weeks)	Zn body burden, tissue Zn levels	1 + 2.5 (r)			>additive	Chmielnicka et al. 1988

<sup>a</sup> First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup> Species code: r = rat

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

Lead, manganese, zinc, and copper were chosen as the subject of this interaction profile based on an analysis of the most frequently occurring binary mixtures in completed exposure pathways at hazardous waste sites. These metals are commonly found in soil. 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 few studies have addressed trinary mixtures of these metals. No conclusions regarding neurological effects of occupational exposure to lead, zinc, and copper can be drawn from the studies of Japanese foundry workers (Araki et al. 1992, 1993a, 1993b; Murata and Araki 1991; Murata et al. 1993) because of limitations in the data analyses and inconsistent results across the studies. Another study of foundry workers exposed to lead at concentrations above the TLV, and zinc and copper below the TLV, reported higher blood lead levels, decreased ALAD activity, and increased FEP in comparison with values in unexposed controls; these effects are characteristic of lead (Antonowicz et al. 1990). Whether some protection was afforded by the coexposure to zinc and copper cannot be determined from the data. A study of the effects of intraperitoneally injected lead, zinc, and copper on the composition of erythrocyte membranes indicated that the mixture was less “toxic” than either lead or copper alone at the same doses as in the mixture (Jehan and Motlag 1995). Zinc alone had no effects. The relevance of intraperitoneal injection and the endpoints to health effects in humans exposed by the oral route is uncertain. A study of wildlife in the vicinity of a zinc smelter, where the environment was contaminated with lead, zinc, and copper (as well as cadmium) found higher levels of lead in bone and lower levels of copper in tissues of those animals as compared with animals from a relatively uncontaminated area (Storm et al. 1994). Thus, zinc and copper did not preclude an increased bone burden of lead; whether they were partially protective cannot be determined from the data. It is possible that decreased copper in the tissues may have been related to zinc inhibition of copper absorption, but no clear conclusions can be drawn from these data.

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

To assess the potential joint toxic action of the components of this mixture, in the absence of adequate whole mixture data or PBPK models, the WOE approach is appropriate (ATSDR 2001a, 2001b). Using

this approach, BINWOE determinations of the plausible direction of interaction and the confidence in the predicted direction are developed.

The selection of target organs or endpoints for 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 joint toxic action data indicate a greater-than-additive interaction may need to be considered.

In the introduction to this document, Table 1 presented an overview of the potential effects of concern from oral exposure to the mixture of lead, manganese, zinc, and copper. The critical effects of two of these metals, lead and manganese, are neurological. The critical effect of a zinc is hematological, mediated through an interference with copper utilization. Hematological effects are also a sensitive effect of lead. The critical effect of copper appears to be hepatic. The pertinent data do not indicate greater-than-additive joint action for effects other than the critical effects of these metals. BINWOE development for neurological toxicity (lead, manganese), hematological toxicity (lead, zinc), and hepatic toxicity (copper) is appropriate, and is supported by the availability of at least some pertinent data, as summarized previously in Table 2.

BINWOE development was undertaken for these endpoints. The BINWOE classification scheme (Figure 1) and detailed rationales for the BINWOE determinations (Tables 12-22) are presented at the end of this section. During the development of this profile, and an interaction profile on another metal mixture, it became apparent that, because mechanistic considerations for the metals are exceedingly complex, the mechanistic understanding is unlikely to be sufficiently clear to support a judgment of direction of interaction with any confidence for pairs of metals lacking any toxicologically relevant joint action data. Therefore, BINWOE development was focused on pairs with some toxicologically relevant joint action data. Thus, the indeterminate BINWOE ratings summarized in this section and in Chapter 3 do not have tables in this section 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. Within the set of BINWOE determinations for each pair of metals, the order of presentation is neurological, followed by hematological, and then hepatic, as appropriate for the particular pair.

For lead and manganese, BINWOEs have been developed for the effect of lead on the neurological toxicity of the manganese, and vice versa (Tables 12 and 13), and for the effect of manganese on the hematological toxicity of lead (Table 14). The BINWOEs for neurological toxicity were additive (no effect) for the effect of lead on manganese (=IICii), and greater than additive for the effect of manganese on lead (>IICii). Confidence, as indicated by the alphanumeric scores, is low to moderate. The BINWOE for the effect of manganese on the hematological toxicity of lead was greater than additive (>IIB2ii) with low to moderate confidence. Manganese does not appear to be hematotoxic, so BINWOE determination for this endpoint is not appropriate for manganese toxicity.

For lead and zinc, BINWOEs have been developed for neurological (Table 15) and hematological effects (Tables 16 and 17). The BINWOE for the effect of zinc on the neurological toxicity of lead was less than additive (<IB), reflecting moderate confidence in the assessment. Zinc is not known to be a neurotoxin, so no BINWOE was developed for zinc for this endpoint. The BINWOEs for hematological toxicity were additive (no effect) for the effect of lead on zinc (=IIB), with moderate confidence, and less than additive for the effect of zinc on lead (<IA), with high confidence, reflecting the quantity and quality of data relevant to this interaction.

For lead and copper, BINWOEs were developed for lead's neurological (Table 18) and hematological (Table 19) toxicity and for copper's hepatic toxicity (Table 20). The BINWOEs for copper's effects on the neurological and hematological toxicity of lead were less than additive (<IC and <IB, respectively) and reflect low to moderate confidence. The BINWOE for the effect of lead on copper hepatotoxicity was additive (no effect) with low confidence (=IIC). As for the previous binary mixtures, BINWOEs were developed only for the established sensitive effects of each metal.

For manganese and zinc or manganese and copper, very little information was located. The available correlation study of metals in urine, occupational study involving elevated exposure to manganese but not copper, and sequential acute parenteral lethality studies were considered inadequate for supporting predictions regarding the joint toxic action of intermediate or chronic coexposure to these binary mixtures. Therefore BINWOEs for these chemical pairs are indeterminate.

For zinc and copper, BINWOEs have been developed for hematological (Table 21) and hepatic (Table 22) toxicity of these metals. The BINWOEs for the effects of copper on the hematological toxicity of zinc (<IIA) and for the effect of zinc on the hepatic toxicity of copper (<IB) were less than additive, and reflect moderate to high confidence. As explained previously, BINWOEs were developed only for the established sensitive effects of each metal.

**Figure 1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions\***

Classification	Factor
<b>Direction of Interaction</b>	
= Additive	0
> Greater than additive	+1
< Less than additive	-1
? Indeterminate	0
<b>Quality of the Data</b>	
<b>Mechanistic Understanding</b>	
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 mechanism(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
<b>Toxicological Significance</b>	
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
<b>Modifiers</b>	
1. Anticipated exposure duration and sequence.	1.0
2. Different exposure duration or sequence.	0.79
a. <i>In vivo</i> data	1.0
b. <i>In vitro</i> data	0.79
i. Anticipated route of exposure	1.0
ii. Different route of exposure	0.79
<i>Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05</i>	
<i>BINWOE = Direction Factor x Weighting Factor: Ranges from -1 through 0 to +1</i>	

\*Source: ATSDR 2001a, 2001b

**Table 12. Effect of Lead on Manganese: Neurological Toxicity for Oral Exposure**

**BINWOE: =IICii (0)**

*Direction of Interaction* - The interaction is predicted to be additive based on the apparent additivity of lead and manganese on learning (conditioned avoidance) in rats and on the lack of effect of lead on brain manganese concentrations in studies in which manganese was administered orally (Chandra et al. 1981). The brain concentration data are not consistent across studies, however, and the data on brain norepinephrine levels and spontaneous motor activity need further investigation before the mode joint toxic action on these endpoints can be determined.

*Mechanistic Understanding* - Intraperitoneal injection of lead and oral exposure to manganese for 14 days did not alter the concentration of manganese in whole brain (Chandra et al. 1981). Intraperitoneal injection of both metals during gestation and lactation did not alter manganese levels in whole brain of the pups (Chandra et al. 1983). In a subsequent study, however, intermediate duration oral exposure to lead and intraperitoneal injection of a low dose of manganese produced higher concentrations of manganese in a few regions of the brain than did manganese alone at the same dose as in the mixture (Shukla and Chandra 1987). At a higher dose of manganese, lead had no effect on brain manganese as compared with the same dose of manganese alone. Binding studies *in vitro* by the same group of investigators showed no influence by the presence of lead on the binding of manganese to brain protein (Kalia et al. 1984). Although the findings of the *in vivo* studies are conflicting, the bulk of the evidence indicates no effect of lead on the distribution or retention of manganese in the brain. This conclusion is supported by the lack of influence of lead on manganese binding to brain protein. Thus additivity is plausible. Because of the ambiguity of the data, a rating of III is appropriate for mechanistic understanding.

*Toxicological Significance* - The critical effects of both lead and manganese are neurological (ATSDR 1999, 2000). Intraperitoneal injection of lead and oral administration of manganese for 7-14 days appeared to adversely affect learning (conditioned avoidance) in an additive manner (Chandra et al. 1981). Spontaneous motor activity and norepinephrine content of the brain were slightly increased by each metal alone, but were significantly decreased by the two metals together at the same doses as tested alone (Chandra et al. 1981). Nervous system toxicants, including lead alone in this study, commonly have excitatory effects at low doses and depressive effects at high doses, so the change in direction of effect is reasonable. Nevertheless, it is not possible to determine the nature of the joint toxic action with regard to norepinephrine and motor activity, due to the study design and results. The results do indicate that to assess potential hazard of the mixture on the basis of each metal separately is likely to underestimate the hazard. Based on additivity seen in the learning test in rats, and the weight of evidence for lack of influence of lead on distribution of manganese to the brain, additivity is predicted as the direction of joint toxic action. Because of the limited database, lack of analysis for deviation from additivity in the studies, and uncertainty regarding the toxicological significance of some of the findings, a classification of C is appropriate.

*Modifying Factors* - A modifying factor for different route of exposure (ii) is applied to account for uncertainties regarding the intraperitoneal route of administration used for one or both of the metals in these studies. Parenteral administration bypasses potential points of interaction for the metals.

*Additional Uncertainties* - The modifying factor for intraperitoneal route does not fully express the uncertainties associated with the applicability of parenteral data to oral exposure for the metals.

**Table 13. Effect of Manganese on Lead: Neurological Toxicity for Oral Exposure**

$$\text{BINWOE: } >\text{ICii } (+1 \times 1.0 \times 0.32 \times 0.79 = +0.25)$$

*Direction of Interaction* - The interaction is predicted to be greater than additive based on the biokinetic finding that manganese increased the concentration of lead in the brains of rats of various ages, including neonatal (Chandra et al. 1981, 1983; Shukla and Chandra 1987). Increased distribution and retention of lead in the brain is anticipated to increase the risk of direct effects of lead on brain tissue. Testing of neurological endpoints (Chandra et al. 1981) suggest additivity for a learning task (conditioned avoidance) and were indeterminate for other endpoints, but suggested that effects of the mixture were more severe than of each metal tested separately at the same dose as in the mixture.

*Mechanistic Understanding* - Intraperitoneal injection of lead and oral exposure to manganese for 14 days increased the concentration of lead in whole brain (Chandra et al. 1981). Intraperitoneal injection of both metals during gestation and lactation also increased lead levels in whole brain of the pups (Chandra et al. 1983). In addition, intermediate duration oral exposure to lead and intraperitoneal injection of low and high doses of manganese produced higher concentrations of lead in five of seven regions of the brain as compared with lead alone at the same dose as in the mixture (Shukla and Chandra 1987). Binding studies *in vitro* by the same group of investigators showed that the presence of manganese increased the binding of lead to brain protein (Kalia et al. 1984). Taken together, the data clearly indicate that manganese increases the distribution and/or retention of lead in the brain. Because lead is thought to affect nervous tissue directly through a variety of mechanisms (ATSDR 1999), increased concentrations of lead in the brain raise the concern for increased risk of adverse effects. A rating of I is appropriate for mechanistic understanding.

*Toxicological Significance* - The critical effects of both lead and manganese are neurological (ATSDR 1999, 2000). Intraperitoneal injection of lead and oral administration of manganese for 7-14 days appeared to adversely affect learning (conditioned avoidance) in an additive manner (Chandra et al. 1981). Spontaneous motor activity and norepinephrine content of the brain were slightly increased by each metal alone, but were significantly decreased by the two metals together at the same doses as tested alone (Chandra et al. 1981). Nervous system toxicants, including lead alone in this study, commonly have excitatory effects at low doses and depressive effects at higher doses, so the change in direction of effect is reasonable. Nevertheless, it is not possible to determine the nature of the joint toxic action with regard to norepinephrine and motor activity, due to the study design and results. The results do indicate that to assess potential hazard of the mixture on the basis of each metal separately is likely to underestimate the hazard. Thus, there is some evidence of additivity, additional evidence of greater effects on neurological endpoints from the mixture than from lead and manganese alone (without a clear indication of the mode of joint action), and brain concentration data that clearly indicate a greater-than-additive effect of manganese on lead distribution/retention in the critical target organ in adult, weanling, and neonatal rats. Thus, the direction of interaction, when one occurs, may be greater than additive. Because of the limited database, lack of analysis for deviation from additivity in the studies, and uncertainty regarding the toxicological significance of some of the data, a rating of C is appropriate.

*Modifying Factors* - A modifying factor for different route of exposure (ii) is applied to account for uncertainties regarding the intraperitoneal route of administration used for one or both of the metals in these studies. Parenteral administration bypasses potential points of interaction for the metals.

*Additional Uncertainties* - The modifying factor for intraperitoneal route does not fully express the uncertainties associated with the applicability of parenteral data to oral exposure for the metals.

**Table 14. Effect of Manganese on Lead: Hematological Toxicity for Oral Exposure**

**BINWOE: >IIB2ii** (+1 x 0.71 x 0.71 x 0.79 x 0.79 = +0.31)

*Direction of Interaction* - The direction of interaction is predicted to be greater than additive based on a single intravenous study in rabbits, in which coadministration of manganese delayed the recovery of blood ALAD activity from lead inhibition (Chiba and Kikuchi 1984b). The delay appeared to be due to the prolongation of the half-life of lead in blood by manganese.

*Mechanistic Understanding* - In rabbits given an intravenous injection of lead and manganese, the half-life of lead in blood was prolonged as compared with the half-life of lead given without manganese (Chiba and Kikuchi 1984b). Lead inhibits blood ALAD, manganese does not, and the *in vitro* addition of manganese to blood from lead-exposed subjects, or from rabbits and mice following parenteral administration of lead, did not restore ALAD activity (Chiba and Kikuchi 1984a). By prolonging the residence of lead in blood, manganese is thought to prolong lead's effects on ALAD. A rating of II reflects the limited amount of relevant mechanistic data.

*Toxicological Significance* - Intravenous injection of lead and manganese resulted in a slower recovery of blood ALAD activity as compared with that seen in a group injected with the same dose of lead alone (Chiba and Kikuchi 1984b). Manganese alone did not affect ALAD. The mechanistic data indicate that manganese prolonged the half life of lead in blood, which could account for the effect on ALAD. A direct effect of manganese on ALAD was not seen (Chiba and Kikuchi 1984a, 1984b). Based on these data from a single group of investigators, the effect of manganese on the hematological toxicity of lead is predicted to be greater than additive. A rating of B is chosen to reflect that this conclusion is based on very little data.

*Modifying Factors* - A modifying factor for duration (2) should be used to reflect uncertainties regarding the applicability of this determination to any duration beyond a single exposure, and for different route of exposure (ii) should be applied to reflect the uncertainties regarding the applicability of intravenous injection to oral exposure.

*Additional Uncertainties* - The modifying factors for duration and route do not fully express the uncertainties associated with the applicability of acute parenteral data to intermediate and chronic oral exposure to the metals.

**Table 15.** Effect of **Zinc** on **Lead**: Neurological Toxicity  
for Oral Exposure

**BINWOE:** <IB (-1 x 1.0 x 0.71 = -0.71)

*Direction of Interaction* - The interaction is predicted to be less than additive. This conclusion is plausible based on zinc's influence on blood and tissue lead levels, influence on lead-inhibited heme biosynthesis, specifically ALAD activity, and induction of proteins that sequester lead. In addition, two intermediate oral studies in animals reported a protective effect of zinc against the neurological effects of lead (Vassilev et al. 1994; Willoughby et al. 1972).

*Mechanistic Understanding* - In humans receiving adequate dietary zinc and a low dietary lead intake, supplemental zinc at the level of the RDA did not affect blood lead or urinary or fecal excretion of lead (Kies and Ip 1990). In rats, supplemental zinc generally did not affect lead absorption or blood and tissue lead from low oral lead doses (Bebe and Panemangelore 1996; Cerklewski and Forbes 1976). At higher lead doses in rats, however, supplemental zinc decreased the gastrointestinal absorption of lead (Cerklewski and Forbes 1976) and decreased blood, bone, liver, kidney, and spleen concentrations of lead (Cerklewski 1979; Cerklewski and Forbes 1976; El-Gazzar et al. 1978; Flora et al. 1982, 1989b, 1991) in intermediate duration studies. Levels of lead in brain were not affected (El-Gazzar et al. 1978; Flora et al. 1989b, 1991), but the evidence in general suggests that oral coexposure to zinc at levels significantly above essentiality decreases lead absorption and body burden at higher lead exposures. Other mechanisms by which zinc may inhibit the neurological effects of lead include zinc protection and reactivation of ALAD activity (a zinc metalloenzyme found in all tissues, which is inhibited by lead) (e.g., Batra et al. 1998; Cerklewski 1979; Cerklewski and Forbes 1976; Flora et al. 1991). The prevention of lead inhibition of ALAD prevents the accumulation of ALA, a substance that appears to act as a GABA agonist at the presynaptic GABA receptors, causing negative-feedback inhibition of GABA release. In addition, ALA undergoes autooxidation, generating free radicals that may contribute to toxicity (ATSDR 1999; EPA 1986). Zinc induces a metallothionein that has been shown to sequester lead *in vitro*, protecting against its cytotoxicity (Goering and Fowler 1987b; Liu et al. 1991). A metallothionein-like protein in erythrocytes binds lead *in vitro* and was present in higher concentrations in lead-exposed workers than in controls, and at exceptionally high levels in a worker with very high blood lead (180 µg/dL) but no signs of lead poisoning, as compared with another worker with high blood lead (160 µg/dL) who was symptomatic (Church et al. 1993a, 1993b). Metallothioneins have been identified in the brain as well as other tissues (Sandstead et al. 2000). An acidic, soluble lead-binding protein in brain (and kidney and erythrocytes) that normally binds zinc is postulated to attenuate lead toxicity to ALAD through lead binding and zinc donation (Fowler 1998). Taken together, the mechanistic data indicate that, under conditions where an interaction occurs, the effect of zinc on the neurotoxicity of lead will be less than additive. A rating of I is chosen to reflect reasonably clear understanding of a number of mechanisms for zinc protection against lead neurotoxicity.

*Toxicological Significance* - Studies of zinc's effect on the neurotoxicity of lead are of marginal quality, and do not include sensitive neurobehavioral endpoints. Intermediate duration oral studies in animals report a protective effect of zinc against inhibition of smooth muscle contractility by lead (Vassilev et al. 1994) and on pharyngeal and laryngeal paralysis caused by lead in young horses (Willoughby et al. 1972), and no effect of zinc on the inhibition of nerve conduction velocity in rabbits (Hietanen et al. 1982). All were high dose studies, and all have significant limitations in their design, reporting, or relevance. Nevertheless, these studies do not provide evidence of potentiation, but rather a protective effect, or no effect, of zinc on lead neurotoxicity. A study in humans using biomarkers of

**Table 15.** Effect of **Zinc** on **Lead**: Neurological Toxicity  
for Oral Exposure (*continued*)

**BINWOE:** <IB (-1 x 1.0 x 0.71 = -0.71)

*Toxicological Significance (continued)* - exposure in orally exposed children indicates a protective effect of zinc on the hematopoietic effects of lead (Chisolm 1981), as do oral studies in animals, particularly at relatively higher lead doses (e.g., Cerklewski and Forbes 1976; Flora et al. 1982, 1991). As discussed in the mechanistic section, oral exposures to supplemental zinc generally result in lower tissue concentrations of lead at higher lead doses and no effect at lower lead doses, although no effect on brain lead was seen at any dose. There are a number of mechanisms by which zinc may affect the toxicity of lead, including decreasing lead absorption, protecting zinc-containing enzymes such as ALAD, and inducing lead-sequestering proteins or zinc donation from these proteins as lead is bound. Based on the joint toxic action studies, supported by the tissue distribution and other mechanistic understanding, the predicted direction of interaction, when one occurs, is less than additive. Because of concerns regarding the quality and relevance of the neurotoxicity data, a classification of B is considered appropriate.

*Additional Uncertainties* - At low exposures to lead, excess zinc (as compared with adequate zinc) may not affect lead neurotoxicity.

**Table 16. Effect of Lead on Zinc: Hematological Toxicity  
for Oral Exposure**

**BINWOE: =IIB (0)**

*Direction of Interaction* - Lead is predicted to have no effect on the toxicity of zinc, based on a general lack of effect of lead on the plasma and tissue levels of zinc in rats fed excess zinc and lead (Cerklewski and Forbes 1976; El-Gazzar et al. 1978), and the lack of effect of high lead on the hematological effects of high zinc in one study in young horses (Willoughby et al. 1972).

*Mechanistic Understanding* - Excess oral zinc interferes with heme synthesis by interfering with copper absorption. The principal mechanism appears to involve zinc induction of metallothionein in the mucosa of the gastrointestinal tract. Copper has a higher affinity for binding to metallothionein, which reduces its absorption into the blood and increases its fecal excretion as the cells of the intestinal mucosa containing copper-metallothionein are exfoliated (ATSDR 1994). Copper is an essential part of several enzymes including ceruloplasmin, which oxidizes ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is critically important to provide iron for hemoglobin synthesis (Friberg et al. 1986). A characteristic and sensitive effect of lead on heme synthesis is the inhibition of ALAD, a zinc-containing enzyme in the heme synthesis pathway (ATSDR 1999). Thus, mechanisms of action of lead and zinc on heme synthesis are different. In studies of the intermediate oral coexposure of rats to lead and supplemental zinc, lead generally did not affect zinc absorption (Cerklewski and Forbes 1976) or plasma or tissue concentrations of zinc (liver, kidney, brain, testes), except for a decrease in plasma zinc (but not in erythrocyte or tissue zinc) in one study using a high lead/zinc ratio (El-Gazzar et al. 1978). Thus, the data generally indicate that lead does not affect the absorption or tissue levels of zinc. Because the data are not totally consistent, a classification of II is appropriate.

*Toxicological Significance* - A single study in young horses fed highly toxic doses of lead and/or zinc reported that zinc resulted in anemia, lead did not, and the combination (at approximately the same doses of each metal as when fed alone) gave the same results as zinc alone (Willoughby et al. 1972), indicating that lead had no effect on zinc hematotoxicity. Limitations of the study include the small number of animals studied, and relevance of results in horses to humans, particularly as the horses treated with lead alone did not develop anemia at blood lead levels of 60 µg/dL. Nevertheless, the study indicates a lack of effect of lead on the hematological effects of zinc. This result, together with general lack of effect of lead on the absorption, plasma, erythrocyte, and tissue levels of zinc, indicate that the direction of interaction is additive (no effect). An appropriate rating is B, reflecting the limitations of the toxicity data (one study, with limitations), and the support provided by the mechanistic data.

*Additional Uncertainties* - Uncertainties are covered in the main classifications above.

**Table 17. Effect of Zinc on Lead: Hematological Toxicity for Oral Exposure**

**BINWOE: <IA (-1 x 1 x 1 = -1)**

*Direction of Interaction* - The direction of interaction is predicted to be less than additive based on a study in orally exposed children indicating a protective effect of zinc on the hematopoietic effects of lead (Chisolm 1981), and several intermediate duration oral studies in rats that show protection by supplemental zinc against a number of hematological effects of lead related to heme synthesis, particularly at higher lead doses (e.g., Cerklewski and Forbes 1976; Flora et al. 1982, 1991). The evidence for zinc inhibition of lead hematotoxicity is clear and toxicologically significant, and is supported by clear mechanistic understanding that excess zinc protects and reactivates lead-inhibited ALAD, decreases the absorption (Cerklewski and Forbes 1976) and tissue distribution of lead, and may induce proteins that sequester lead and donate zinc to ALAD and for other tissue needs.

*Mechanistic Understanding* - A characteristic and sensitive effect of lead is the inhibition of ALAD, a zinc-containing enzyme in the heme synthesis pathway (ATSDR 1999). Zinc protects the enzyme from inactivation by lead *in vivo* (see toxicological significance) and *in vitro* (e.g., Abdulla et al. 1979; Davis and Avran 1978; Murras and Allain 1979; Tomukuni 1979), as assayed directly, or by the appearance of greater amounts of ALA in the urine. Supplemental zinc decreased the gastrointestinal absorption of lead (Cerklewski and Forbes 1976) and decreased blood, bone, liver, kidney, and spleen concentrations of lead (Cerklewski 1979; Cerklewski and Forbes 1976; El-Gazzar et al. 1978; Flora et al. 1982, 1989b, 1991) in rats in intermediate duration studies; this effect was seen at higher but not lower doses, and was not seen in humans at lower doses of both metals than used in the rat studies. The evidence in general suggests that oral coexposure to zinc at levels significantly above essentiality decreases lead absorption and body burden at higher lead exposures. Zinc induces a metallothionein that has been shown to sequester lead *in vitro*, protecting against its cytotoxicity (Goering and Fowler 1987b; Liu et al. 1991). A metallothionein-like protein in erythrocytes binds lead *in vitro* and was present in higher concentrations in lead-exposed workers than in controls, and at exceptionally high levels in a worker with very high blood lead (180 µg/dL), but no signs of lead poisoning, as compared with another worker with high blood lead (160 µg/dL) who was symptomatic (Church et al. 1993a, 1993b). An acidic, soluble lead-binding protein in erythrocytes (and brain and kidney) that normally binds zinc is postulated to attenuate lead toxicity to ALAD through lead binding and zinc donation (Fowler 1998). The mechanistic data indicate that, under conditions where an interaction occurs, the effect of zinc on the hematological toxicity of lead will be less than additive. The understanding warrants a classification of I.

*Toxicological Significance* - Although a study in occupationally exposed men given a low oral dose of zinc did not provide evidence of an effect on blood lead or urinary ALA, the inhalation route of exposure for lead circumvents potential interactions at the level of absorption, and the dose of zinc was very low (equivalent to the RDA). An increase in urinary ALA above the normal range was significantly associated with a decrease in the chelatable zinc/lead ratio to 18.45 or less in children given chelation therapy for lead poisoning (Chisolm 1981). Supplemental zinc protected against the inhibiting effects of lead on ALAD activity, and against lead-induced increases in zinc protoporphyrin and urinary ALA excretion in rats given both metals orally for intermediate durations (Cerklewski and Forbes 1976; El-Gazzar et al. 1978; Flora et al. 1982, 1989b, 1991). These protective effects were seen at higher but not lower lead doses, and when basal levels of zinc in the diet were adequate. The clear data regarding a protective effect of zinc on lead hematotoxicity, supported by the clear mechanistic understanding, warrants a classification of A.

*Additional Uncertainties* - At low exposures to lead and low supplemental zinc (as compared with adequate zinc), zinc may not affect lead hematotoxicity.

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

**BINWOE:**  $<IC (-1 \times 1.0 \times 0.32 = -0.32)$

*Direction of Interaction* - The interaction is predicted to be less than additive. This conclusion is plausible based on copper's protective influence on blood and tissue lead levels (Flora et al. 1982, 1989a; Kies and Ip 1990), protective influence on lead-inhibited heme biosynthesis, specifically ALAD activity (Flora et al. 1989a), and induction of metallothionein which can sequester lead.

*Mechanistic Understanding* - In humans receiving adequate dietary copper and a low dietary lead intake, supplemental copper at a level about five times the RDA decreased blood lead and had a protective effect on lead balance (i.e., copper changed lead balance from positive to negative) (Kies and Ip 1990). In rats receiving both metals orally, supplemental copper generally did not affect lead absorption or blood and liver, kidney, and bone lead concentrations at lower copper doses and copper/lead dose ratios (Cerklewski and Forbes 1977). At higher supplemental copper doses and higher copper/lead dose ratios in rats, however, supplemental copper decreased blood, liver, and kidney concentrations of lead (Flora et al. 1982, 1989a) in intermediate duration studies. Levels of lead in brain were not affected (Flora et al. 1989a) at the higher copper and copper/lead doses, or in an intermediate duration study of intraperitoneally injected copper and oral lead in rats (Malhotra et al. 1982). The evidence in general suggests that oral coexposure to copper at levels significantly above essentiality decreases lead absorption and body burden. Another mechanism by which copper may influence the neurotoxicity of lead is the attenuation by copper of the hematopoietic effects of lead (including lead's inhibition of ALAD) in an intermediate oral study in rats (Flora et al. 1989a). The prevention of lead inhibition of ALAD prevents the accumulation of ALA, a substance that appears to act as a GABA agonist at the presynaptic GABA receptors, causing negative-feedback inhibition of GABA release. In addition, ALA undergoes autooxidation, generating free radicals that may contribute to toxicity (ATSDR 1999; EPA 1986). Another potential mechanism involves metallothionein, which can sequester lead. Copper, like zinc, induces metallothionein (ATSDR 1990, 1994). Whether copper may inhibit the absorption and toxicity of lead through induction of metallothionein is unclear. Such a mechanism appears to account in part for the protective effect of zinc against lead toxicity (Church et al. 1993a, 1993b; Goering and Fowler 1987b; Liu et al. 1991). Metallothioneins have been identified in the brain as well as other tissues (Sandstead et al. 2000). Taken together, the mechanistic data indicate that, under conditions where an interaction occurs, the effect of copper on the neurotoxicity of lead may be less than additive. A rating of I is chosen to reflect reasonably clear understanding of a number of mechanisms for copper protection against lead neurotoxicity.

*Toxicological Significance* - A single case-control study indicates that >20 years of occupational exposure to copper and lead may increase the risk of Parkinson's disease (Gorell et al. 1997, 1999). This study does not provide information suitable for determining the type of joint toxic action of these metals, and these metals are not established as etiological agents for Parkinson's disease. Experimental studies of copper's effect on the neurotoxicity of lead are limited to studies of neurotransmitter concentrations in brain. Copper did not affect the lead-induced decrease in brain concentration of dopamine in rats following intermediate oral exposure (Flora et al. 1989a). Lead did not affect norepinephrine or influence copper's effect on this transmitter, and neither metal affected serotonin in this study. Entirely different results were obtained for the single metals and the mixture in another intermediate duration study of brain concentrations of these neurotransmitters in rats (Malhotra et al. 1982), but the dose of copper was higher and was administered through intraperitoneal injection, which bypasses homeostatic mechanisms for copper in the gastrointestinal tract and also

**Table 18.** Effect of **Copper** on **Lead**: Neurological Toxicity  
for Oral Exposure (*continued*)

**BINWOE:** <IC (-1 x 1.0 x 0.32 = -0.32)

*Toxicological Significance (continued)* - potential points of interaction with lead. Although confidence is greater in the oral study, which showed no effect of copper on a lead-induced effect on dopamine, the inconsistency across studies increases the uncertainty regarding the toxicological significance of this finding. In addition, no studies of copper's influence on sensitive neurobehavioral effects of lead are available. As discussed in the mechanistic section, supplemental copper (at about 5 times RDA) decreased blood lead had a protective effect on lead balance in humans exposed to low dietary lead. Intermediate duration oral exposures in rats to supplemental copper and to lead generally resulted in lower blood and tissue concentrations of lead at higher copper doses and copper/lead dose ratios, and no effect at lower copper doses, in comparison with lead alone. No effect on brain lead was seen. An additional mechanism by which copper may affect the toxicity of lead is by inducing metallothionein, which may sequester lead. These considerations support the prediction that the direction of interaction, when one occurs, may be less than additive. Because this prediction is based on mechanistic data, and the toxicity data are conflicting, a classification of C is appropriate.

*Additional Uncertainties* - At low supplemental copper doses or copper/lead dose ratios, excess copper (as compared with adequate copper) may not affect lead neurotoxicity.

**Table 19. Effect of Copper on Lead: Hematological Toxicity for Oral Exposure**

**BINWOE: <IB** ( $-1 \times 1.0 \times 0.71 = -0.71$ )

*Direction of Interaction* - The predicted direction of interaction is less than additive, based on the protection by supplemental copper against lead-induced hematopoietic effects in an intermediate duration oral study in rats (Flora et al. 1989a), supported by mechanistic data that indicate coexposure to excess copper decreases lead absorption and body burden (Flora et al. 1982, 1989a; Kies and Ip 1990), and induces metallothionein, which can sequester lead.

*Mechanistic Understanding* - Lead and copper appear to act on different components related to hematopoietic 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 1999). Excess copper inhibits as glucose-6-phosphatase, which leads to hemolysis, a high-dose affect (Barceloux 1999). It is unclear how excess copper would affect lead's hematological toxicity. In humans receiving adequate dietary copper and a low dietary lead intake, supplemental copper at a level about five times the RDA decreased blood lead and had a protective effect on lead balance (changed lead balance from positive to negative) (Kies and Ip 1990). In rats receiving both metals orally, supplemental copper generally did not affect lead absorption or blood and liver, kidney, and bone lead concentrations at lower copper doses and copper/lead dose ratios (Cerklewski and Forbes 1977). At higher supplemental copper doses and higher copper/lead dose ratios doses in rats, however, supplemental copper decreased blood, liver, and kidney concentrations of lead (Flora et al. 1982, 1989a) in intermediate-duration studies. The evidence in general suggests that oral coexposure to copper at levels significantly above essentiality decreases lead absorption and body burden. Another potential mechanism involves metallothionein, which can sequester lead. Copper, like zinc, induces metallothionein (ATSDR 1990, 1994). Whether copper may inhibit the absorption and toxicity of lead through induction of metallothionein is unclear. Such a mechanism appears to account in part for the protective effect of zinc against lead toxicity (Church et al. 1993a, 1993b; Goering and Fowler 1987b; Liu et al. 1991). Taken together, the mechanistic data indicate that, under conditions where an interaction occurs, the effect of copper on the hematotoxicity of lead may be less than additive due to coppers protective effect on absorption and body burden of lead. A classification of I is appropriate.

*Toxicological Significance* - Supplemental copper protected against lead-induced hematopoietic effects (inhibition of ALAD, increase in zinc protoporphyrin, and increase in urinary ALA) in an intermediate oral study in rats (Flora et al. 1989a), but did not, at a lower copper dose and lower copper/lead ratio, affect the lead-induced decrease in hemoglobin in rats in a similar study (Flora et al. 1982). These results indicate that the effect of copper on lead hematotoxicity, when it occurs, will be less than additive. This prediction is supported by the mechanistic data regarding the effects of copper on blood and tissue levels of lead, and mechanistic predictions regarding copper induction of metallothionein discussed under mechanistic understanding. A classification of B is appropriate to reflect that only one study has demonstrated the interaction.

*Additional Uncertainties* - At low supplemental copper doses or copper/lead dose ratios, excess copper (as compared with adequate copper) may not affect lead hematotoxicity.

**Table 20.** Effect of **Lead** on **Copper**: Hepatic Toxicity for Oral Exposure

**BINWOE: =IIC (0)**

*Direction of Interaction* - Lead is predicted to have no effect on the hepatic toxicity of copper, based on the lack of effect of lead on liver concentrations of copper and on ceruloplasmin levels in rats following intermediate duration oral exposure to lead and excess copper (Cerklewski and Forbes 1977; Flora et al. 1982, 1989a).

*Mechanistic Understanding* - Copper at excessive levels inhibits enzymes such as glucose-6-phosphatase and glutathione reductase, by binding to enzyme sulfhydryl groups, thus interfering with their protection of cells from free radical damage. Whether these mechanisms are involved in liver toxicity is not clear, but inhibition of glucose-6-phosphatase leads to hemolysis. Metallothionein, a cysteine-rich, low-molecular-weight protein that binds copper in the gastrointestinal mucosa and other body tissues, provides some homeostatic regulation of copper absorption and protects against copper toxicity; its synthesis is induced by copper (Barceloux 1999). Lead also binds to metallothionein, but does not appear to induce its synthesis. Among the mechanisms for lead toxicity are binding to sulfhydryl and other functional groups and acting as a calcium agonist. The above mechanistic considerations do not lead to an understanding of a plausible direction of interaction. Tissue distribution studies, discussed under toxicological significance, indicate that oral coexposure to lead and excess copper generally does not affect tissue concentrations of copper, particularly in the critical target organ, the liver. The data, however, are not entirely consistent. Therefore, a prediction of additive is appropriate for mechanistic understanding, with a classification of III to reflect data inconsistency and limited understanding.

*Toxicological Significance* - No direct toxicological data regarding the effect of lead on liver toxicity of copper were located. In rats exposed orally for intermediate durations to lead and supplemental copper, lead generally did not affect hepatic copper levels (Cerklewski and Forbes 1977; Flora et al. 1982, 1989a), renal copper levels (Flora et al 1982), or serum ceruloplasmin levels (Cerklewski and Forbes 1977). A decrease in renal copper and increase in brain copper was seen in one study (Flora et al. 1989a). The reason for the discrepancy in renal copper levels (no effect versus decrease) is uncertain. Lead had no effect on copper-induced increases in brain norepinephrine (Flora et al 1989a), but copper is not known to be a neurotoxicant, so the toxicological significance of this finding is unclear. Intermediate duration coadministration to rats of copper intraperitoneally and lead orally resulted in a decrease in brain copper levels, relative to copper alone, and different results on neurotransmitter levels (Malhotra et al. 1982) than had been seen when both metals were given orally (Flora et al. 1989a). Based on the results obtained from oral coexposure, which are considered more relevant, lead did not affect the concentration of copper in the critical target organ, the liver, or affect serum concentrations of ceruloplasmin, a key copper metalloenzyme that provides iron in the appropriate valence state for heme synthesis. Therefore, it is expected that joint toxic action will be additive (no effect of lead on copper hepatotoxicity), but confidence in this prediction is low because of limitations in the database and lack of consistency or understanding in some of the endpoints. A classification of C is appropriate.

*Additional Uncertainties* - Uncertainties are covered in the primary ratings above.

**Table 21.** Effect of **Copper** on **Zinc**: Hematological Toxicity for Oral Exposure

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

*Direction of Interaction* - Copper is predicted to protect against the hematological toxicity of zinc, which is mediated through zinc's interference with copper absorption (ATSDR 1994). Excess copper is expected to protect against zinc-induced copper deficiency. Two intermediate duration oral studies in rats demonstrated a protective effect of excess copper against zinc-induced hemoglobinemia (Magee and Matrone 1960; Smith and Larson 1946).

*Mechanistic Understanding* - Excess oral zinc interferes with heme synthesis by interfering with copper absorption. The principal mechanism appears to involve zinc induction of metallothionein in the mucosa of the gastrointestinal tract. Copper has a higher affinity than does zinc for binding to metallothionein. The binding of copper to metallothionein reduces copper absorption into the blood and increases its fecal excretion as the cells of the intestinal mucosa containing copper-metallothionein are exfoliated (ATSDR 1994). Copper is an essential part of several enzymes including ceruloplasmin, which oxidizes ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is critically important to provide iron for hemoglobin synthesis (Friberg et al. 1986). Thus, excess copper may overcome the deficiency caused by excess zinc, protecting against the hematological effects of zinc. Studies of absorption in the vascularly perfused rat intestine following *in vivo* oral exposure at lower doses than used in the studies reviewed under toxicological significance showed no effect of copper on the absorption of zinc in this system (Ostreicher and Cousins 1985). Intermediate-duration oral studies of the effect of excess copper on the concentration of zinc in various tissues following coexposure to excess zinc tend to show no effect (Magee and Matrone 1960; Reinstein et al. 1984), except for a decrease in fetal hepatic zinc (Reinstein et al. 1984). A subcutaneous injection study (Chmielnicka et al. 1988), which showed an increased body burden of zinc when zinc and copper were injected together, is considered of questionable relevance to exposure by ingestion because injection circumvents homeostatic mechanisms and known points of interaction for oral exposure. Thus, mechanistic understanding predicts that an interaction, when it occurs, is likely to be less than additive. A classification of II is appropriate because there is little direct mechanistic data to support the prediction.

*Toxicological Significance* - Intermediate-duration oral coexposure to excess copper attenuated the decrease in blood hemoglobin caused by high oral zinc in rats in two studies, one using a relatively modest supplemental dose of copper (Smith and Larson 1946), and the other a higher dose (Magee and Matrone 1960). These results are toxicologically significant, and are supported by mechanistic considerations. A rating of A is considered appropriate.

*Additional Uncertainties* - At relatively low zinc and copper doses, zinc may not affect copper absorption.

**Table 22. Effect of Zinc on Copper: Hepatic Toxicity for Oral Exposure**

$$\text{BINWOE: } <\text{IB } (-1 \times 1.0 \times 0.71 = -0.71)$$

*Direction of Interaction* - The interaction is predicted to be less than additive. Coexposure to excess oral zinc reduced the accumulation of copper in the liver and increased fecal excretion of copper in animals given excess oral copper (Jenkins and Hidioglou 1989; Ogiso et al. 1974; Reinstein et al. 1984). In intermediate duration studies, excess zinc given orally protected against liver damage, growth depression, and lethality in calves due to excess oral copper (Jenkins and Hidioglou 1989), and excess zinc given intraperitoneally protected against depression of immune function in rats resulting from excess oral copper (Pocino et al. 1990).

*Mechanistic Understanding* - Zinc is expected to protect against the toxicity of excess copper by interfering with copper absorption. Zinc induces metallothionein in the mucosa of the gastrointestinal tract. Metallothionein has a higher affinity for copper than for zinc. Binding to metallothionein reduces copper absorption into the blood and increases its fecal excretion as the cells of the intestinal mucosa containing copper-metallothionein are exfoliated (ATSDR 1994). Exposure to excess zinc reduced the concentration of copper in the livers of rats coexposed to excess copper in an acute-duration oral study (Ogiso et al. 1974). Similar results were obtained in calves orally exposed to excess zinc and excess copper for an intermediate duration (Jenkins and Hidioglou 1989). Although coexposure to excess zinc and copper did not affect copper concentrations in the livers of pregnant rats in an intermediate duration oral study, copper levels in livers of the fetuses were decreased (Reinstein et al. 1984). Excess oral zinc increased the fecal excretion of copper in calves exposed to excess oral copper (Jenkins and Hidioglou 1989). Studies of absorption in the vascularly perfused rat intestine following *in vivo* oral exposure at relative low supplemental doses showed no effect of zinc on the absorption of copper in this system (Ostreicher and Cousins 1985). Overall, the mechanistic understanding indicates that the direction of interaction is likely to be less than additive, and warrants a classification of I.

*Toxicological Significance* - Two intermediate-duration studies in animals provide evidence that excess zinc alleviates the toxicity of excess copper. Excess oral zinc partially alleviated severe growth depression, and protected against lethality, liver damage (as evidenced by plasma transaminase levels), jaundice, and clinical signs of hemolysis in calves exposed to high levels of copper orally (Jenkins 1989; Jenkins and Hidioglou 1989). Intraperitoneal injections of zinc restored copper-inhibited immune responses to control levels in mice exposed orally to excess copper (Pocino et al. 1990). Zinc deficiency depresses immune function. These results, together with the mechanistic data showing a decrease in hepatic copper from coexposure to zinc, support a prediction of less than additive for the influence of zinc on copper toxicity. Although depression of immune function has not been established as an effect of excess copper, it is a known effect of zinc deficiency (ATSDR 1994), suggesting that the administration of copper may have caused zinc deficiency, which was ameliorated by intraperitoneal injection of zinc. Similarly, copper effects on growth may be related to copper-induced zinc deficiency. The protection by zinc against copper-related liver damage in calves is direct toxicological evidence of a less-than-additive interaction. Because of uncertainties regarding the relevance of results in calves, whose digestive systems are considerably different from humans, and the applicability of immune function parameters to copper hepatotoxicity, a classification of B is selected for toxicological significance.

*Additional Uncertainties* - At low supplemental zinc and copper, zinc may not affect copper absorption.

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

As presented previously, the mixture of lead, manganese, zinc, and copper was chosen as the subject of this interaction profile based on an analysis of the most frequently occurring binary mixtures in completed exposure pathways at hazardous waste sites. These metals are commonly found in soil. The exposure scenario of greatest concern for this mixture is long-term, low-level oral exposure. The components of this mixture vary in concentration and in proportion to each other from one hazardous waste site to another, and from 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 physiologically based pharmacokinetic/pharmacodynamic (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 few occupational and environmental exposure studies of the trinary mixture of lead, zinc, and copper are available, but are not adequate to serve as the basis for any conclusions regarding the toxicity of this submixture due to deficiencies in their design and inconsistencies in results across studies by the same group of investigators (Antonowicz et al. 1990; Araki et al. 1992, 1993a, 1993b; Murata and Araki 1991; Murata et al. 1993; Storm et al. 1994). In general, the effects seen during coexposure to lead, zinc, and copper were characteristic of lead toxicity. Whether coexposure to zinc and copper provided partial protection against these effects cannot be determined from the data.

Because suitable data, joint action models, and PBPK models are lacking for the complete mixture, and because there are two sensitive endpoints in common to components of the mixture, the recommended approach for the exposure-based assessment of joint toxic action of this mixture, consistent with ATSDR (2001a) guidance, is to estimate endpoint-specific hazard indexes for the neurotoxicity of lead and manganese and for the hematotoxicity of lead and zinc in order to screen for noncancer health hazards from potential additivity. The qualitative WOE method is used to assess the potential impact of interactions of the mixture components with regard to neurotoxicity and hematotoxicity. Copper hepatotoxicity is assessed using the hazard quotient for copper, and applying the qualitative WOE method to assess the potential impact of the other metals on copper's hepatotoxicity.

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 conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

The health guidance values to be used in estimating hazard quotients and endpoint-specific hazard indexes for these effects are provided in Table 23. More complete explanations of these values are provided in Chapter 1 and in the appendices. The values for lead are target-organ toxicity doses (TTDs), adopted because ATSDR (1999) does not recommend a specific health guideline value for lead. The TTDs are the CDC (1991) blood lead level of concern (see Appendix A). The value for manganese is the upper end of the ESADDI range, recommended as a guidance value by ATSDR (2000), and adopted as a TTD. The value for copper also is a TTD, developed because ATSDR (1990) did not recommend a specific health guideline value for copper, and because an RDA and UL have recently been derived by the Institute of Medicine (2001). The UL provides a reasonable provisional value to use until the toxicological profile for copper is updated.

**Table 23. MRLs and TTDs for Chronic Oral Exposure to Chemicals of Concern.**  
See Appendices A, B, C, and D for Details.

Endpoint	Chemical			
	Lead PbB $\mu\text{g}/\text{dL}$	Manganese ( $\text{mg}/\text{kg}/\text{day}$ )	Zinc ( $\text{mg}/\text{kg}/\text{day}$ )	Copper ( $\text{mg}/\text{kg}/\text{day}$ )
Neurological	10 <sup>a</sup>	0.07 <sup>b</sup>	NA	NA
Hematological	10 <sup>a</sup>	NA	0.3 <sup>c</sup>	NA
Hepatic	NA	NA	NA	0.14 <sup>d</sup>

<sup>a</sup>CDC (1991) PbB level of concern, adopted as TTD

<sup>b</sup>Upper end of ESADDI range, recommended as guidance value (ATSDR 2000), adopted as TTD

<sup>c</sup>Intermediate oral MRL, adopted as chronic MRL (ATSDR 1994)

<sup>d</sup>UL (Institute of Medicine 2001), adopted as TTD

NA = not applicable

BINWOE determinations for the critical effects of the mixture components-neurological (the critical effect of lead and manganese), hematological (the critical effect of zinc and a sensitive effect of lead), and hepatic (the critical effect of copper)-are summarized in Table 24. Of the 15 BINWOE determinations, two are greater than additive (for the effects of manganese on the neurological and hematological effects of lead). Six of the BINWOEs are less than additive, three are additive, and four are indeterminate.

For neurological effects, the BINWOE(s) for the effect of manganese on lead is greater than additive, for the effects of zinc on lead and of copper on lead are less than additive, and for lead on manganese is additive (no effect). Confidence in these assessments ranges from low to high-moderately. The effects of zinc and copper on manganese neurotoxicity are indeterminate. Thus, the predicted impact of interactions on the hazard for neurological effects will be to increase the hazard for mixtures in which manganese and lead predominate, and decrease the hazard for mixtures with relatively low manganese and higher zinc, copper, and lead (relative to health guidance values for these metals).

For hematological effects, the BINWOE(s) for the effect of manganese on lead is greater than additive, for the effects of zinc and copper on lead are less than additive, for the effect of lead on zinc is additive, and for the effect of copper on zinc is less than additive. Confidence in these assessments generally ranges from low-moderate to high-moderate, but for the effect of zinc on lead is high (<1A). Similar to the case for neurological effects, the predicted impact of interactions on the hazard for hematological effects will be to increase the hazard for mixtures in which manganese and lead predominate, and decrease the hazard for mixtures with relatively low manganese and higher zinc, copper, and lead (relative to health guidance values for these metals).

For hepatic effects, the BINWOE for the effect of lead on copper is additive with low confidence (=IIC), for the effect of zinc on copper is less than additive with high-moderate confidence (<IB), and for the effect of manganese on copper is indeterminate. The predicted impact of interactions on the hazard for hepatic effects will be to decrease the hazard for mixtures in which zinc and copper predominate.

**Table 24. Matrix of BINWOE Determinations for Neurological, Hematological, and Hepatic Toxicity of Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern**

		ON TOXICITY OF			
		Lead	Manganese	Zinc	Copper
E F F E C T O F	Lead		=IIICii (0) n	=IIB (0) h	=IIIC (0) p
	Manganese	>ICii (+0.25) n >IIB2ii (+0.31) h		? (0) h	? (0) p
	Zinc	<IB (-0.71) n <IA (-1.0) h	? (0) n		<IB (-0.71) p
	Copper	<IC (-0.32) n <IB (-0.71) h	? (0) n	<IIA (-0.71) h	

n = neurological, h = hematological, p = hepatic

The BINWOE determinations were explained in Section 2.3. No pertinent interactions data were available for the pairs of metals classified as indeterminate (?), and mechanistic information appeared inadequate or ambiguous, so indeterminate ratings were assigned to these pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (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).

Estimation of hazard quotients for lead is problematic because of the lack of an oral MRL or reference dose (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 1999). 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), which is appropriate for both neurological and hematological effects (Appendix A).

Proceeding with the estimation of endpoint-specific hazard indexes involves calculating these values for neurological effects and for hematological effects, as described in Section 2.3.2 and Figure 2 of ATSDR 2001a. For example, a hazard index for neurological effects of this mixture is calculated as follows:

$$HI_{NEURO} = \frac{E_{Pb}}{CDC\ PbB_{Pb\ NEURO}} + \frac{E_{Mn}}{TTD_{Mn\ NEURO}}$$

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 1999; CDC 1991), and  $E_{Mn}$  is the exposure to manganese (as the oral intake in the same units as the corresponding TTD, mg/kg/day), and  $TTD_{Mn\ NEURO}$  is the upper end of the ESADDI range, recommended as a guidance value by ATSDR (2000), and adopted as the TTD for neurological effects. A similar procedure is used to calculate the endpoint-specific hazard index for hematological effects.

If one or both 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 hazard quotient for the hepatotoxicity of copper exceeds one, it provides preliminary evidence that copper may constitute a health hazard. Coexposure to lead is predicted to have no effect, and coexposure to zinc may be protective against copper's hepatic toxicity. The impact of coexposure to manganese is

indeterminate. Depending on the magnitude of the hazard quotient for copper, and of exposure to the other components of the mixture, further evaluation using methods described by ATSDR (1992) may be needed.

## 4. Conclusions

No pertinent health effects data or PBPK models were available for the mixture of lead, manganese, zinc, and copper. 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, hematological, and hepatic effects. The recommendations for assessing the potential hazard to public health of the joint toxic action of this mixture include the estimation of endpoint-specific hazard indexes for neurological effects of lead and manganese and for hematological effects of lead and zinc. 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 indexes. The hazard quotient for copper's hepatic toxicity (critical effect for oral exposure) is estimated separately and the qualitative WOE is used to predict the impact of interactions on this hazard quotient. The impact of interactions on the endpoint-specific hazard indexes and the copper hazard quotient are discussed below in terms of the WOE approach.

*Neurological:* The predicted direction of joint toxic action for neurological effects, an endpoint common to two components, is greater than additive for the effect of manganese on lead, less than additive for the effects of zinc and copper on lead, additive (no effect) for the effect of lead on manganese, and indeterminate for the effects of zinc and copper on manganese. The combined WOE score (sum of the BINWOE scores) is -0.78, indicating that the potential health hazard may be less than estimated by the endpoint-specific hazard index for neurological effects, particularly for waste sites with relatively high hazard quotients for lead, copper, and zinc, and a lower hazard quotient for manganese. The indeterminate ratings for two of the BINWOEs (zinc and copper on manganese) are a source of uncertainty in assessments where manganese accounts for a great portion of the apparent neurological hazard.

*Hematological:* The potential health hazard for hematological effects is likely to be lower than indicated by the endpoint-specific hazard index for mixtures where lead, zinc, and copper predominate, because three of the BINWOEs for combinations of these metals were less than additive with moderate to high confidence, and the remaining one was additive. The BINWOE for manganese on lead was greater than additive with low-moderate confidence, for lead on manganese was additive, and for manganese on zinc was indeterminate. The combined WOE score is -2.11.

*Hepatic:* The predicted effects of the other mixture components on the hepatic toxicity of copper are less than additive for zinc with high-moderate confidence (-0.71), additive for lead (0), and indeterminate (0) for manganese. Thus, the available data indicate the potential health hazard for hepatic effects may be less than predicted by the hazard quotient for mixtures where zinc and copper predominate. There is uncertainty with regard to the potential effect of manganese due to the lack of pertinent information.

## 5. List of References

- Abdel-Mageed AB, Oehme FW. 1990. A review on biochemical roles, toxicity and interactions of zinc, copper and iron: IV. Interactions. *Vet Hum Toxicol* 32:456-458.
- \*Abdulla M, Svensson S, Haeger-Aronsen B. 1979. Antagonistic effects of zinc and aluminum on lead inhibition of  $\delta$ -aminolevulinic acid dehydratase. *Arch Environ Health* 34(6):464-469.
- Acuna-Castillo C, Morales B, Huidobro-Toro J. 1999. Zinc and copper modulate differentially the P2X<sub>4</sub> receptor. *J Neurochem* 74:1529-1537.
- Alexander BH, Checkoway H, Costa-Mallen P, et al. 1998. Interaction of blood lead and  $\delta$ -aminolevulinic acid dehydratase genotype on markers of heme synthesis and sperm production in lead smelter workers. *Environ Health Perspect* 106:213-216.
- \*Anderson LA, Hakojarvi SL, Boudreau SK. 1998. Zinc acetate treatment in Wilson's disease. *Ann Pharmacother* 32:78-87.
- \*Antonowicz J, Andrzejak R, Smolik R. 1990. Influence of heavy metal mixtures on erythrocyte metabolism. *Int Arch Occup Environ Health* 62:195-198.
- Anwer J, Ali S, Mehrotra NK. 1988. Antagonistic effect of zinc in lead treated developing chick embryos. *Drug Chem Toxicol* 11(1):85-95.
- \*Araki S, Murata K, Uchida E, et al. 1993a. Radial and median nerve conduction velocities in workers exposed to lead, copper, and zinc: A follow-up study for 2 years. *Environ Res* 61:308-316.
- \*Araki S, Murata K, Yokoyama K, et al. 1992. Auditory event-related potential (P300) in relation to peripheral nerve conduction in workers exposed to lead, zinc, and copper: Effects of lead on cognitive function and central nervous system. *Am J Ind Med* 21(4):539-547.
- \*Araki S, Yokoyama K, Murata K. 1993b. Assessment of the effects of occupational and environmental factors on all faster and slower large myelinated nerve fibers: A study of the distribution of nerve conduction velocities. *Environ Res* 62:325-332.
- Ashraf MH, Fosmire GJ. 1985. Effects of marginal zinc deficiency on subclinical lead toxicity in the rat neonate. *J Nutr* 115:334-346.
- \*ATSDR. 1990. Toxicological profile for copper. Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- \*ATSDR. 1992. Public health assessment guidance manual. Atlanta, GA: Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services.
- \*ATSDR. 1994. Toxicological profile for zinc. Atlanta, GA: Agency for Toxic Substances and Disease Registry

---

\*Cited in text

- \*ATSDR. 1999. Toxicological profile for lead. Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- \*ATSDR. 2000. Toxicological profile for manganese. Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- \*ATSDR. 2001a. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- \*ATSDR. 2001b. Guidance manual for the preparation of an interaction profile. Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- Axelsson O, Wingren G. 1992. Epidemiological studies of occupational cancer as related to complex mixtures of trace elements. *J Trace Elem Exp Med* 5(2):86.
- \*Baldwin M, Mergler D, Larribe F, et al. 1999. Bioindicator and exposure data for a population based study of manganese. *Neurotoxicology* 20(2-3):343-354.
- Baranowska-Dutkiewicz B, Dutkiewicz T. 1991. Evaluation of simultaneous industrial and environmental exposure to metals. *Sci Total Environ* 101:149-151.
- \*Barceloux DG. 1999. Copper. *Clin Toxicol* 37(2):217-230.
- Basavaraju SR, Jones TD. 1998. Atherosclerotic risks from chemicals: Part I. Toxicological observations and mechanisms of atherosclerosis. *Arch Environ Contam Toxicol* 35:152-164.
- \*Batra N, Nehru B, Bansal MP. 1998. The effect of zinc supplementation on the effects of lead on the rat testis. *Reprod Toxicol* 12(5):535-540.
- \*Bebe FN, Panemangalore M. 1996. Modulation of tissue trace metal concentrations in weanling rats fed different levels of zinc and exposed to oral lead and cadmium. *Nutr Res* 16(8):1369-1380.
- Beck BD. 1992. Symposium overview: An update on exposure and effects of lead. *Fundam Appl Toxicol* 18:1-16.
- Bernstein IA, Vaughan FL. 1999. Cultured keratinocytes in in vitro dermatotoxicological investigation: A review. *J Toxicol Environ Health* 2(Part B):1-30.
- Blumenthal S, Lewand D, Sochanik A, et al. 1994. Inhibition of Na<sup>+</sup>-glucose cotransport in kidney cortical cells by cadmium and copper: Protection by zinc. *Toxicol Appl Pharmacol* 129:177-187.
- \*Border EA, Cantrell AC, Kilroe-Smith TA. 1976. The *in vitro* effect of zinc on the inhibition of human  $\delta$ -aminolevulinic acid dehydratase by lead. *Br J Ind Med* 33:85-87.
- Bremner I, Beattie JH. 1995. Copper and zinc metabolism in health and disease: Speciation and interactions. *Proc Nutr Soc* 54:489-499.
- \*Brewer GJ. 2000. Recognition, diagnosis, and management of Wilson's disease. *Proc Soc Exp Biol Med* 223:39-46.

\*Brewer GJ, Dick RD, Schall W, et al. 1992. Use of zinc acetate to treat copper toxicosis in dogs. *J Am Vet Med Assoc* 201(4):564-568.

Brewer GJ, Hill GM, Dick RD, et al. 1985. Interactions of trace elements: Clinical significance. *J Am Coll Nutr* 4:33-38.

Bruinink A, Faller P, Sidler C, et al. 1998. Growth inhibitory factor and zinc affect neural cell culture in a tissue specific manner. *Chem Biol Interact* 115:167-174.

Bush AI, Pettingell WH, Multhaup G, et al. 1994. Rapid induction of alzheimer A $\beta$  amyloid formation by zinc. *Science* 265:1464-1467.

\*Bushnell PJ, Levin ED. 1983. Effects of zinc deficiency on lead toxicity in rats. *Neurobehav Toxicol Teratol* 5:283-288.

Camerino D, Cassitto MG, Gilioli R. 1993. Prevalence of abnormal neurobehavioral scores in populations exposed to different industrial chemicals. *Environ Res* 61:251-257.

Cantrell AC, Kilroe-Smith TA, Simoes MM, et al. 1977. The effect of zinc and pH on the behaviour of aminolevulinic acid dehydratase activity in baboons exposed to lead. *Br J Ind Med* 34:110-113.

Carfagna MA, Ponsler GD, Muhoberac BB. 1995. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. *Chem Biol Interact* 100:53-65.

Carpentieri U, Myers J, Daeschner CW, et al. 1988. Effects of iron, copper, zinc, calcium, and magnesium on human lymphocytes in culture. *Biol Trace Elem Res* 16:165-176.

\*CDC. 1991. Preventing lead poisoning in young children. Atlanta, GA: U.S. Department of Health and Human Services. Public Health Service. Center for Disease Control and Prevention.

\*Cerklewski FL. 1979. Influence of dietary zinc on lead toxicity during gestation and lactation in the female rat. *J Nutr* 109:1703-1709.

Cerklewski FL. 1984. Post-absorptive effect of increased dietary zinc on toxicity and removal of tissue lead in rats. *J Nutr* 114:550-554.

\*Cerklewski FL, Forbes RM. 1976. Influence of dietary zinc on lead toxicity in the rat. *J Nutr* 106:689-696.

\*Cerklewski FL, Forbes RM. 1977. Influence of dietary copper on lead toxicity in the young male rat. *J Nutr* 107:143-146.

Chan S, Gerson B, Subramaniam S. 1998. The role of copper, molybdenum, selenium, and zinc in nutrition and health. *Toxicology* 18(4):673-685.

\*Chandra SV, Mohd M, Saxena DK, et al. 1981. Behavioral and neurochemical changes in rats simultaneously exposed to manganese and lead. *Arch Toxicol* 49:49-56.

- \*Chandra SV, Murthy RC, Saxena DK. 1983. Effects of pre- and postnatal combined exposure to Pb and Mn on brain development in rats. *Ind Health* 21:273-279.
- Cheek DB, Smith RM, Spargo RM, et al. 1981. Zinc, copper and environmental factors in the aboriginal peoples of the North West. *Aust N Z J Med* 11:508-512.
- \*Chiba M, Kikuchi M. 1984a. The *in vitro* effects of zinc and manganese on  $\delta$ -aminolevulinic acid dehydratase activity inhibited by lead or tin. *Toxicol Appl Pharmacol* 73:388-394.
- \*Chiba M, Kikuchi M. 1984b. The *in vivo* effects of manganese and zinc on  $\delta$ -aminolevulinic acid dehydratase activity inhibited by lead. *Toxicol Lett* 20:143-147.
- \*Chisolm JJ Jr. 1981. Dose-effect relationships for lead in young children: Evidence in children for interactions among lead, zinc, and iron. In: Lynam DR, Piantanida LG, Cole JF, eds. *Environmental Lead: Proceedings of the 2nd International Symposium on Environmental Lead Research*. Ecotoxicology and environmental quality series. NY: Academic Press, 1-7.
- \*Chmielnicka J, Komsta-Szumaska E, Zareba G. 1988. Effects of interaction between  $^{65}\text{Zn}$ , cadmium, and copper in rats. *Biol Trace Elem Res* 17:285-292.
- \*Church HJ, Day JP, Braithwaite RA, et al. 1993a. Binding of lead to a metallothionein-like protein in human erythrocytes. *J Inorg Biochem* 49(1):55-68.
- \*Church HJ, Day JP, Braithwaite RA, et al. 1993b. The speciation of lead in erythrocytes in relation to lead toxicity. Case studies of two lead-exposed workers [Abstract]. *Neurotoxicology* 14(2-3):359-364.
- Colt JS, Blair A. 1998. Parental occupational exposures and risk of childhood cancer. *Environ Health Perspect* 106(Suppl. 3):909-925.
- Crow JP, Sampson JB, Zhuang Y, et al. 1997. Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite. *J Neurochem* 69:1936-1944.
- Cuajungco MP, Goldstein LE, Nunomura A, et al. 2000. Evidence that the  $\beta$ -amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of A $\beta$  by zinc. *J Biol Chem* 275(26):19439-19442.
- Cunnane SC. 1981. Zinc and copper interact antagonistically in the regulation of linoleic acid metabolism. *Prog Lipid Res* 20:601-603.
- Dameron CT, Harrison MD. 1998. Mechanisms for protection against copper toxicity. *Am J Clin Nutr* 67:1091S-1097S.
- Davis CD, Feng Y. 1998. Dietary copper, manganese and iron affect the formation of aberrant crypts in colon of rats administered 3,2'-dimethyl-4-aminobiphenyl. *J Nutr* 129:1060-1067.
- Davis JR, Avram MJ. 1977. A comparison of the stimulatory effects of cadmium and zinc on normal and lead-inhibited human erythrocytic  $\delta$ -aminolevulinic acid dehydratase activity *in vitro*. *Toxicol Appl Pharmacol* 44:181-190.

\*Davis JR, Avram MJ. 1978. A comparison of the stimulatory effects of cadmium and zinc on normal and lead-inhibited human erythrocytic  $\delta$ -Aminolevulinic acid dehydratase activity *in vitro*. *Toxicol Appl Pharmacol* 44:181-190.

\*Delves HT, Clayton BE, Bicknell J. 1973. Concentration of trace metals in the blood of children. *Brit J Prev Soc Med* 27:100-107.

Dieter MP. 1993. Fate, transport, and interactions of metals. *Environ Health Perspect* 101(4):344-345.

Domingo JL, Gomez M, Jones MM. 1998. Concurrent administration of D-penicillamine and zinc has no advantages over the use of either single agent on copper excretion in the rat. *Toxicology* 126:195-201.

Dorward A, Yagminas AP. 1994. Activity of erythrocyte  $\delta$ -aminolevulinic acid dehydratase in the female cynomolgus monkey (*macaca fascicularis*): kinetic analysis in control and lead-exposed animals. *Comp Biochem Physiol* 108B(2):241-252.

Dutkiewicz B, Dutkiewicz T, Milkowska G. 1979. The effect of mixed exposure to lead and zinc on ALA level in urine. *Int Arch Occup Environ Health* 42:341-348.

DuVal GE, Fowler BA. 1989. Preliminary purification and characterization studies of a low molecular weight, high affinity cytosolic lead-binding protein in rat brain. *Biochem Biophys Res Commun* 159:177-184.

\*El-Gazzar RM, Finelli VN, Boiano J, et al. 1978. Influence of dietary zinc on lead toxicity in rats. *Toxicol Lett* 1:227-234.

\*Emsley CL, Gao S, Li Y, et al. 2000. Trace element levels in drinking water and cognitive function among elderly Chinese. *Am J Epidemiol* 151:913-920.

\*EPA. 1986. Air quality criteria for lead. Vol. IV. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. EPA 600/8-83-028dF, 12-34-12-37.

Farant JP, Wigfield DC. 1987. Interaction of divalent metal ions with normal and lead-inhibited human erythrocytic porphobilinogen synthase *in vitro*. *Toxicol Appl Pharmacol* 89:9-18.

Farant JP, Wigfield DC. 1990. The effects of copper, zinc, mercury, and cadmium on rabbit erythrocytic porphobilinogen synthase *in vivo*. *J Anal Toxicol* 14:222-226.

Ferm VH. 1972. The teratogenic effects of metals on mammalian embryos. *Adv Teratol* 5:51-75.

Finelli VN. 1977. Lead, zinc and  $\delta$ -aminolevulinic acid dehydratase. In: Lee SD, ed. *Biochemical effects of environmental pollutants*. Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 351-363.

Finelli VN, El-Gazzar RM. 1977. Interaction of lead and zinc on the prothrombin activity in rats. *Toxicol Lett* 1:33-39.

- \*Finelli VN, Klauder DS, Karaffa MA, et al. 1975. Interaction of zinc and lead and  $\delta$ -aminolevulinatase dehydratase. *Biochem Biophys Res Commun* 65(1):303-311.
- Fischer PWF, Giroux A, L'Abbe MR. 1981. The effect of dietary zinc on intestinal copper absorption. *Am J Clin Nutr* 34:1670-1675.
- Flanagan PR, Chamberlain MJ, Valberg LS. 1982. The relationship between iron and lead absorption in humans. *Am J Clin Nutr* 36:823-829.
- Fleming DEB, Chettle DR, Wetmur JG, et al. 1998. Effect of the  $\delta$ -aminolevulinatase dehydratase polymorphism on the accumulation of lead in bone and blood in lead smelter workers. *Environ Res* 77:49-61.
- Flora SJS, Bhattacharya R, Sachan SRS. 1994. Dose-dependent effects of zinc supplementation during chelation of lead in rats. *Pharmacol Toxicol* 74:330-333.
- \*Flora SJS, Coulombe RA, Sharma RP, et al. 1989a. Influence of dietary protein deficiency on lead-copper interaction in rats. *Ecotoxicol Environ Saf* 18:75-82.
- \*Flora SJS, Jain VK, Behari JR, et al. 1982. Protective role of trace metals in lead intoxication. *Toxicol Lett* 13:51-56.
- \*Flora SJS, Kumar D, Gupta D. 1991. Interaction of zinc, methionine or their combination with lead at gastrointestinal or post-absorptive level in rats. *Pharmacol Toxicol* 68:3-7.
- \*Flora SJS, Singh S, Tandon SK. 1989b. Thiamine and zinc in prevention or therapy of lead intoxication. *J Int Med Res* 17:68-75.
- \*Fowler BA. 1998. Roles of lead-binding proteins in mediating lead bioavailability. *Environ Health Perspect* 106(Suppl. 6):1585-1587.
- Fowler BA, Oskarsson A, Woods JS. 1987. Metal- and metalloid-induced porphyrinurias. *Ann NY Acad Sci* 514:172-182.
- \*Friberg L, Nordberg GF, Vouk VB. 1986. Handbook on the toxicology of metals. Vol. II: Specific Metals. 2<sup>nd</sup> ed. Amsterdam: Elsevier Science Publishers B.V., 242.
- Fujita H, Yamamoto R, Sato K, et al. 1985. *In vivo* regulation of  $\delta$ -aminolevulinatase dehydratase activity. *Toxicol Appl Pharmacol* 77:66-75.
- Gerhardsson L, Nordberg GF. 1993. Lung cancer in smelter workers -Interactions of metals as indicated by tissue levels. *Scand J Work Environ Health* 19(Suppl.1):90-94.
- Gochfeld M. 1997. Factors influencing susceptibility to metals. *Environ Health Perspect* 105(Suppl.4):817-822.
- Goering PL. 1993. Lead-protein interactions as a basis for lead toxicity. *Neurotoxicology* 14:45-60.

Goering PL, Fowler BA. 1985. Mechanisms of renal lead-binding protein protection against lead-inhibition of  $\delta$ -aminolevulinic acid dehydratase. *J Pharmacol Exp Ther* 234:365-371.

Goering PL, Fowler BA. 1987a. Kidney zinc-thionein regulation of  $\delta$ -aminolevulinic acid dehydratase inhibition by lead. *Arch Biochem Biophys* 253(1):48-55.

\*Goering PL, Fowler BA. 1987b. Metal constitution of metallothionein influences inhibition of  $\delta$ -aminolevulinic acid dehydratase (porphobilin synthase) by lead. *Biochem J* 245:339-345.

Goering PL, Fowler BA. 1987c. Regulatory roles of high-affinity metal-binding proteins in mediating lead effects on  $\delta$ -aminolevulinic acid dehydratase. *Ann N Y Acad Sci* 514:235-247.

\*Gorell JM, Johnson CC, Rybicki BA, et al. 1997. Occupational exposure to metals as risk factors for Parkinson's disease. *Neurology* 48:650-658.

\*Gorell JM, Johnson CC, Rybicki BA, et al. 1999. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicology* 20(2-3):239-248.

Goyer RA. 1995. Nutrition and metal toxicity. *Am J Clin Nutr* 61(Suppl.):646S-650S.

Goyer RA. 1997. Toxic and essential metal interactions. *Annu Rev Nutr* 17:37-50.

\*Grant-Frost DR, Underwood EJ. 1958. Zinc toxicity in the rat and its interrelation with copper. *Austral J Exp Biol* 36:339-346.

Guilarte TR, Miceli RC. 1992. Age-dependent effects of lead on [ $^3$ H]MK-801 binding to the NMDA receptor-gated ionophore: In vitro and in vivo studies. *Neurosci Lett* 148:27-30.

Haeger-Aronsen B, Schutz A, Abdulla M. 1976. Antagonistic effect in vivo of zinc on inhibition of  $\delta$ -aminolevulinic acid dehydratase by lead. *Arch Environ Health* 31(4):215-220.

Haghdoust NR, Newman LM, Johnson EM. 1997. Multiple chemical exposures: Synergism vs. individual exposure levels. *Reprod Toxicol* 11:9-27.

Hanas JS, Rodgers JS, Bantle JA, et al. 1999. Lead inhibition of DNA-binding mechanism of Cys<sub>2</sub>His<sub>2</sub> zinc finger proteins. *Pharm Exp Ther* 56:982-988.

\*Hardman JF, Limbird LE, eds. 1996. Goodman & Gilman's the pharmacological basis of therapeutics. New York, NY: McGraw-Hill, 120, 123-124, 252.

Hashemzadeh-Gargari H, Guilart TR. 1999. Divalent cations modulate *N*-methyl-D-aspartate receptor function at the glycine site. *J Pharmacol Exp Ther* 290:1356-1362.

\*Hietanen E, Aitio A, Koivusaari U, et al. 1982. Tissue concentrations and interaction of zinc with lead toxicity in rabbits. *Toxicology* 25:113-127.

\*Hsu FS, Krook L, Pond WG, et al. 1975. Interactions of dietary calcium with toxic levels of lead and zinc in pigs. *J Nutr* 105:112-118.

Hussain T, Ali MM, Chandra SV. 1987. The combined effect of  $Pb^{2+}$  and  $Mn^{2+}$  on monoamine uptake and  $Na^+$ ,  $K^+$ -ATPase in striatal synaptosomes. *J Appl Toxicol* 7(4):277-280.

Hutton M. 1983. The effects of environmental lead exposure and *in vitro* zinc on tissue  $\delta$ -aminolevulinic acid dehydratase in urban pigeons. *Comp Biochem Physiol* 74C(2):441-446.

\*Institute of Medicine. 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 7-1-7-27. [Http://www.nap.edu/books/0309072794/html/](http://www.nap.edu/books/0309072794/html/). December 19, 2001.

IRIS. 2001. Integrated Risk Information System. U.S. Environmental Protection Agency. [Http://www.epa.gov/iris/subst/index.htm](http://www.epa.gov/iris/subst/index.htm). April 17, 2001.

\*Jehan ZS, Motlag DB. 1995. Metal induced changes in the erythrocyte membrane of rats. *Toxicol Lett* 78:127-133.

\*Jenkins KF. 1989. Effect of copper loading of preruminant calves on intracellular distribution of hepatic copper, zinc, iron, and molybdenum. *J Dairy Sci* 72:2346-2350.

\*Jenkins KJ, Hidirolou M. 1989. Tolerance of the calf for excess copper in milk replacer. *J Dairy Sci* 72(1):150-156.

\*Johnson MA, Flagg EW. 1986. Effects of sucrose and cornstarch on the development of copper deficiency in rats fed high levels of zinc. *Nutr Res* 6:1307-1319.

\*Johnson NE, Tenuta K. 1979. Diets and lead blood levels of children who practice pica. *Environ Res* 18:369-376.

\*Joselow MM, Tobias E, Koehler R, et al. 1978. Manganese pollution in the city environment and its relationship to traffic density. *Am J Public Health* 68:557-560.

Kaiser J. 1994. Alzheimer's: Could there be a zinc link? *Science* 265:1365.

Kaji T, Takata M, Miyahara T, et al. 1990. Interaction of zinc with cadmium and copper on ossification of embryonic chick bone in tissue culture. *Arch Environ Contam Toxicol* 19:653-656.

\*Kalia K, Chandra SV, Viswanathan PN. 1984. Effect of  $^{54}Mn$  and lead interaction on their binding with tissue proteins: *In vitro* studies. *Ind Health* 22:207-218.

Kang YJ. 1999. The antioxidant function of metallothionein in the heart. *Proc Soc Exp Biol Med* 222:263-273.

Kasarskis EJ. 1990. Metal interactions of lead with zinc in the postnatally lead exposed rat. *Neurotoxicol Teratol* 12(5):562.

\*Kies C, Ip SW. 1990. Lead bioavailability to humans from diets containing constant amounts of lead: Impact of supplemental copper, zinc and iron. *Trace Subst Environ Health* 24:177-184.

- \*Kies C, Umoren J. 1989. Inhibitors of copper bioutilization: Fiber, lead, phytate and tannins. *Adv Exp Med Biol* 258:81-93.
- \*Klauder DS, Petering HG. 1975. Protective value of dietary copper and iron against some toxic effects of lead in rats. *Environ Health Perspect* 12:77-80.
- \*Klauder DS, Petering HG. 1977. Anemia of lead intoxication. A role for copper. *J Nutr* 107:1779-1785.
- \*Klauder DS, Murthy L, Petering HG. 1972. Effect of dietary intake of lead acetate on copper metabolism in male rats. *Trace Subst Environ Health* 6:131-136.
- Koizumi S, Yamada H, Suzuki K, et al. 1992. Zinc-specific activation of a HeLa cell nuclear protein which interacts with a metal responsive element of the human metallothionein-II<sub>A</sub> gene. *Eur J Biochem* 210:555-560.
- Kubena KS, McMurray DN. 1996. Nutrition and the immune system: A review of nutrient-nutrient interactions. *J Am Diet Assoc* 96:1156-1164.
- \*Lasley SM, Gilbert ME. 1999. Lead inhibits the rat *N*-methyl-D-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. *Toxicol Appl Pharmacol* 159:224-233.
- \*Lauwerys R, Roels H, Buchet JP, et al. 1983. The influence of orally-administered vitamin C or zinc on the absorption of and the biological response to lead. *J Occup Med* 25(9):668-678.
- Levander OA. 1979. Lead toxicity and nutritional deficiencies. *Environ Health Perspect* 29:115-125.
- Lin JL, Tan DT, Hsu KH, et al. 2001. Environmental lead exposure and progressive renal insufficiency. *Arch Intern Med* 161:264-271.
- \*Liu J, Kershaw WC, Klaassen CD. 1991. The protective effect of metallothionein on the toxicity of various metals in rat primary hepatocyte culture. *Toxicol Appl Pharmacol* 197:27-34.
- \*Magee AC, Matrone G. 1960. Studies on growth, copper metabolism and iron metabolism of rats fed high levels of zinc. *J Nutr* 72:233-242.
- \*Mahaffey KR. 1985. Factors modifying susceptibility to lead toxicity. In: Mahaffey KR, ed. *Dietary and environmental lead. Human health effects*. Cincinnati, OH: Elsevier Science Publishers B.V., 373-419.
- Mahaffey KR, Capar SG, Gladen BC, et al. 1981. Concurrent exposure to lead, cadmium, and arsenic: Effects on toxicity and tissue metal concentrations in the rat. *J Lab Clin Med* 98:463-481.
- \*Malhotra KM, Shukla GS, Chandra SV. 1982. Neurochemical changes in rats coexposed to lead and copper. *Arch Toxicol* 49:331-336.
- \*Markowitz ME, Rosen JF. 1981. Zinc (Zn) and copper (Cu) metabolism in CaNa<sub>2</sub>EDTA-treated children with plumbism. *Pediat Res* 15:635.

- Marshall EG, Gensburg LJ, Geary NS, et al. 1997. Maternal residential exposure to hazardous wastes and risk of central nervous system and musculoskeletal birth defects. *Arch Environ Health* 52(6):416-425.
- \*Mauras Y, Allain P. 1979. Inhibition of delta-aminolevulinic acid dehydratase in humans by lead and activation by zinc or cysteine. *Enzyme* 24:181-187.
- Milanino R, Deganello A, Marrella M, et al. 1992. Oral zinc as initial therapy in Wilson's disease: Two years of continuous treatment in a 10-year-old child. *Acta Paediatr* 81:163-166.
- Miller GD, Massaro TF, Massaro EJ. 1990. Interactions between lead and essential elements: A review. *Neurotoxicology* 11:99-120.
- \*Miniuk K, Moniuszko-Jakoniuk JM, Kulikowska E, et al. 1989. The interactions of copper, lead and ethanol in rats: Effects on some biochemical parameters of blood. *Pol J Pharmacol Pharm* 41:273-280.
- Mizutani T, Yamamoto K, Tajima K. 1983. Isotope effects on the metabolism and pulmonary toxicity of butylated hydroxytoluene in mice by deuteration of the 4-methyl group. *Toxicol Appl Pharmacol* 69:283-290.
- Monteiro HP, Bechara EJH, Abdalla DSP. 1991. Free radicals involvement in neurological porphyrias and lead poisoning. *Mol Cell Biochem* 103:73-83.
- \*Murata K, Araki S. 1991. Autonomic nervous system dysfunction in workers exposed to lead, zinc, and copper in relation to peripheral nerve conduction: A study of R-R interval variability. *Am J Ind Med* 20(5):663-671.
- \*Murata K, Araki S, Yokoyama K, et al. 1993. Assessment of central, peripheral, and autonomic nervous system functions in lead workers: Neuroelectrophysiological studies. *Environ Res* 61:323-326.
- Murgueytio AM, Evans RG, Sterling D, et al. 1998. Behaviors and blood lead levels of children in a lead-mining area and a comparison community. *Environ Health*:14-20.
- Myloie AA, Boseman A, Kyle J. 1986. Metabolic interactions between lead and copper in rats ingesting lead acetate. *Biol Trace Elem Res* 9:221-231.
- \*NRC. 1989. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press. National Research Council.
- \*NRC. 1995. Nutrient requirements of laboratory animals. 4<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council, 11-102.
- \*NRC. 1998. Nutrient requirements of laboratory animals. 4<sup>th</sup> ed. National Academy Press. National Research Council. 47-49, 110-111, 115-116, 121-122.
- \*Ogiso T, Moriyama K, Sasaki S, et al. 1974. Inhibitory effect of high dietary zinc on copper absorption in rats. *Chem Pharm Bull* 22:55-60.

- Ogiso T, Ogawa N, Miura T. 1979. Inhibitory effect of high dietary zinc on copper absorption in rats. II. Binding of copper and zinc to cytosol proteins in the intestinal mucosa. *Chem Pharm Bull* 27(2):515-521.
- \*Ostreicher P, Cousins RJ. 1985. Copper and zinc absorption in the rat: Mechanism of mutual antagonism. *J Nutr* 115:159-166.
- Panemangalore M. 1993. Interaction among zinc, copper and cadmium in rats: Effects of low zinc and copper diets and oral cadmium exposure. *J Trace Elem Exp Med* 6(3):125-139.
- \*Papaioannou R, Sohler A, Pfeiffer CC. 1978. Reduction of blood lead levels in battery workers by zinc and vitamin C. *J Orthomol Psychiatr* 7(2):94-106.
- Platt B, Busselberg D. 1994. Combined actions of  $Pb^{2+}$ ,  $Zn^{2+}$ , and  $Al^{3+}$  on voltage-activated calcium channel currents. *Cell Mol Neurobiol* 14(6):831-840.
- \*Pocino M, Malave I, Baute L. 1990. Zinc administration restores the impaired immune response observed in mice receiving excess copper by oral route. *Immunopharmacol Immunotoxicol* 12(4):697-713.
- Pohl HR, Roney N, Fay M, et al. 1999. Site-specific consultation for a chemical mixture. *Toxicol Ind Health* 15:470-479.
- Poirier J, Donaldson J, Barbeau A. 1985. The specific vulnerability of the substantia nigra to MPTP is related to the presence of transition metals. *Biochem Biophys Res Commun* 128(1):25-33.
- \*Prasad AS, Brewer GJ, Schoemaker EB, et al. 1978. Hypocupremia induced by zinc therapy in adults. *JAMA* 240:2166-2168.
- Qian Y, Mikeska G, Harris ED, et al. 1999. Effect of lead exposure and accumulation on copper homeostasis in cultured C6 rat glioma cells. *Toxicol Appl Pharmacol* 158:41-49.
- Quinn MR, Harris CL. 1995. Lead inhibits  $Ca^{2+}$ -stimulated nitric oxide synthase activity from rat cerebellum. *Neurosci Lett* 196:65-68.
- Quintanilla-Bega B, Smith DR, Kahng MW, et al. 1995. Lead-binding proteins in brain tissue of environmentally lead-exposed humans. *Chem Biol Interact* 98:193-209.
- \*Reinstein NH, Lonnerdal B, Keen CL, et al. 1984. Zinc-copper interactions in the pregnant rat: Fetal outcome and maternal and fetal zinc, copper and iron<sup>1,2</sup>. *J Nutr* 114:1266-1279.
- Rifkin RJ. 1965. *In vitro* inhibition of  $Na^{+}$ - $K^{+}$  and  $Mg^{2+}$  ATPases by mono, di and trivalent cations. *Proc Soc Exp Biol Med* 120:802-804.
- Rodriguez RE, Kasprzak KS. 1989. Antagonists to metal carcinogens. *J Am Coll Toxicol* 8(7):1265-1269.

- \*Roels H, Lauwerys R, Buchet JP, et al. 1987. Epidemiological survey among workers exposed to manganese: Effects of lung, central nervous system, and some biological indices. *Am J Emerg Med* 11:307-327.
- Rosen JF. 1981. Zinc (Zn) and copper (Cu) metabolism in CaNa<sub>2</sub>EDTA-treated children with plumbism. *Pediatr Res* 15:635.
- Rowles TK, Womac C, Bratton GR, et al. 1989. Interaction of lead and zinc in cultured astroglia. *Metab Brain Dis* 4(3):187-200.
- Roughead ZK, Johnson LK, Hunt JR. 1999. Dietary copper primarily affects antioxidant capacity and dietary iron mainly affects iron status in a surface response study of female rats fed varying concentrations of iron, zinc and copper. *J Nutr* 129:1368-1376.
- Sagripanti JL, Goering PL, Lamanna A. 1991. Interaction of copper with DNA and antagonism by other metals. *Toxicol Appl Pharmacol* 110:477-485.
- \*Sandstead HH, Frederickson CJ, Penland JG. 2000. History of zinc as related to brain function. *J Nutr* 130:496S-502S.
- Sandstrom B. 1998. Toxicity considerations when revising the nordic nutrition recommendations. *J Nutr* 128:372-374.
- Saxena DK, Murthy RC, Singh C, et al. 1989. Zinc protects testicular injury induced by concurrent exposure to cadmium and lead in rats. *Res Commun Chem Pathol Pharmacol* 64:317-329.
- Schmitt N, Anderson TW, Philion JJ, et al. 1996. Could zinc help protect children from lead poisoning? *Can Med Assoc J* 154(1):13-14.
- Schulte S, Muller WE, Priedberg KD. 1995. In vitro and in vivo effects of lead on specific <sup>3</sup>H-PN2---110 binding to dihydropyridine receptors in the frontal cortex of the mouse brain. *Toxicology* 97:113-121.
- Shackelford ME, Collins TFX, Black TN, et al. 1994. Mineral interactions in rats fed ain-76A diets with excess calcium. *Food Chem Toxicol* 32(3):255-263.
- Shafiq-ur-Rehman, Chandra O. 1984. Regional interrelationships of zinc, copper, and lead in the brain following lead intoxication. *Bull Environ Contam Toxicol* 32:157-165.
- Sharonova IN, Vorobjev VS, Haas HL. 2000. Interaction between copper and zinc at GABA<sub>A</sub> receptors in acutely isolated cerebellar Purkinje cells of the rat. *Br J Pharmacol* 130:851-856.
- Shaw WHR. 1961. Cation toxicity and the stability of transition-metal complexes. *Nature* 192:754-755.
- \*Shukla GS, Chandra SV. 1987. Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. *Arch Environ Contam Toxicol* 16:303-310.

\*Simons TJB. 1997. Interactions between lead and metals in cellular toxicity. In: Yasui M, Strong MJ, Ota K et al., eds. Mineral and metal neurotoxicology. New York, NY. CRC Press, 243-251.

Skoczynska A, Smolik R. 1994. The effect of combined exposure to lead and cadmium on serum lipids and lipid peroxides level in rats. *Int J Occup Med Environ Health* 7(3):263-271.

Skoczynska A, Smolik R, Jelen M. 1993. Lipid abnormalities in rats given small doses of lead. *Arch Toxicol* 67:200-204.

Skoczynska A, Smolik R, Milian A. 1994. The effect of combined exposure to lead and cadmium on the concentration of zinc and copper in rat tissues. *Int J Occup Med Environ Health* 7(1):41-49.

Smith JW, Tokach MD, Goodband RD, et al. 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. *J Anim Sci* 75:1861-1866.

\*Smith SE, Larson EJ. 1946. Zinc toxicity in rats: Antagonistic effects of copper and liver. *J Biol Chem* 103:29-38.

Snyder RD, Friedman MB. 1998. Enhancement of cytotoxicity and clastogenicity of L-DOPA and dopamine by manganese and copper. *Mutat Res* 405:1-8.

\*Sohler A, Kruesi M, Pfeiffer CC. 1977. Blood lead levels in psychiatric outpatients reduced by zinc and vitamin C. *J Orthomol Psychiatr* 6:272-276.

Spencer JPE, Jenner A, Aruoma OI, et al. 1994. Intense oxidative DNA damage promoted by L-DOPA and its metabolites implications for neurodegenerative disease. *FEBS Lett* 353:246-250.

Srivastava AK, Gupta BN, Bihari V, et al. 1992. Clinical studies in workers engaged in maintenance of watermark moulds in a paper mill. *Int Arch Occup Environ Health* 64:141-145.

Srivastava L, Tandon, SK. 1984. Effect of zinc on lead-induced changes in brain lysosomal enzymes in the chick embryo. *Toxicol Lett* 20:111-114.

Steinbach OM, Wolterbeek HT. 1994. Role of cytosolic copper, metallothionein and glutathione in copper toxicity in rat hepatoma tissue culture cells. *Toxicology* 92:75-90.

Stohs SJ, Bagchi D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med* 18(2):321-336.

\*Storm GL, Fosmire GJ, Bellis ED. 1994. Heavy metals in the environment: Persistence of metals in soil and selected vertebrates in the vicinity of the Palmerton zinc smelters. *J Environ Qual* 23:508-514.

Sukumar A, Subramanian R. 1992. Trace elements in scalp hair of manufacturers of fireworks from Sivakasi, Tamil Nadu. *Sci Total Environ* 114:161-168.

Summerfield AL, Steinberg FU, Gonzalez JG. 1992. Morphologic findings in bone marrow precursor cells in zinc-induced copper deficiency anemia. *Am J Clin Pathol* 97:665-668.

Sunderman PW. 1979. Carcinogenicity and anticarcinogenicity of metal compounds. In: Emmelot P, Kriek E, ed. Environmental Carcinogenesis. Amsterdam: Elsevier/North-Holland Biomedical Press, 165-225.

\*Sziraki I, Sziraki I, Rauhala P, et al. 1999. Implications for atypical antioxidative properties of manganese in iron-induced brain lipid peroxidation and copper-dependent low density lipoprotein conjugation. *Neurotoxicology* 20(2-3):455-466.

Taves DR. 1974. Rapid assignment of patients by minimization in clinical trials. *Pharmacologist* 16(2):252.

\*Thawley DG, Pratt SE, Selby LA. 1978. Antagonistic effect of zinc on increased urine  $\delta$ -aminolevulinic acid excretion in lead-intoxicated rats. *Environ Res* 15:218-226.

\*Thawley DG, Willoughby RA, McSherry BJ, et al. 1977. Toxic interactions among Pb, Zn, and Cd with varying levels of dietary Ca and vitamin D: Hematological system. *Environ Res* 14:463-475.

\*Thomasino JA, Zuroweste E, Brooks SM, et al. 1977. Lead, zinc, and erythrocyte  $\delta$ -aminolevulinic acid dehydratase: Relationships in lead toxicity. *Arch Environ Health* 32:244-247.

Tom K, Chen M, Goettlich-Riemann W, et al. 1977. Collagen and elastin metabolism in relation to dietary zinc and copper in the chick and rat. *Nutr Rep Int* 15(2):191-198.

\*Tomokuni K. 1979. Interaction of zinc and other metal on the activity of erythrocyte  $\delta$ -amino-levulinic acid dehydratase in vitro. *J Toxicol Sci* 4:11-18.

Van Campen DR. 1966. Effects of zinc, cadmium, silver and mercury on absorption and distribution of copper-64 in rats. *J Nutr* 88:125-130.

Van Campen DR. 1969. Copper interference with the intestinal absorption of zinc-65 by rats. *J Nutr* 97:104-108.

\*Vassilev PP, Venkova K, Pencheva N, et al. 1994. Changes in the contractile responses to carbachol and in the inhibitory effects of verapamil and nitrendipine on isolated smooth muscle preparations from rats subchronically exposed to  $Pb^{2+}$  and  $Zn^{2+}$ . *Pharmacol Toxicol* 75:129-135.

\*Velez-Pardo C, Rio MJD, Ebinger G, et al. 1995. Manganese and copper promote the binding of dopamine to "serotonin binding proteins" in bovine frontal cortex. *Neurochem Int* 26(6):615-622.

Vescovi A, Facheris L, Zaffaroni A, et al. 1991. Dopamine metabolism alterations in a manganese-treated pheochromocytoma cell line (PC12). *Toxicology* 67:129-142.

Victory W, Soifer NE, Weiss JS, et al. 1981. Acute effects of lead on the renal handling of zinc in dogs. *Toxicol Appl Pharmacol* 61:358-367.

Walsh CT, Sandstead HH, Prasad AS, et al. 1994. Zinc: Health effects and research priorities for the 1990s. *Environ Health Perspect* 102(Suppl. 2):5-46.

\*Watanabe T, Iwami O, Nakatsuka H, et al. 1991. Correlation of cadmium, copper, manganese, and zinc levels in the urine of people in nonpolluted areas. *J Toxicol Environ Health* 33:263-272.

White AR, Bush AI, Beyreuther K, et al. 1999. Exacerbation of copper toxicity in primary neuronal cultures depleted of cellular glutathione. *J Neurochem* 72:2092-2098.

Wiegant FAC, Souren JEM, Van Wijk R. 1999. Stimulation of survival capacity in heat shocked cells by subsequent exposure to minute amounts of chemical stressors; role of similarity in hsp-inducing effects. *Hum Exp Toxicol* 18:460-470.

\*Willoughby RA, MacDonald E, McSherry BJ, et al. 1972. Lead and zinc poisoning and the interaction between Pb and Zn poisoning in the foal. *Can J Comp Med* 36:348-359.

\*Xu B, Chia S-E, Ong C-N. 1994. Concentrations of cadmium, lead, selenium, and zinc in human blood and seminal plasma. *Biol Trace Elem Res* 40:49-57.

\*Yadrick MK, Kenney MA, Winterfeldt EA. 1989. Iron, copper, and zinc status: Response to supplementation with zinc or zinc and iron in adult females<sup>1-3</sup>. *Am J Clin Nutr* 49:145-150.

\*Yoshikawa H, Ohta H. 1982. Interaction of metals and metallothionein. In: Foulkes EC, ed. *Biological roles of metallothionein*. Amsterdam: Elsevier North Holland Inc., 11-23.

\*Zawia NH, Crumpton T, Brydie M, et al. 2000. Disruption of the zinc finger domain. A common target that underlies many of the effects of lead. *Neurotoxicology* 21(6):1069-1080.

Zhang ZX, Anderson DW, Roman MN. 1996. Motor neuron disease on Guam: Geographic and familial occurrence, 1956-85. *Acta Neurol Scand* 94:51-59.

\*Zielhuis RL, del Castillo PD, Herber RFM, et al. 1978. Levels of lead and other metals in human blood: Suggestive relationships, determining factors. *Environ Health Perspect* 25:103-109.



## 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 bone, 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 1999).

### 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  $\mu\text{g}/\text{dL}$ . In addition, associations between biomarkers of lead exposure and increased problem behavior in the classroom have been reported (ATSDR 1999; Marlowe et al. 1985). In adult humans, slowing of nerve conduction velocity occurs at PbBs of  $\geq 30 \mu\text{g}/\text{dL}$ ; 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 1999).

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  $\mu\text{g}/\text{dL}$ . 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  $\mu\text{g}/\text{dL}$ , and the more recent literature suggest that PbB concentrations <40  $\mu\text{g}/\text{dL}$  also may be associated with adverse effects on sperm counts and morphology (ATSDR 1999).

### **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 1999).

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 ( $\approx 3 \mu\text{g/dL}$ ). 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  $40 \mu\text{g/dL}$ . As a result of the inhibition of ALAD and stimulation of ALAS, ALA accumulates in blood, urine, and soft tissues, including brain. ALA is structurally similar to GABA, an inhibitory neurotransmitter. ALA appears to act as a GABA agonist at the presynaptic GABA receptors, causing negative-feedback inhibition of GABA release. In addition, ALA undergoes autooxidation, generating free radicals that may contribute to toxicity, and ALA promotes oxyhemoglobin oxidation. At relatively high levels of lead exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Decreases in tissue heme pools can have deleterious effects throughout the body, not only because heme is a constituent of hemoglobin, but also because heme is a prosthetic group of cytochrome P450 and the cytochromes of cellular energetics (ATSDR 1999; EPA 1986). 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  $25 \mu\text{g/dL}$ . 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 1999). Other potential mechanisms of neurotoxicity include lead acting as a calcium agonist in a number of processes (ATSDR 1999), and lead inhibition of receptor binding to the NMDA receptor channel, which does not appear to occur at the zinc allosteric site and is relatively insensitive (Lasley and Gilbert 1999).

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 1999).

Lead has been shown to interfere with the DNA binding properties of zinc-finger regions of transcription factors, and this interference could potentially elicit multiple responses, but consequences have not yet been defined (Zawia et al. 2000).

#### A.4 Health Guidelines

ATSDR (1999) has not derived MRLs for lead. ATSDR (1999) 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  $>10 \mu\text{g}/\text{dL}$  are to be considered elevated (ATSDR 1999; 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.,  $10 \mu\text{g}/\text{dL}$ ) in children (EPA 1994a) or in fetuses (EPA 1996). This approach is supported by human epidemiological studies that have associated PbBs exceeding  $10 \mu\text{g}/\text{dL}$  with impairment or delays in neurobehavioral development and other effects on children (e.g., blood enzymes). EPA estimates lead risk in children using the Integrated Exposure Uptake Biokinetic (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." The International Agency for Research on Cancer (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). American Conference of Governmental Industrial Hygienists (ACGIH 2001) classified lead and certain inorganic lead compounds as A3 *carcinogens-confirmed animal carcinogen with unknown relevance to humans*. Lead chromate, assessed on the basis of both lead and chromate, was classified by ACGIH (2001) 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, manganese, zinc, and copper mixture that is the subject of this Interaction Profile. The relevant endpoints for lead and this mixture include neurological and hematological. Relevant endpoints for another metal mixture also included renal, cardiovascular, and testicular. The TTDs derived for those endpoints are retained in this Appendix, but are not relevant for the present mixture. Chronic oral TTDs for lead are derived below, using the methods described in ATSDR (2001a, 2001b). 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 (1999), and particularly Section 2.2.1 (Effects in Humans Based on Blood Lead (PbB) Levels), Section 2.5 (Relevance to Public Health), and Section 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  $\geq 30$   $\mu\text{g}/\text{dL}$  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  $10$   $\mu\text{g}/\text{dL}$  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  $20$   $\mu\text{g}/\text{dL}$  in children (IPCS 1995; Needleman and Gatsonis 1990; Pocock et al. 1994; Schwartz 1994). The CDC (1991) determined that blood lead levels of  $>10$   $\mu\text{g}/\text{dL}$  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 ( $10$   $\mu\text{g}/\text{dL}$ ) in children or

fetuses (EPA 1994a, 1996). The CDC level of concern for lead of 10  $\mu\text{g}/\text{dL}$  is adopted as the TTD for neurological effects ( $\text{TTD}_{\text{NEURO}}$ ).

### **Renal Effects**

Chronic nephropathy is associated with PbB levels of 40->100  $\mu\text{g}/\text{dL}$  in humans exposed to lead occupationally. There are some indications of renal damage in a study of children whose mean PbB was 34.2  $\mu\text{g}/\text{dL}$  (increased -acetyl- $\beta$ -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  $\mu\text{g}/\text{dL}$ , is taken as the TTD for renal effects ( $\text{TTD}_{\text{RENAL}}$ ).

### **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 (1999). The correlation between PbB and blood pressure is apparent at relatively low PbBs extending through 10  $\mu\text{g}/\text{dL}$  (e.g., Schwartz 1995). Therefore, the CDC level of concern, 10  $\mu\text{g}/\text{dL}$ , is adopted as the TTD for cardiovascular effects ( $\text{TTD}_{\text{CARDIO}}$ ).

### **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 1999). Accordingly, the CDC PbB of concern, 10  $\mu\text{g}/\text{dL}$  (CDC 1991), is selected as the TTD for hematological effects ( $\text{TTD}_{\text{HEMATO}}$ ).

## Testicular Effects

Adverse effects of the testes and sperm have been reported in occupationally exposed men with PbBs of 40-50  $\mu\text{g}/\text{dL}$  in some studies, but not in others, and are well-established at higher levels of exposure (PbBs  $\geq 66 \mu\text{g}/\text{dL}$ ) (ATSDR 1999). The point of departure for increased risk of below normal sperm and total sperm count was 40  $\mu\text{g}/\text{dL}$  (Alexander et al. 1996). This value is selected as the TTD for testicular effects (TTD<sub>TESTIC</sub>).

## Summary (TTDs for Lead)

TTD<sub>NEURO</sub> = 10  $\mu\text{g}/\text{dL}$  PbB = CDC level of concern

TTD<sub>RENAL</sub> = 34  $\mu\text{g}/\text{dL}$  PbB

TTD<sub>CARDIO</sub> = 10  $\mu\text{g}/\text{dL}$  PbB

TTD<sub>HEMATO</sub> = 10  $\mu\text{g}/\text{dL}$  PbB

TTD<sub>TESTIC</sub> = 40  $\mu\text{g}/\text{dL}$  PbB

## A.6 References

ACGIH. 2001. 2001 TLVs and BEIs. Threshold limit values for chemical substances and physical agents. Biological exposure indices. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Alexander BH, Checkoway H, van Netten C, et al. 1996. Semen quality of men employed at a lead smelter. *Occup Environ Med* 53:411-416. (As cited in ATSDR 1999).

ATSDR. 1999. Toxicological profile for lead. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR. 2001a. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR. 2001b. Guidance manual for the preparation of an interaction profile. Atlanta, GA. Agency for Toxic Substances and Disease Registry.

CDC. 1991. Preventing lead poisoning in young children. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. (As cited in ATSDR 1999).

EPA. 1986. Air quality criteria for lead. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. EPA 600/8-83-028F, 12-34 to 12-37.

EPA. 1994a. Guidance manual for the integrated exposure uptake biokinetic model for lead in children. U.S. Environmental Protection Agency. EPA/540/R-93/081. PB93-963510. (As cited in ATSDR 1999).

EPA. 1994b. Technical support document: Parameters and equations used in integrated exposure uptake biokinetic model for lead in children. (v0.99d). U.S. Environmental Protection Agency. EPA/540/R-94/040. PB94-963505. (As cited in ATSDR 1999).

EPA. 1996. Bioavailability of lead in soil samples from the Jasper County, Missouri superfund site. U.S. Environmental Protection Agency Region 8. Document Control No. 04800-030-0161. (As cited in ATSDR 1999).

IARC. 1987. IARC monographs on the evaluation of carcinogenic risk to humans. International Agency for Research on Cancer, World Health Organization. Supplement 7, Vols 1 to 47.

IPCS. 1995. Inorganic lead. Environmental Health Criteria 165 ed. Geneva: World Health Organization. International Programme on Chemical Safety. (As cited in ATSDR 1999).

IRIS. 2001. Integrated Risk Information System. U.S. Environmental Protection Agency. [Http://www.epa.gov/iris/subst/index.htm](http://www.epa.gov/iris/subst/index.htm). April 17, 2001.

Lasley SM and Gilbert ME. 1999. Lead inhibits the rat N-methyl-D-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. *Toxicol Appl Pharmacol* 159:224-233.

Marlowe M, Cossairt A, Moon C, et al. 1985. Main and interaction effects of metallic toxins on classroom behavior. *J Abnorm Child Psychol* 13(2):185-198.

Needleman HL, Gatsonis CA. 1990. Low-level lead exposure and the IQ of children: A meta-analysis of modern studies. *J Am Med Assoc* 263(5):673-678.

NTP. 2001. 9<sup>th</sup> report on carcinogens. Research Triangle Park, NC: U.S. Department of Health and Human Services. National Toxicology Program. <http://ehis.niehs.nih.gov/roc/toc9.html>. September 11, 2001.

Pocock SJ, Smith M, Baghurst P. 1994. Environmental lead and children's intelligence: A systematic review of the epidemiological evidence. *Br Med J* 309:1189-1197. (As cited in ATSDR 1999).

Schwartz J. 1994. Low-level lead exposure and children's IQ: A meta-analysis and search for a threshold. *Environ Res* 65:42-55. (As cited in ATSDR 1999).

Schwartz J. 1995. Lead, blood pressure, and cardiovascular disease in men. *Arch Environ Health* 50:31-37. (As cited in ATSDR 1999).

Verberk MM, Willems TE, Verplanke AJ, et al. 1996. Environmental lead and renal effects in children. *Arch Environ Health* 51(1):83-87. (As cited in ATSDR 1999).

Zawia NH, Crumpton T, Brydie M, et al. 2000. Disruption of the zinc finger domain: A common target that underlies many of the effects of lead. *Neurotoxicology* 21(6):1069-1080.

## Appendix B: Background Information for Manganese

Manganese is an element that exists naturally in the environment primarily as salts or oxides. Inorganic manganese in the (II), (III), and (IV) oxidation states are the forms most often encountered in the environment and in the workplace. The available information is insufficient to characterize any differences in toxicity for different manganese oxidation states, and they may be interconvertible in the body (ATSDR 2000).

One limitation of dose or exposure data for both human and animal studies is that the contribution to body burden from the diet generally is not precisely known, and frequently is not even discussed in the studies. Manganese is an essential element that occurs naturally in the human diet, and is included in all commercial animal chows, but levels of intake are often not precisely known, even for animal diets. Therefore, reported doses in experimental studies discussed in the following sections are for the intentionally administered manganese, consistent with the documentation by ATSDR (2000).

### B.1 Toxicokinetics

Absorption of manganese across the gastrointestinal tract typically averages about 3-5% in humans. Absorption may be age-dependent, with infants, especially the premature, retaining a higher proportion of manganese than do adults, although it is uncertain whether the higher retention results from higher absorption or from differences in excretion, or to interactions with iron intake, which is inversely related to manganese absorption. Studies of absorption in animals generally support the human data.

Absorption of manganese through the gastrointestinal tract may occur through nonsaturable simple diffusion through the mucosal layer of brush border membranes, or by an active-transport mechanism that is high-affinity, low capacity, and rapidly saturable. Dietary manganese, absorbed into the portal circulation as manganese(II), is bound to  $\alpha_2$ -macroglobulin or albumin in the plasma. In the liver, the major portion of manganese(II) is secreted into the bile, but some is thought to be oxidized by ceruloplasmin to Mn(III), which enters circulation conjugated with plasma transferrin (ATSDR 2000).

Manganese is a normal component of the body. In humans, highest concentrations of manganese occur in the liver, pancreas, and kidney; lowest concentrations occur in bone and fat. In humans with health conditions (liver disease or dysfunction), excess manganese uptake has occurred following oral exposure. In these cases, excess manganese preferentially accumulated in the basal ganglia, especially the globus

pallidus and the substantia nigra. Similar findings, with accumulation of manganese particularly in the globus pallidus, have been reported in humans exposed occupationally (primarily by inhalation). In monkeys given manganese intravenously, accumulation of manganese in the globus pallidus and substantia nigra also was observed. One study in rats demonstrated that continuing exposure to high levels of manganese in the diet resulted in large increases in tissue levels of manganese compared with controls over the first 24 days, but that levels tended to decrease towards the control levels as exposure was continued through 224 days. This finding is thought to be the result of homeostatic mechanisms that lead to decreased absorption and/or increased excretion of manganese when manganese intakes are high. Absorbed manganese is removed from the blood by the liver, where it conjugates with bile and is excreted into the intestine. Although some of manganese in the intestine is reabsorbed through entero-hepatic circulation, biliary secretion followed by fecal excretion is the main excretion pathway for gastrointestinally absorbed manganese. Small amounts of manganese are excreted in urine, sweat, and milk. Biliary/fecal excretion in the neonate may be different than in adults, but data for humans indicate greater neonatal excretion, and in animals indicate less neonatal excretion (ATSDR 2000).

## **B.2 Health Effects**

Manganese is an essential element for humans and animals. Manganese acts both as a constituent of metalloenzymes and as an enzyme activator. Manganese plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from free radicals, and the formation of glycosaminoglycans. Metalloenzymes containing manganese include arginase, pyruvate carboxylase, and Mn superoxide dismutase. Manganese has been shown to activate numerous other enzymes including transferases, decarboxylases, and hydrolases (ATSDR 2000).

The NRC (1989) has established ESADDIs for manganese. The ESADDIs are 2-5 mg/day for adolescents (>11 years) and adults. For children, the ESADDIs are 0.3-0.6 mg/day for infants from birth to 6 months, 0.6-1.0 mg/day for infants from 6 months to 1 year, 1.0-1.5 mg/day for children from 1 to 3 years, 1.5-2.0 mg/day for children from 4 to 6 years, and 2.0-3.0 mg/day for children 7 to 10 years of age.

Effects of manganese deficiency in humans are not well-defined. Limited information indicates that dermatitis, and possibly decreased levels of clotting proteins, decreased serum cholesterol, reddening of black hair, and slowed growth of hair and nails may be consequences of manganese deficiency. Effects

of manganese deficiency in animals include impaired growth, skeletal abnormalities, testicular degeneration in males, impaired reproductive function in females, ataxia, altered carbohydrate and lipid metabolism, and increased oxidation of mitochondrial membranes, and reduced high density lipoprotein (HDL) cholesterol (ATSDR 2000)

Exposure to manganese above essential levels can have toxic consequences. Chronic occupational (inhalation) exposure to manganese has been linked to neurological deficits, evidenced as deficits in the ability to perform rapid hand movements and some loss of coordination and balance, and increased mild symptoms such as forgetfulness, anxiety, or insomnia. Very high chronic inhalation exposure, as in former manganese miners, resulted in permanent neurological damage, evidenced by a syndrome of neurological effects called manganism, which includes mask-like facial expression, slow and clumsy gait, fine tremor, and sometimes psychiatric disturbances. Ultimately, patients develop severe hypertonia and muscle rigidity and may be completely disabled. Although some of these symptoms also occur in Parkinson's disease, the two diseases are different (ATSDR 2000). Unlike Parkinson's patients, manganism patients generally do not respond to levo-dopa treatment, indicating that degeneration of the receptors and neurons that normally respond to this drug (and to dopamine) may have occurred.

The evidence for neurological effects in humans from oral exposure is more limited, but collectively, the studies suggest an association between ingestion of water and/or food containing increased levels of manganese and adverse neurological effects. Some of the studies reported symptoms and signs similar to those associated with inhalation exposure. Two of the studies focused on children, and associated increased oral intakes of manganese and increased hair manganese with poorer performance in school and on neurobehavioral exams. Other studies have reported higher manganese concentrations in the hair of learning disabled children than in normal children (ATSDR 2000).

Oral neurotoxicity studies in animals have been conducted over a wide range of doses. The lowest dose of manganese tested, 1 mg/kg/day by gavage, produced neuronal degeneration and increased monoamine oxidase in neonatal rats during intermediate duration studies, but no clinical or behavioral signs of neurotoxicity. This dose was considered a serious lowest-observed-adverse-effect level (LOAEL). Neurobehavioral effects were seen in neonatal rats at higher gavage doses of manganese: increased pulse-elicited startle reflex at 11 mg/kg/day and increased spontaneous motor activity at 22 mg/kg/day. In more mature rats given manganese orally in drinking water or food, increased motor activity has been observed at 40 and 140 mg/kg/day, and decreased motor activity at  $\geq 284$  mg/kg/day. Many other studies in rodents

focused on neurotransmitter levels in the brain. Monkeys given 25 mg/kg/day of manganese by gavage for 18 months developed weakness, muscular rigidity, and neuronal loss and depigmentation of the substantia nigra (ATSDR 2000)

Occupational exposure has resulted in male reproductive effects, including decreased libido and sperm count and viability. Intratracheal instillation of manganese, which may result in gastrointestinal as well as respiratory absorption, resulted in degeneration of the seminiferous tubules and loss of spermatogenesis in rabbits. In young male rodents given relatively high doses of manganese ( $\geq 1,050$  mg/kg/day), delayed growth and maturation of the testes were reported and appeared to be due to decreased testosterone secretion (ATSDR 2000).

### **B.3 Mechanisms of Action**

Although the central nervous system is the primary target of manganese toxicity, a mechanism for the neurotoxicity of manganese has not been clearly established. One suggested mechanism is that manganese enhances the autooxidation or turnover of various intracellular catecholamines (dopamine, norepinephrine, epinephrine), leading to increased production of free radicals, reactive oxygen species, and other cytotoxic metabolites, accompanied by a depletion of cellular antioxidant defense mechanisms. Other potential mechanisms include the ability of manganese(II) to substitute for calcium under physiological conditions, the possibility that the dopamine reuptake carrier is linked to a transport mechanism for manganese, inhibition by manganese of brain mitochondrial oxidative phosphorylation, or manganese involvement in complex interactions with other minerals such as iron, copper, selenium, zinc, and calcium (ATSDR 2000). In humans with manganism, neuropathological changes are detectable in the basal ganglia; the specific area of injury and accumulation of manganese appears to be primarily in the globus pallidus. The substantia nigra is sometimes affected, but generally to a lesser extent. Studies in monkeys given manganese intravenously have produced similar results.

### **B.4 Health Guidelines**

ATSDR (2000) did not derive acute or intermediate inhalation MRLs manganese due to lack of suitable data.

Using the benchmark dose (BMD) approach, ATSDR (2000) derived a chronic inhalation MRL of  $4 \times 10^{-5}$  mg/m<sup>3</sup> for manganese based on the 95% lower confidence limit for an increased risk of 10% (BMDL<sub>10</sub>) of 74 µg Mn/m<sup>3</sup> (respirable dust) for neurobehavioral effects in workers exposed for an average duration of 5.3 years (Roels et al. 1992). The MRL was calculated by converting this concentration from intermittent to continuous exposure (using factors of 5/7 for days per week and 8/24 for hours per day), and applying uncertainty factors of 10 for human variability, 10 for potential differences in toxicity among different manganese forms and other limitations in the database including lack of developmental testing and reproductive testing in females, and a modifying factor of 5 for potential increased susceptibility in children based on differential pharmacokinetics.

ATSDR (2000) did not derive oral MRLs for manganese because no clear threshold level for neurological effects could be determined from the acute and intermediate duration data, and because no firm conclusions were considered possible regarding a critical effect level of chronic intake versus essential dietary levels of chronic intake of manganese. ATSDR (2000) derived a provisional guidance value for total dietary intake of 0.07 mg Mn/kg/day, based on the upper end of the ESADDI range (5 mg/day, divided by 70 kg, the weight of an adult), to be used in ATSDR human health assessments. The chronic inhalation MRL for manganese is based on neurological effects.

The NRC (1989) concluded that given the apparent lack of manganese deficiency in adults, the U.S. dietary intakes of manganese (2.2-2.7 mg/day for women and men, respectively) appear to satisfy the need for this element. Therefore, the NRC established an estimated safe and adequate daily dietary intake for manganese of 2.0-5.0 mg/day for adults. An RDA could not be determined because data were insufficient to determine the manganese needs of healthy persons.

EPA (IRIS 2001) derived an RfC of  $5 \times 10^{-5}$  mg/m<sup>3</sup> for manganese based on a LOAEL<sub>HEC</sub> of 0.05 mg Mn/m<sup>3</sup> for neurobehavioral effects in workers exposed to manganese for an average of 5.3 years (Roels et al. 1992). The uncertainty factor used in this derivation was 1,000 (10 for human variability, 10 for the use of a LOAEL, and 10 for database limitations including the less-than chronic exposure period and the lack of developmental data, and potential differences in the toxicity of various forms of manganese).

EPA (IRIS 2001) derived a chronic RfD of 0.14 mg Mn/kg/day for manganese based on a NOAEL of 0.14 mg/kg/day for central nervous system effects, and an uncertainty factor of 1. This NOAEL was based on studies and analyses of human dietary consumption of manganese, which indicated that normal

intakes of as high as, and even higher than 10 mg Mn/day (0.14 mg/kg/day) occur and are without adverse consequences. A modifying factor of 3 is to be applied when assessing the potential hazard of exposure to manganese from drinking water or soil. (Application of this factor results in a value of 0.047 mg/kg/day.)

The National Toxicology Program (NTP 2001) does not list manganese as a substance known or reasonably anticipated to be a human carcinogen and IARC has not published a monograph on the potential carcinogenicity of manganese. EPA (IRIS 2001) has classified manganese in Group D-*not classifiable as to human carcinogenicity*, based on a lack of human data and inadequate animal data.

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

A TTD for chronic oral exposure to manganese was derived for the endpoint affected by manganese and one or more of the other chemicals in the lead, manganese, zinc, and copper mixture that is the subject of this Interaction Profile, using the methods described in ATSDR (2001a, 2001b). The relevant endpoints for this mixture include neurological and hematological. Manganese is neurotoxic, but the available data for oral exposure were considered inadequate for MRL derivation by ATSDR (2000) because no clear threshold level for neurological effects could be determined from the acute- and intermediate-duration data, and because no firm conclusions were considered possible regarding a critical effect level of chronic intake versus essential dietary levels of chronic intake of manganese. Therefore, ATSDR (2000) recommended a provisional guidance value for total dietary intake of 0.07 mg Mn/kg/day, based on the upper end of the ESADDI range (5 mg/day, divided by 70 kg, the weight of an average adult), to be used in ATSDR human health assessments. This value is suitable as a TTD for neurological effects.

#### **Summary (TTD for Manganese)**

$$\text{TTD}_{\text{NEURO}} = 0.07 \text{ mg/kg/day}$$

## B.6 References

ATSDR. 2000. Toxicological profile for manganese. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR. 2001a. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR. 2001b. Guidance manual for the preparation of an interaction profile. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

IRIS. 2001. Integrated Risk Information System. U.S. Environmental Protection Agency. [Http://www.epa.gov/iris/subst/index.htm](http://www.epa.gov/iris/subst/index.htm). April 17, 2001.

NRC. 1989. Recommended dietary allowances. 10<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council. 205-213, 224-235, 284-285.

NTP. 2001. 9<sup>th</sup> Report on carcinogens. Research Triangle Park, NC: U.S. Department of Health and Human Services. National Toxicology Program. [Http://ehis.niehs.nih.gov/roc/toc9.html](http://ehis.niehs.nih.gov/roc/toc9.html). September 11, 2001.

Roels HA, Ghyselen P, Buchet JP, et al. 1992. Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. Br J Ind Med 49:25-34. (As cited in ATSDR 2000).



## Appendix C: Background Information for Zinc

### C.1 Toxicokinetics

Zinc is absorbed through the respiratory system and skin as evidenced by increased blood, urine, and tissue levels, but quantitative estimates of respiratory or dermal absorption were not located. The absorption of zinc from the gastrointestinal tract has been studied extensively. Absorption from the gastrointestinal tract is homeostatically regulated; 20-30% of ingested zinc is absorbed under normal physiological conditions. Intestinal absorption is saturable, suggesting that it may be enzyme- or carrier-mediated. Alternatively, it has been proposed that excess zinc ions neutralize membrane charges, interfering with membrane binding and subsequent uptake into the mucosal cells. Zinc induces metallothionein synthesis in the mucosal cells. The metallothionein may contribute to zinc homeostasis by sequestering some zinc in the intestinal mucosal cells until the cells are sloughed and excreted in the feces. Dietary constituents influence zinc absorption, with protein facilitating absorption and fiber or calcium decreasing absorption. In the plasma, the carrier for zinc is primarily albumin, with lesser amounts of zinc bound to  $\alpha_2$ -macroglobulin and amino acids. Zinc is initially concentrated in the liver and then distributed throughout the body, with high concentrations in the prostate, retina, sperm, gastrointestinal tract, kidney, brain, skin, lung, heart, and pancreas. The highest percentages of the body burden of zinc are found in muscle ( $\approx 63\%$ ) and bone ( $\approx 28\%$ ). Zinc is excreted primarily in the feces; fecal excretion includes unabsorbed zinc, zinc excreted in the bile, and zinc in exfoliated intestinal mucosal cells. In addition, zinc is excreted in the urine and in sweat (ATSDR 1994). Zinc does not appear to accumulate in the body with age (Walsh et al. 1994).

One limitation of dose or exposure data for both human and animal studies is that the contribution to body burden from the diet generally is not precisely known, and frequently is not even discussed in the studies. Zinc is an essential element that occurs naturally in the human diet, and is included in all commercial animal chows, but levels of intake are often not precisely known, even for animal diets. Therefore, reported doses in experimental studies discussed in the following sections are for the intentionally administered zinc, unless otherwise specified, consistent with ATSDR (1994).

## C.2 Health Effects

Zinc is an essential element for humans and animals due to its role as a constituent of metalloenzymes such as alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, Cu/Zn superoxide dismutase, and DNA and ribonucleic acid (RNA) polymerases, as a constituent of zinc-finger regions of transcription factors, and as a constituent of or requirement for ALAD. Thus, zinc is required for cell division, metabolism, growth, and repair (ATSDR 1994; Dreosti 2001; IRIS 2001; NRC 1989). Zinc occurs in only one oxidation state, Zn(II), and tends to have a stabilizing role in its binding with sulfhydryl groups and maintenance of protein conformation (Bremner and Beattie 1995). Zinc released from vesicles in presynaptic terminals of certain glutaminergic neurons is thought to modulate postsynaptic NMDA receptors for glutamate (Sandstead et al. 2000). Zinc deficiency causes loss of appetite, growth retardation, dermatitis, impaired wound healing, impaired immunological function, hypogonadism (including testicular atrophy), impaired reproductive capacity, depressed mental function, and increased incidences of congenital malformations (ATSDR 1994; Dreosti 2001; IRIS 2001; NRC 1989). The RDA for zinc is 15 mg/day for adolescent and adult males and 12 mg/day for adolescent and adult females, equivalent to  $\approx 0.2$  mg/kg/day. The RDA for infants is 5 mg/day, for children is 10 mg/day, and for lactating women is 16-19 mg/day (ranging from  $\approx 0.3$  to 0.8 mg/kg/day for these 3 groups) (NRC 1989). The RfD for zinc is 0.3 mg/kg/day, which supplies adequate zinc for adolescents and adults, but not for infants, children, or possibly for lactating women (IRIS 2001).

The NRC (1995) has estimated that zinc requirements in the dry diet for rats are 12 ppm for maintenance and growth and 25 ppm for reproduction including lactation, and for mice are 10 ppm for maintenance and growth and 30 ppm for reproduction including lactation. These estimates represent minimal requirements determined in experiments with purified diets and do not include a margin of safety. The NRC (1995) notes that higher concentrations of zinc-18 ppm for maintenance and growth-are required when the diet includes ingredients that contain phytate, such as soybean meal. The concentrations of zinc in the NIH-07 and NIH-31 natural-ingredient fixed-formula diets provided by the addition of a mineral premix are 18 and 11 ppm, respectively, but additional zinc may be contributed by the other constituents of the diet, and these diets also contain soybean meal and other potential sources of phytate. These diets are used for maintaining rat and mouse breeding colonies at the National Institutes of Health (NIH) and are fed in NTP toxicology and carcinogenicity studies. The concentration of zinc in the commonly used purified diet AIN-76A for rats and in the more recent purified diets AIN-93G and AIN-93M for rats and mice is 30 ppm (NRC 1995). Debilitating deficiency has been reported with zinc

concentrations in the diet of <1 ppm and milder deficiency at 2 ppm (Bushnell and Levin 1983). Assuming 100% of the zinc intake is from the diet and using food intake and body weight values for F344 and Sprague-Dawley rats (EPA 1988), levels of 12 and 30 ppm zinc in the diet corresponds to an intake of 2 and 4-5 mg/kg/day for weanling rats, and 1 and 2-3 mg/kg/day for 1-year-old rats and rats in subchronic and chronic studies.

In humans and animals, exposure to zinc at levels above normal dietary requirements causes anemia, gastrointestinal irritation, pancreatic and adrenal abnormalities, impaired immune function, and decreased levels of serum HDL cholesterol, serum ferritin, and erythrocyte superoxide dismutase. Neurological signs have been reported in two human case reports of zinc ingestion and mild histopathological changes were seen in brains of animals treated with zinc, but are not as well documented (ATSDR 1994; IRIS 2001). The worsening of cognition in patients with Alzheimer's disease within two days of starting on zinc supplementation has been noted (Bush et al. 1994), but details, including the supplemental zinc dose, were not published, and no clear evidence has emerged subsequently to indicate that zinc supplementation hastens the progression of Alzheimer's disease. A later publication by Bush and colleagues (Cuajungco et al. 2000) postulated that the detrimental effects in Alzheimer's patients may have been due to gastrointestinal effects. Additional effects of high levels of zinc oral intake, seen only in animal studies, included renal effects in rats fed 191 mg Zn/kg/day in the diet for 3 months. Reproductive effects including altered sperm chromatin structure in male rats that received 25 mg/kg/day in the diet for 8 weeks, and developmental effects, including fetal resorption, decreased fetal weight, and increased stillbirths at  $\geq 200$  mg/kg/day for 15-36 days. The adverse effects of zinc generally occur at levels of intake elevated by an order of magnitude or more over the RDA or animal dietary requirement (ATSDR 1994; IRIS 2001). Exceptions are the decreases in serum ferritin and erythrocyte superoxide dismutase, a slight decrease in hematocrit (Yadrick et al. 1989), and decreases in HDL cholesterol, which occur at about 5 times the RDA (ATSDR 1994; IRIS 2001).

### **C.3 Mechanisms of Action**

The anemia and possibly the effect on HDL cholesterol are thought to be caused by zinc-induced copper deficiency. High oral intakes of zinc induce the synthesis of metallothionein in the intestinal mucosal cells, which provides a possible mechanism for zinc induction of copper deficiency. Copper binds to metallothionein with a higher affinity than does zinc and will tend to replace zinc; although some of the metallothionein-bound copper in the intestinal mucosal cells is released to the blood, much of the copper-

metallothionein is excreted in the feces when the intestinal mucosal cells are exfoliated. Copper is an essential part of several enzymes including ceruloplasmin, which oxidizes ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is critically important to provide iron for hemoglobin synthesis (ATSDR 1994; Friberg et al. 1986; IRIS 2001). Decreases in another copper metalloenzyme, erythrocyte superoxide dismutase, are considered a more sensitive and reliable indicator of altered copper status than are ceruloplasmin levels or tissue or plasma copper levels (ATSDR 1994; IRIS 2001).

#### **C.4 Health Guidelines**

ATSDR (1994) did not derive inhalation MRLs for any duration of exposure to zinc or an oral MRL for acute exposure to zinc due to lack of suitable studies.

ATSDR (1994) derived an intermediate oral MRL of 0.3 mg/kg/day for zinc based on a LOAEL for hematological effects of 1 mg/kg/day in humans. The LOAEL was determined by adding the estimate of dietary zinc intake for women (20-30 years old) of 9.72 mg Zn/day (0.16 mg/kg/day) from a Food and Drug Administration (FDA) Total Diet study for 1982-1986 (Pennington et al. 1989) to 50 mg Zn/day (0.83 mg/kg/day), the reported supplemental zinc dose associated with decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase activity in women given this dose of zinc (as zinc gluconate) for 20 weeks (Yadrick et al. 1989). An uncertainty factor of 3 was applied, based on the use of a minimal LOAEL from a study of the most sensitive humans, and the consideration of zinc's essentiality.

The intermediate oral MRL was adopted as the chronic oral MRL due to the lack of adequate chronic studies. ATSDR (1994) noted that the chronic oral MRL is expected to be without adverse effects from either excess zinc or from nutritional zinc deficiency in healthy, nonpregnant, adult humans ingesting the average American diet.

The NRC (1989) has established an RDA for zinc of 15 mg/day for adolescent and adult males and 12 mg/day for adolescent and adult females, equivalent to  $\approx 0.2$  mg/kg/day.

EPA derived an RfD of 0.3 mg/kg/day based on the LOAEL of 1 mg Zn/kg/day for the decrease in erythrocyte superoxide dismutase (indicative of altered copper status) in women due to 50 mg/day of

supplemental zinc (Yadrick et al. 1989), combined with the dietary zinc estimates of 9.72 mg/day from the FDA Total Diet Study for 1982-1986 (Pennington et al. 1989).

The National Toxicology Program (NTP 2001) does not list zinc as a substance known or reasonably anticipated to be a human carcinogen and IARC has not published a monograph on the potential carcinogenicity of manganese. EPA (IRIS 2001) has classified zinc in Group D-*not classifiable as to human carcinogenicity*, based on inadequate data in humans and animals.

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

Derivation of a TTD for chronic oral exposure to zinc would be appropriate for sensitive endpoints affected by zinc and one or more of the other chemicals in the lead, manganese, zinc, and copper mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include neurological and hematological. Zinc is hematotoxic, and ATSDR has derived an MRL for intermediate oral exposure based on hematological effects, and has adopted that MRL for chronic oral exposure as well.

#### Summary (TTD for Zinc)

$MRL_{\text{HEMATO}} = 0.3 \text{ mg/kg/day}$

### C.6 References

ATSDR. 1994. Toxicological profile for zinc. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Bremner I, Beattie JH. 1995. Copper and zinc metabolism in health and disease: Speciation and interactions. Proc Nutr Soc 54:489-499.

Bush AI, Pettingell WH, Multhaup G, et al. 1994. Rapid induction of Alzheimer A $\beta$  amyloid formation by zinc. Science 265:1464-1467.

Bushnell PJ, Levin ED. 1983. Effects of zinc deficiency on lead toxicity in rats. Neurobehav Toxicol Teratol 5:283-288.

Cuajungco MP, Goldstein LE, Nunomura A, et al. 2000. Evidence that the  $\beta$ -amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of A $\beta$  by zinc. J Biol Chem 275(26):19439-19442.

Dreosti IE. 2001. Review: Zinc and the gene. *Mutat Res* 475:161-167.

EPA. 1988. Recommendations for and Documentation of Biological Values for Risk Assessment. U.S. Environmental Protection Agency. NTIS PB88-179874. EPA/600/6-87/008. Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.

Friberg L, Nordberg GF, Vouk VB, eds. 1986. Handbook on the toxicology of metals. 2<sup>nd</sup> ed, Vol. II: Specific Metals. Amsterdam: Elsevier Science Publishing Co. Inc, 242, 668, 671.

IRIS. 2001. Integrated Risk Information System. U.S. Environmental Protection Agency. <http://www.epa.gov/iris/subst/index.htm>. April 17, 2001.

NRC. 1989. Recommended dietary allowances. 10<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council, 205-213, 224-235, 284-285.

NRC. 1995. Nutrient requirements of laboratory animals. 4<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council, 11-102.

NTP. 2001. 9<sup>th</sup> report on carcinogens. Research Triangle Park, NC: U.S. Department of Health and Human Services. National Toxicology Program. <http://ehis.niehs.nih.gov/roc/toc9.html>. September 11, 2001.

Pennington JAT, Young BE, Wilson D. 1989. Nutritional elements in U.S. diets: Results from the total diet study, 1982 to 1986. *J Am Diet Assoc* 89(5):659-664. (As cited in ATSDR 1994).

Sandstead HH, Frederickson CJ, Penland JG. 2000. History of zinc as related to brain function. *J Nutr* 130:496S-502S.

Walsh CT, Sandstead HH, Prasad AS, et al. 1994. Zinc: Health effects and research priorities for the 1990s. *Environ Health Perspect* 102(Suppl 2):5-46.

Yadrick MK, Kenney MA, Winterfeldt EA. 1989. Iron, copper, and zinc status: Response to supplementation with zinc or zinc and iron in adult females<sup>1-3</sup>. *Am J Clin Nutr* 49:145-150.

## Appendix D: Background Information for Copper

Copper is a naturally occurring element that exists in the environment as the free metal, and in the (I) and (II) oxidation states. Because the biological availability and toxicity of copper are related to the copper(II) oxidation state, ATSDR (1990) has focused on that form of copper.

One limitation of dose or exposure data for both human and animal studies is that the contribution to body burden from the diet generally is not discussed or known. Copper is an essential element that occurs naturally in the human diet, and is included in all commercial animal chows. Precise levels of intake are often not known, even for animal diets. Therefore, reported doses in experimental studies discussed in the following sections are for the intentionally administered copper, consistent with ATSDR (1990).

### D.1 Toxicokinetics

Copper is readily absorbed from the stomach and small intestine. Gastrointestinal absorption in humans has been estimated at 60%; the site of maximal absorption appears to be the stomach and upper intestine in humans and rats, and the lower small intestine in hamsters. The predominant mechanism of absorption is mucosal uptake and binding of copper to metallothionein. The copper bound to metallothionein is slowly released to the blood or excreted when the mucosal cells are sloughed off. Copper induces the synthesis of metallothionein. Thus, metallothionein appears to play a homeostatic role in copper bioavailability. Following release to the blood, copper is loosely bound to albumin and amino acids, and transported to the liver, where it is incorporated into ceruloplasmin and released into the plasma. In addition, excess absorbed copper is stored in the liver or excreted through the bile. Concentrations of copper in healthy adults are highest in the hair and nails, followed by the liver and brain (ATSDR 1990). In the fetus, however, copper is accumulated in the liver to 6- to 10-fold higher concentrations than in adult liver, while circulating concentrations of copper in the fetus are low (EPA 1987). This accumulated hepatic copper is then incorporated into ceruloplasmin by the neonatal liver and released into the circulation (EPA 1987). The major pathway for excretion of absorbed copper is through the bile, followed by fecal excretion. Reabsorption of biliary copper is negligible. Additional contributions to fecal copper excretion is from unabsorbed copper and copper from desquamated mucosal cells (ATSDR 1990).

## D.2 Health Effects

Copper is an essential element that is incorporated into numerous enzymes involved in hemoglobin formation, carbohydrate metabolism, catecholamine synthesis, and cross-linking of collagen, elastin, and hair keratin (ATSDR 1990). Copper can exist in two oxidation states Cu(I) to Cu(II). Copper is present in many oxidases where its ability to change oxidation states is integral to catalytic activity (Bremner and Beattie 1995). One of these enzymes is ceruloplasmin, a ferroxidase that oxidize ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is critically important to provide iron for hemoglobin synthesis (Friberg et al. 1986). Other copper metalloenzymes include cytochrome c oxidase, Zn/Cu superoxide dismutase, dopamine  $\beta$ -hydroxylase, tyrosinase (tyrosine hydroxylase), and ascorbic acid oxidase (ATSDR 1990; Institute of Medicine 2001). Dopamine  $\beta$ -hydroxylase transforms dopamine to norepinephrine, tyrosinase metabolizes tyrosine to dopa (precursor of dopamine), and monoamine oxidase is important in the metabolic degradation of serotonin, epinephrine, norepinephrine, and dopamine (Hardman and Limbird 1996).

The NRC (1989) has established ESADDIs for copper. The ESADDIs are 1.5-2.5 mg/day for adolescents (>11 years) and 1.5-3.0 mg/day adults. For children, the ESADDIs are 0.4-0.6 mg/day for infants from birth to 6 months, 0.6-0.7 mg/day for infants from 6 months to 1 year, 0.7-1.0 for children from 1 to 3 years, 1.0-1.5 mg/day for children from 4 to 6 years, and 1.0-2.0 mg/day for children 7 to 10 years of age. An RDA of 0.9 mg/day is reported in a prepublication document (Institute of Medicine 2001); the final version has not been published as of December 2001.

The dietary requirements for copper in rats is 5 ppm for growth and maintenance and 8 ppm for pregnancy and lactation (NRC 1995), and for growing pigs ranges from 6 ppm in 3-5 kg pigs to 3 ppm in 80-120 kg pigs (NRC 1998).

Little information is available regarding the toxicity of ingested copper to humans. Case reports of adverse effects following ingestion of large amounts of copper(II) in contaminated water or as suicide attempts include acute gastrointestinal distress, acute hemolytic anemia in a young child, hepatic micronodular cirrhosis in two infant siblings, hepatic centrilobular necrosis in adults, renal tubular necrosis or clinical evidence of renal tubular damage in a child and in adults. Reliable information on doses was not available (ATSDR 1990).

Wilson's disease, an autosomal recessive disorder that affects normal copper homeostasis, gives some information regarding potential targets of copper toxicity in humans. The disease is characterized by excessive retention of copper in the liver, decreased concentration of plasma ceruloplasmin, impaired biliary copper excretion, and hypercupruria. Adverse health effects seen in Wilson's disease patients are hepatic and renal lesions and hemolytic anemia, similar to the effects observed in human case reports summarized in the previous paragraph. In addition, neurological effects, including poor coordination, tremor, and psychological impairment have been observed in this disease. These neurological manifestations have not been seen in normal humans who ingested high doses of copper or in animals (ATSDR 1990).

In animals exposed to oral intakes of copper well above normal dietary requirements for intermediate durations, effects included gastrointestinal effects (forestomach hyperplasia) at 28 mg/kg/day in rats and 155 mg/kg/day in mice, decreased hemoglobin and hematocrit in rats at  $\geq 40$  mg/kg/day and pigs at 14.6 mg/kg/day, increased systolic blood pressure (one study) in rats at 10 mg/kg/day, hepatic damage in rats (increased serum glutamic-oxaloacetic transaminase (SGOT) at 7.9 mg/kg/day and necrosis at  $\geq 40$  mg/kg/day) and pigs (increased SGOT levels at 36 mg/kg/day), renal tubular damage in rats (hyaline-like droplets at 14 mg/kg/day and necrosis at  $\geq 100$  mg/kg/day necrosis), and developmental effects in mice (increased fetal mortality at  $\geq 104$  mg/kg/day and developmental abnormalities at  $\geq 155$  mg/kg/day). In addition, there is a single report of potential testicular effects: an increase in testes weight in rats at 130 mg/kg/day. The toxicological significance of this effect is uncertain because no histopathological examinations were performed. The rats appeared to develop tolerance to the hepatic and renal effects, as evidenced by regeneration of tissue. Information regarding potential neurotoxicity was limited. No neurobehavioral effects or changes in dopamine and norepinephrine were seen in rats at 12.5 mg/kg/day. In another study, a 25% decrease in 3,4-dihydroxyphenylacetic acid (dopamine metabolite), but no change in dopamine was seen in rats at 175 mg/kg/day. Thus, no clear evidence of neurological effects is available for copper (ATSDR 1990). Additional information on neurotoxicity has been reviewed in Section 2.2.4 of this Interaction Profile and includes inconsistent changes in neurotransmitter levels in the brain of rats exposed orally (Flora et al. 1989a) versus intraperitoneally (Malhotra et al. 1982) to excess copper, and an increased odds ratio for development of Parkinson's disease in workers exposed to copper for more than 20 years (Gorell et al. 1997, 1999). These findings are not adequate to establish that copper causes neurological effects.

Decreased body weight gain has been reported in several studies in rats ingesting  $\geq 100$  mg/kg/day, in pigs ingesting 14.6 mg/kg/day, and in mice consuming 4.2 mg/kg/day (ATSDR 1990). It is possible that copper interference with zinc may account for this observation.

Data on potential neurological effects are inadequate.

An assessment by the Institute of Medicine (2001) concluded that there is little evidence to indicate that chronic exposure to copper (above essential levels) results in systemic effects other than liver damage. Gastrointestinal effects were considered of more concern for exposure through drinking water or beverages than from food, and for acute exposure than for chronic, as there is evidence of development of tolerance.

### **D.3 Mechanisms of Action**

Copper is essential for heme synthesis, as described in Section D.2, and as part of Zn/Cu superoxide dismutase, protects against free radical damage. Copper at excessive levels, however, inhibits enzymes such as glucose-6-phosphatase and glutathione reductase, by binding to enzyme sulfhydryl groups, thus interfering with their protection of cells from free radical damage. Inhibition of glucose-6-phosphatase leads to hemolysis. Copper can damage the proximal renal tubule directly, or indirectly as a consequence of hypotension or hemolysis. Metallothionein, a cysteine-rich, low-molecular-weight protein that binds copper in the gastrointestinal mucosa and other body tissues, provides some protection against copper toxicity; its synthesis is induced by copper (Barceloux 1999). In addition, copper is exported from mammalian cells via a copper-translocating adenosine triphosphatase (ATPase) (Dameron and Harrison 1998). Acute oral poisoning causes irritation and erosion of the epithelial lining of the gastrointestinal tract (Barceloux 1999).

### **D.4 Health Guidelines**

ATSDR (1990) did not derive any inhalation MRLs for copper because of a lack of suitable data. Oral MRLs were not derived for copper because of the lack of human data, lack of NOAEL values in the animal studies, development of tolerance in rats, and because of the essentiality of copper. Nor did ATSDR (1990) suggest a suitable guidance value in this relatively early toxicological profile.

The NRC (1989) derived an estimated safe and adequate daily dietary intake for copper of 1.5-3 mg/day for adults. The NRC noted that copper balance studies in humans indicate that 1.6 mg/day is needed to replace fecal, urinary, and body surface losses of copper, but many U.S. diets provide less than 1.6 mg of copper per day, yet anemia or neutropenia ascribable to copper deficiency is not observed in adults consuming typical U.S. diets, suggesting either a homeostatic adaptation to low dietary copper, or an incorrect estimate of dietary copper intake. For these reasons, the NRC concluded that an RDA for copper cannot be established. More recently, an RDA has been estimated at 0.9 mg/day ( $\approx 0.013$  mg/kg/day) (Institute of Medicine 2001: prepublication document, final version not published as of December 2001). The critical effect for overexposure to copper was considered to be liver damage, and an UL of 10 mg/day ( $\approx 0.14$  mg/kg/day) of copper was established for adults ( $\geq 19$  years old).

EPA (IRIS 2001) does not list an RfC or RfD for copper.

The National Toxicology Program (NTP 2001) does not list copper as a substance known or reasonably anticipated to be a human carcinogen and IARC has not published a monograph on the potential carcinogenicity of copper. EPA (IRIS 2001) has classified copper in Group D-*not classifiable as to human carcinogenicity*, based on a lack of human data, inadequate animal data, and equivocal mutagenicity data.

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

A TTD for the hepatic effects of oral exposure to copper was derived to serve as a provisional value until such time as the toxicological profile on copper is updated. An RDA has been estimated at 0.9 mg/day ( $\approx 0.013$  mg/kg/day) by the Institute of Medicine (2001: prepublication document, final version not published as of December 2001). In addition, an UL of 10 mg/day ( $\approx 0.14$  mg/kg/day) of copper was established for adults ( $\geq 19$  years old). The critical effect for overexposure to copper was considered to be liver damage. Pending publication of the final version of the documentation by the Institute of Medicine, this UL appears suitable for use as a  $TTD_{HEPATIC}$  for copper.

### Summary (TTD for Copper)

$$TTD_{HEPATIC} = 0.14 \text{ mg/kg/day}$$

## D.6 References

- ATSDR. 1990. Toxicological profile for copper. Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- Barceloux DG. 1999. Copper. *Clin Toxicol* 37(2):217-230.
- Bremner I, Beattie JH. 1995. Copper and zinc metabolism in health and disease: Speciation and interactions. *Proc Nutr Soc* 54:489-499.
- Dameron CT, Harrison MD. 1998. Mechanisms for protection against copper toxicity. *Am J Clin Nutr* 67:1091S-1097S.
- EPA. 1987. Summary review of the health effects associated with copper. Health Issue Assessment. Cincinnati, OH: Environmental Criteria and Assessment Office. U.S. Environmental Protection Agency, 29.
- Flora SJS, Coulombe RA, Sharma RP, et al. 1989a. Influence of dietary protein deficiency on lead-copper interaction in rats. *Ecotoxicol Environ Saf* 18:75-82.
- Friberg L, Nordberg GF, Vouk VB, eds. 1986. Handbook on the toxicology of metals. 2<sup>nd</sup> ed, Vol. II: Specific Metals. Amsterdam: Elsevier Science Publishing Co. Inc, 242, 668, 671.
- Gorell JM, Johnson CC, Rybicki BA, et al. 1997. Occupational exposure to metals as risk factors for Parkinson's disease. *Neurology* 48:650-658.
- Gorell JM, Johnson CC, Rybicki BA, et al. 1999. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicology* 20(2-3):239-248.
- Hardman JF, Lmbird LE, eds. 1996. Goodman & Gilman's the pharmacological basis of therapeutics. New York, NY: McGraw-Hill, 120, 123-124, 252.
- Institute of Medicine. 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 7-1 - 7-27. [Http://www.nap.edu/books/0309072794/html/](http://www.nap.edu/books/0309072794/html/). December 19, 2001.
- IRIS. 2001. Integrated Risk Information System. U.S. Environmental Protection Agency. [Http://www.epa.gov/iris/subst/index.htm](http://www.epa.gov/iris/subst/index.htm). April 17, 2001.
- Malhotra KM, Shukla GS, Chandra SV. 1982. Neurochemical changes in rats co-exposed to lead and copper. *Arch Toxicol* 49:331-336.
- NRC. 1989. Recommended dietary allowances. 10<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council, 205-213, 224-235, 284-285.
- NRC. 1995. Nutrient requirements of laboratory animals. 4<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council, 11-102.

NRC. 1998. Nutrient requirements of swine. 10<sup>th</sup> ed. Washington, DC: National Academy Press. National Research Council, 47-49, 110-111, 115-116, 121-122.

NTP. 2001. 9<sup>th</sup> report on carcinogens. Research Triangle Park, NC: U.S. Department of Health and Human Services. National Toxicology Program. [Http://ehp.niehs.nih.gov/roc/toc9.html](http://ehp.niehs.nih.gov/roc/toc9.html). September 11, 2001.

