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 under-estimate 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 ≈40 µg/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 combina-

tion. The greater effects in the combination group could be due to the greater total dose of metals rather than to an interaction. In addition, the effects seen during lactation could have been secondary effects due to decreased quality or quantity of milk produced by the dams as a result of the toxic insult. As in the previous study by these investigators, coadministration of lead had no affect 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 μmole/kg of lead (1 mg/kg) plus 5 μmole/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, β-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 intraperi-toneally (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[a] (r)[b]		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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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	Greater than additive	Additive/no effect	Less than additive	Conclusions	References
		Results				
		Greater than additive	Additive/no effect	Less than additive	Conclusions	References
		Oral exposure (mg/kg/day)				
Intermediate	Blood and Hepatic Pb levels			1–4 i.p. + 2[a] (r)[b]	<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)[b]		additive	Chandra et al. 1981
Acute (7–14 days)	Neurological (brain norepinethrine, 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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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 ≈50 µg/dL throughout the study. In addition, zinc supplementation had no effect on urinary excretion of ALA and β_2-microglobulin (indicators of hemato-logical 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 ≈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 µg/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 µg/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 µg/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, ≈0.6 mg/kg/day), adequate (35 ppm, ≈2.7 mg/kg/day), and excess (200 ppm, ≈15.6 mg/kg/day) zinc as the carbonate in the diet for 3–7 weeks, and/or low (50 ppm, ≈3.9 mg/kg/day) and high (200 ppm, ≈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 ≈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 (≈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, ≈2, and 10 mg Zn/kg/day) as the sulfate and/or lead at 20 mg/L in the drinking water (≈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, ≈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 deceased 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, ≈430 mg Pb/kg/day) and zinc carbonate (6,300 ppm Zn, ≈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 (≈44 mg/kg/day) as lead acetate with or without 4,000 ppm zinc (≈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 ≈75% (to ≈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 (≈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 μg/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 µg/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 µg/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 Greater than additive	Results Additive/no effect	Results Less than additive	Conclusions	References
		Oral exposure (mg/kg/day)				
Intermediate	Hematological (anemia)		103 + 187[a] (s)[b]		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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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 Greater than additive	Results Additive/no effect	Results Less than additive	Conclusions	References
Inhalation (occupational) exposure (Zn mg/kg/day + PbB µg/DL)						
Intermediate	Hematopoietic (urinary ALA)		0.9 oral + 50[a] (ha)[b]		additive	Lauwerys et al. 1983
Intermediate	Renal (urinary β_2-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	Hematopoitic (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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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[a] (ha)[b]		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 — Greater than additive	Results — Additive/no effect	Results — Less than additive	Conclusions	References
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

[a] First dose listed is for the chemical influencing the other chemical's tissue concentrations.
[b] 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 ≥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 (≈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 µg/dL during two 7-day periods without supplemental copper and 4.11 µg/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, ≈1.8 mg/kg/day) as the chloride, nor lead (200 ppm, ≈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, ≈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 (≈14 mg/kg/day) as the acetate in drinking water and/or 100 ppm copper (≈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 affect 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 (≈20 mg/kg/day) as the acetate, and/or copper intraperitoneally at 2 mg/rat/day (≈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 hematopoetic toxicity. Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, and inhibiting the insertion of iron into protoporphyrin, mediated by ferrochelatase. As a result of alterations in the activity of ALAS and ALAD, ALA accumulates in blood, urine, and soft tissues. 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 β-hydroxylase, tyrosinase (tyrosine hydroxylase), and ascorbic acid oxidase (ATSDR 1990; Institute of Medicine 2001). Dopamine β-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[a] (r)[b]		additive	Flora et al. 1989a
Intermediate	Serum cerulo-plasmin, 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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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			$0.071 + 0.0036^a$ (ha)[b]	<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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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 dopamininergic 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 favorable 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, ≈897 mg/kg/day) as zinc carbonate, with or without supplemental oral copper (0.2 mg/day, ≈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 (≈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; ≈15 mg/kg/day) with and without excess zinc (1,000 ppm dry weight; ≈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 Hidiroglou 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 immuno-logical 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, ≈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 gastro-intestinal 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[a] (r)[b]	<additive	Ogiso et al. 1974
Acute (7 days)	Absorption (vascularly perfused rat intestine after *in vivo* 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 Hidiroglou 1989
Intermediate	Hematological (hematocrit, clinical signs of hemolyis, jaundice)			15 + 15 (q)	<additive	Jenkins and Hidiroglou 1989
Intermediate	Hepatic (plasma GOT; jaundice)			15 + 15 (q)	<additive	Jenkins and Hidiroglou 1989
Intermediate	Body weight gain			15 + 15 (q)	<additive	Jenkins and Hidiroglou 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 Hidiroglou 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 Hidiroglou 1989

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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 *in vivo* exposure)		2.9[a] + 14.4 (r)[b]		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

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] 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 (=IIICii), 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 (=IIIC). 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
Direction of Interaction	**Direction**
= Additive	0
> Greater than additive	+1
< Less than additive	–1
? Indeterminate	0

Quality of the Data	Weighting
Mechanistic Understanding	
I. Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II. Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely 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
Toxicological Significance	
A. The toxicological significance of the interaction has been directly demonstrated.	1.0
B. The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C. The toxicological significance of the interaction is unclear.	0.32
Modifiers	
1. Anticipated exposure duration and sequence.	1.0
2. Different exposure duration or sequence.	0.79
a. *In vivo* data	1.0
b. *In vitro* data	0.79
i. Anticipated route of exposure	1.0
ii. Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from –1 through 0 to +1

*Source: ATSDR 2001a, 2001b

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

BINWOE: =IIICii (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). Intra-peritoneal 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

BINWOE: >ICii (+1 x 1.0 x 0.32 x 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 under-estimate 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. It 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 hematoxicity. 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; Mauras 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 x 1.0 x 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 doses 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. It 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 hematopoetic toxicity. Lead alters heme synthesis by stimulating mitochondrial ALAS, directly inhibiting ALAD, and inhibiting the insertion of iron into protoporphyrin, mediated by ferrochelatase. As a result of alterations in the activity of ALAS and ALAD, ALA accumulates in blood, urine, and soft tissues (ATSDR 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: =IIIC (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 neuro-transmitter 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 ($-1 \times 0.71 \times 1.0 = -0.71$)

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

BINWOE: <IB (–1 x 1.0 x 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 Hidiroglou 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 Hidiroglou 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 Hidiroglou 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 Hidiroglou 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 Hidiroglou 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.