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

As presented previously, the mixture of lead, manganese, zinc, and copper was chosen as the subject of this interaction profile based on an analysis of the most frequently occurring binary mixtures in completed exposure pathways at hazardous waste sites. These metals are commonly found in soil. The exposure scenario of greatest concern for this mixture is long-term, low-level oral exposure. The components of this mixture vary in concentration and in proportion to each other from one hazardous waste site to another, and from one point of exposure to another. The ideal basis for the assessment of joint toxic action of this (or other) environmental mixtures would be data and models of joint toxic action for the toxicity and carcinogenicity of the complete mixture or validated physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models that would support prediction of the effects of different doses and proportions of mixture components.

As discussed in Section 2.3, no adequate epidemiological or toxicological studies and no PBPK models are available for the quaternary mixture. A few occupational and environmental exposure studies of the trinary mixture of lead, zinc, and copper are available, but are not adequate to serve as the basis for any conclusions regarding the toxicity of this submixture due to deficiencies in their design and inconsistencies in results across studies by the same group of investigators (Antonowicz et al. 1990; Araki et al. 1992, 1993a, 1993b; Murata and Araki 1991; Murata et al. 1993; Storm et al. 1994). In general, the effects seen during coexposure to lead, zinc, and copper were characteristic of lead toxicity. Whether coexposure to zinc and copper provided partial protection against these effects cannot be determined from the data.

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

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

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

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

Endpoint	Chemical			
	Lead PbB µg/dL	Manganese (mg/kg/day)	Zinc (mg/kg/day)	Copper (mg/kg/day)
Neurological	10[a]	0.07[b]	NA	NA
Hematological	10[a]	NA	0.3[c]	NA
Hepatic	NA	NA	NA	0.14[d]

[a]CDC (1991) PbB level of concern, adopted as TTD
[b]Upper end of ESADDI range, recommended as guidance value (ATSDR 2000), adopted as TTD
[c]Intermediate oral MRL, adopted as chronic MRL (ATSDR 1994)
[d]UL (Institute of Medicine 2001), adopted as TTD
NA = not applicable

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

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

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

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

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

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

n = neurological, h = hematological, p = hepatic

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

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

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

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).

TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).

MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii: different route of exposure (0.79).

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

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

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

where HI_{NEURO} is the hazard index for neurological toxicity, E_{Pb} is the exposure to lead (as predicted PbB in µg/dL), $CDC\ PbB_{NEURO}$ is the CDC PbB of concern (10 µg/dL) for the neurological toxicity of lead (ATSDR 1999; CDC 1991), and E_{Mn} is the exposure to manganese (as the oral intake in the same units as the corresponding TTD, mg/kg/day), and $TTD_{Mn\ NEURO}$ is the upper end of the ESADDI range, recommended as a guidance value by ATSDR (2000), and adopted as the TTD for neurological effects. A similar procedure is used to calculate the endpoint-specific hazard index for hematological effects.

If one or both of the endpoint-specific hazard indexes exceed one, they provide preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of the components on that endpoint (ATSDR 2001a). The qualitative WOE method is then used to estimate the potential impact of interactions on the endpoint-specific hazard indexes (Figure 2, ATSDR 2001a), using the BINWOEs developed in this profile. As discussed in ATSDR (2001a), when the endpoint-specific hazard index is greater than unity and/or when the qualitative WOE indicates that joint toxic action may be greater than additive, further evaluation using methods described by ATSDR (1992) is needed. Similarly, if the hazard quotient for the hepatoxicity of copper exceeds one, it provides preliminary evidence that copper may constitute a health hazard. Coexposure to lead is predicted to have no effect, and coexposure to zinc may be protective against copper's hepatic toxicity. The impact of coexposure to manganese is indeterminate. Depending on the magnitude of the hazard quotient for copper, and of exposure to the

other components of the mixture, further evaluation using methods described by ATSDR (1992) may be needed.