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

Benzene, toluene, ethylbenzene, and xylenes frequently occur together at hazardous waste sites. The four chemicals are volatile and have good solvent properties. Toxicokinetic studies in humans and animals indicate that these chemicals are well absorbed, distribute to lipid-rich and highly vascular tissues such as the brain, bone marrow, and body fat due to their lipophilicity, and are rapidly eliminated from the body (Appendices A, B, C, and D). Metabolism of the four chemicals is dose-dependent and generally extensive at dose levels that do not saturate the first metabolic step of each compound, which involves cytochrome P-450-dependent mixed function oxidases. The predominant cytochrome P-450 isozyme involved in the metabolism of each chemical is CYP2E1. As discussed in Chapter 2 and the Appendices, all four chemicals can produce neurological impairment via parent compound-induced physical and chemical changes in nervous system membranes. Exposure to benzene can additionally cause hematological effects including aplastic anemia, with subsequent manifestation of acute myelogenous leukemia, via the action of reactive metabolites.

No data are available on toxic or carcinogenic responses to whole mixtures of BTEX. To conduct exposure-based assessments of possible health hazards from BTEX in the absence of these data, a component-based approach that considers both the shared (neurologic) and unique (hematologic/ immunologic/carcinogenic) critical effects of the chemicals is recommended. In particular, as explained below, it is advised that (1) the Hazard Index approach be used to assess the joint neurotoxic hazard of the four mixture components, and (2) the hematological and carcinogenic hazards be assessed on a benzene-specific basis. As discussed by ATSDR (1992, 2001a), exposure-based health assessments are used, in conjunction with evaluation of community-specific health outcome data, consideration of community health concerns, and biomedical judgement, to assess the degree of public health hazard presented by mixtures of hazardous substances released into a community.

Neurotoxicity is the critical noncancer effect of concern for BTEX mixtures. Neurological impairment forms the basis for 9 of the 13 MRLs for the component chemicals, including 6 of 8 inhalation MRLs, as summarized in Table 2-11. The six neurotoxicity-based inhalation MRLs are for benzene (intermediate MRL), toluene (acute and chronic MRLs), and mixed isomers of xylene (acute, intermediate, and chronic MRLs). The two other inhalation MRLs are based on immunotoxicity (the acute MRL for benzene) and developmental toxicity (the intermediate MRL for ethylbenzene). These MRLs do not imply that there is a low neurotoxic potential for acute exposure to benzene and intermediate exposure to ethylbenzene, but rather that there is insufficient information on sensitive neurological effects at low levels of exposure.

Available exposure-response data on neurological effects for these chemicals and duration categories mainly reflect relatively insensitive endpoints, such as overt symptoms and signs of central nervous system toxicity (ATSDR 1997, 1999b). Although these data clearly show that the nervous system is a target of benzene and ethylbenzene (also see information summarized in Appendices A and C), more studies are needed to identify thresholds for neurotoxicity and to better characterize the relative sensitivity of neurological, immunological, and developmental endpoints. Because neurotoxicity is likely to be as sensitive an effect as immunotoxicity and developmental toxicity for these chemicals as discussed in Section 2.3, it is reasonable to assume that the acute MRL for benzene and the intermediate MRL for ethylbenzene are also protective of neurological effects, indicating that these MRLs can also be used as risk guidance values for neurotoxicity.

Derivations of a chronic MRL for benzene, an intermediate MRL for toluene, and acute and chronic MRLs for ethylbenzene were precluded by insufficient inhalation exposure-response data for neurotoxicity and other health endpoints (ATSDR 1995, 1997, 2000). Although no data were suitable for deriving an intermediate-duration inhalation MRL for toluene, ATSDR (2000) concluded that the neurotoxicity-based chronic MRL would also be protective for intermediate-duration exposures. A neurotoxicity-based guidance value can be estimated for chronic exposure to benzene by applying a duration uncertainty factor of 10 to the intermediate MRL for benzene. A guidance value for chronic exposure to ethylbenzene can be similarly estimated by applying a factor of 10 to the neurotoxicity-based intermediate guidance value for ethylbenzene. The intermediate-duration guidance value for ethylbenzene can additionally be used as a guidance value for acute exposure. Considering the available inhalation MRLs and risk guidance values for benzene, toluene, ethylbenzene, and xylene discussed above and in the preceding paragraph, all 12 possible values are plausibly based on neurotoxicity as summarized in Table 3-1.

The hazard index method is recommended for assessing the joint neurotoxic hazard of BTEX because this approach is most appropriately applied to mixture components that cause the same effect by the same mechanism of action (ATSDR 2001a). A hazard index is calculated for each exposure scenario of concern by first determining a hazard quotient for each of the mixture components. A hazard quotient is the ratio of an exposure estimate to the appropriate MRL, reference dose (RfD)/reference concentration (RfC), or guidance value. Available inhalation MRLs and guidance values for BTEX components are summarized in Table 3-1. The assessment should proceed if two or more of the individual components

Table 3-1. Inhalation MRLs and Risk Guidance Values for Neurological Effects of BTEX

Chemical	Exposure Duration		
	Acute	Intermediate	Chronic
Benzene	0.05 ppm (guidance value)[a]	0.004 ppm (MRL)	0.0004 ppm (guidance value)[b]
Toluene	1 ppm (MRL)	0.08 ppm (guidance value)[c]	0.08 ppm (MRL)
Ethylbenzene	1 ppm (guidance value)[d]	1 ppm (guidance value)[e]	0.1 ppm (guidance value)[b]
Xylenes (mixed)	1 ppm (MRL)	0.7 ppm (MRL)	0.1 ppm (MRL)

[a]The immunotoxicity-based acute MRL is used as a guidance value for neurotoxicity.
[b]Estimated by dividing the intermediate-duration value by an uncertainty factor of 10 to adjust for chronic exposure.
[c]ATSDR (2000) concluded that the neurotoxicity-based chronic inhalation MRL for toluene will also be protective for intermediate-duration exposures.
[d]The intermediate-duration guidance value is assumed to be protective for acute exposures.
[e]The developmental toxicity-based intermediate-duration MRL is used as a guidance value for neurotoxicity.

have hazard quotients equaling or exceeding ratios of 0.1 (see Figure 2 in the *Guidance Manual for the Assessment of the Joint Toxic Action of Chemical Mixtures*, ATSDR 2001a). If only one or if none of the components 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 a significant health hazard. As exposure levels approach threshold levels for toxic effects, a hazard index approach is likely to give a more complete assessment of health hazards than an approach that only examines hazard quotients for individual components in a mixture.

Under conditions for proceeding with the hazard index approach, the hazard quotients are summed to derive the hazard index for neurological effects as follows:

$$HI_{NEURO} = \frac{E_B}{MRL_B} + \frac{E_T}{MRL_T} + \frac{E_E}{MRL_E} + \frac{E_X}{MRL_X}$$

where *HI* is the hazard index, *E* represents the exposure estimates for the individual components, MRL represents the appropriate minimal risk level or guidance value for the components, and B, T, E, and X represent benzene, toluene, ethylbenzene, and xylene. A different hazard index is derived for each duration of exposure (acute, intermediate, and chronic) and exposure route of concern. The calculated indexes will provide indicators of the hazard for neurotoxicity from exposure to the BTEX mixture. Preliminary evidence that exposure to the mixture may constitute a hazard for neurological impairment is provided if the hazard index for a particular exposure scenario exceeds one. As the value of the hazard index increases above one, there is increased concern for the possibility of a health hazard as well as the need for further evaluation using methods described by ATSDR (1992).

The addition of hazard quotients for a particular exposure scenario assumes that the mixture components additively act on a common toxicity target by a common mechanism or mode of action, and that less-than-additive (e.g., antagonistic interactions) or greater-than-additive (e.g., synergism or potentiation) interactions do not occur among the components of the mixture. A primary objective of this profile was to assess available information on modes of joint toxic action of benzene, toluene, ethylbenzene, and xylenes. As discussed in Section 2.3, information from PBPK modeling studies of BTEX and ternary (toluene/ethylbenzene/xylene) and quinary (DBTEX) mixtures of its components, supported by data on neurotoxic interactions in binary component mixtures, can be used to evaluate the possible influence of component interactions in the overall neurotoxicity of BTEX. The PBPK studies are particularly relevant because they provide information on exposure levels of the mixture of concern and corresponding

predicted blood concentrations of the parent compounds after possible metabolic interactions (i.e., net blood levels of the form of the chemicals expected to act on target neuronal membranes).

Based on the results of PBPK model simulations and experimental exposures with BTEX in rats and the ternary and quinary component mixtures in humans and rats (Haddad et al. 1999a, 1999b, 2000, 2001; Tardif et al. 1997), it was concluded that inhalation exposure to mixtures of approximately 20 ppm each of benzene, toluene, ethylbenzene, and xylene is unlikely to result in biologically significant increases in blood levels of these chemicals in humans compared to individual chemical exposure. Although the interactions threshold could be better defined for benzene and the other mixture components by using the human PBPK model for DBTEX (Haddad et al. 2001) to conduct simulations of BTEX (i.e., by using it as a human BTEX model), the available predictions clearly indicate that metabolic interactions are probably negligible at $\leq$20 ppm of each component, which implies that environmental exposures to BTEX are well below the threshold for interactions. Competitive metabolic inhibition is the most plausible mechanism of interaction among the BTEX components based on the PBPK studies as well as *in vitro* and *in vivo* metabolism and toxicity studies for some of the binary component mixtures, as discussed in Section 2.3. Therefore, due to the apparent lack of competitive metabolic interactions in BTEX mixtures below approximately 20 ppm of each component, it is plausible that joint neurotoxic actions among the chemicals will be additive at environmental levels of exposure. Exposure to higher concentrations of BTEX components (i.e., above the threshold for metabolic inhibition) would be expected to lead to greater than additive increases in blood levels of parent compounds and, consequently, increased concern for neurotoxicity. However, as discussed in Section 2.3, it is unclear whether the PBPK model descriptions are adequate for predicting interactions from inhalation of BTEX mixtures above approximately 200 ppm of each component, or if they are appropriate for oral exposures. Studies that directly examined the joint toxic action of BTEX chemicals on the nervous system are essentially limited to a few human and animal inhalation studies of some binary mixtures of components, particularly benzene/toluene, toluene/xylene, and ethylbenzene/xylene (Dudek et al. 1990; Frantik and Vodickova 1995; Frantik et al. 1988; Korsak et al. 1988, 1992; Toftgard and Nilsen 1981, 1982). As discussed in Section 2.3, the neurotoxicity studies of the binary mixtures provide no data that conflict with the predictions of the PBPK studies (i.e., that joint action is expected to be additive at BTEX concentrations below approximately 20 ppm of each component). In summary, based on evidence from PBPK and neurotoxicity studies supporting the plausibility of additive joint action at the shared target of toxicity at relatively low levels of exposure, the hazard index approach is recommended for assessing possible neurotoxic health hazards from environmental exposures to BTEX. This is a conservative approach for assessing BTEX due to the protective nature of the MRLs and guidance values on which it is based, the

data indicating that greater-than-additive interactions are unlikely at component doses that would not otherwise be overtly toxic, and because the neurotoxicity of the mixture would be decreased, not increased, if interactions were less than additive at low levels of exposure.

Hematotoxicity and carcinogenicity are additional concerns for exposure to BTEX based on strong evidence that benzene induces these health effects in humans and that ethylbenzene is carcinogenic in animals. It is well established that long-term exposure to benzene can cause damage to the human hematopoietic system, resulting in effects that include aplastic anemia with subsequent development of leukemia, through the action of its metabolites (see Appendix A). Reactive metabolites also appear to be involved in the induction of kidney, liver, testicular, and other tumors in rats and mice exposed to ethylbenzene (see Appendix C). The carcinogenic potential of benzene is recognized by its consensus classification as a human carcinogen by NTP (2001), EPA (IRIS 2001), and IARC (1987). IARC (2000) has classified ethylbenzene as possibly carcinogenic to humans on the basis of the positive animal data. Ethylbenzene was not determined to be a known or anticipated human carcinogen by NTP (2001) or classifiable as to human carcinogenicity by EPA (IRIS 2001), but these assessments predate the animal data used as the basis of the IARC classification and precluded the derivation of a cancer risk value by EPA. The lack of evidence for the carcinogenicity of the other BTEX chemicals is reflected by the classification of toluene and xylenes as not classifiable as to human carcinogenicity by EPA (IRIS 2001) and IARC (1999a, 1999b).

The evaluation of possible hematotoxic and carcinogenic hazards from exposure to BTEX is best approached by evaluating benzene as a single component. PBPK model predictions indicate that toluene, ethylbenzene, and xylene are unlikely to influence the hematotoxicity or carcinogenicity of benzene, and benzene, toluene, and xylene are unlikely to affect the carcinogenicity of ethylbenzene, at environmental levels of exposure. Exposure to relatively high concentrations of BTEX (above approximately 20 ppm of each component) would be expected to result in reduced blood levels of benzene and ethylbenzene metabolites (compared to exposure to benzene and ethylbenzene alone) due to competitive metabolic interactions (Haddad et al. 1999a, 1999b, 2001; Tardif et al. 1997), thereby decreasing the potential for hematotoxicity and carcinogenicity. Binary interaction studies in animals similarly indicate that toluene can inhibit the hematological effects of benzene, although data are insufficient to conclude that the protective interaction would occur at low doses (see Section 2.2.3). Considering the causal relationship between the noncancer hematological effects of benzene and subsequent manifestation of leukemia, as well as the lack of a cancer risk value for ethylbenzene, it is recommended that an overall assessment of the hematotoxic and carcinogenic hazards of BTEX be conservatively based on benzene cancer risk as

discussed in Section 2.3. Benzene, therefore, should be evaluated as a single component using ATSDR (1992) public health assessment guidance, which indicates that exposure will be a concern if the estimated risk of cancer equals or exceeds $1x10^{-6}$. Increased lifetime cancer risks for inhalation exposure are estimated by multiplying the unit risk for benzene by the estimated exposure (air concentration).