INTERACTION PROFILE FOR:
Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX)

U.S. Department of Health and Human Services
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PREFACE

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

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

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

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

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

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

SUMMARY

Benzene, toluene, ethylbenzene, and xylenes frequently co-occur at hazardous waste sites. Various combinations of these chemicals are among the most frequently found binary mixtures in completed exposure pathways at hazardous waste sites. Media contaminated with these chemicals include air, water, and soil. Contamination of groundwater can result in volatilization into indoor air when the groundwater is used as household water. In addition, contamination of groundwater and subsurface soil can result in migration of these chemicals into basements as soil gas. The purposes of this profile are: (1) to evaluate data on the toxicology of mixtures of benzene, toluene, ethylbenzene, and xylenes (BTEX); (2) to evaluate data on the joint toxic actions (e.g., additive, less-than-additive, or greater-than-additive joint actions) of these chemicals in producing health hazards; and (3) to make recommendations for exposure-based assessments of the potential impact of joint toxic action of the mixture on public health.

No studies are available that directly characterize health hazards and dose-response relationships for exposures to "whole" mixtures of BTEX. Exposure to each of the individual chemicals can produce neurological impairment via parent chemical-induced changes in neuronal membranes. Benzene can additionally cause hematological effects, which may ultimately lead to aplastic anemia and acute myelogenous leukemia, and there is evidence that ethylbenzene is carcinogenic in other tissues. No studies were located that directly examined joint toxic actions of benzene, toluene, ethylbenzene, and xylenes on the nervous system, but additive joint neurotoxic action is plausible for environmental exposures based on predictions from physiologically based pharmacokinetic (PBPK) modeling studies with BTEX and a ternary mixture of its components, and supporting data from neurotoxicity interaction studies of binary component mixtures.

In the absence of data on toxic or carcinogenic responses to the whole mixture, possible health hazards from exposures to BTEX are best assessed using a component-based approach that considers both the shared (neurologic) and unique (hematologic/carcinogenic) critical effects of the constituent chemicals. A hazard index approach that assumes additive joint action and uses ATSDR Minimal Risk Levels (MRLs) and guidance values based on neurological impairment is recommended for exposure-based assessments of possible neurotoxic health hazards from the four components. The possible hematotoxic and carcinogenic hazards of BTEX exposures should be evaluated on the basis of benzene alone due to the causal relationship between the noncancer hematological effects of benzene and the development of leukemia, and the lack of a cancer risk value for ethylbenzene. It therefore is recommended that the cancer unit risk value for benzene be used to jointly assess possible hematotoxic and carcinogenic hazards from exposures to BTEX.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienist
AML	acute myelogenous leukemia
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	areas under the blood concentration curves
B	benzene
BEI	biological exposure index
BHIs	biological hazard indexes
BINWOE	binary weight-of-evidence
BTEX	benzene, toluene, ethylbenzene, and xylenes
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CI	confidence interval
cm	centimeters
CYP	cytochrome P-450
DA	dopamine
DBTEX	dichloromethane, benzene, toluene, ethylbenzene, and xylenes
DNA	deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DT	Division of Toxicology
E	ethylbenzene
EC_{50}	effective concentration, 50%
EPA	Environmental Protection Agency
FQPA	Food Quality Protection Act
HI	hazard index
5-HIAA	5-hydroxyindoleacetic acid
5-HT	indoleamine serotonin
HVA	homovanillic acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
kg	kilogram
L	liter
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m^3	cubic meters
mg	milligram
mL	milliliter
mm	millimeter
MRI	magnetic resonance imagery
MRL	Minimal Risk Level
NADH	nicotinamide adenine dinucleotide phosphate (reduced form)
NADPH	nicotinamide adenine dinucleotide phosphate (oxidized form)
NE	norepiephrine
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OPT	olfactory perception thresholds
PBPK	physiologically based pharamacokinetic
ppm	parts per million
RD_{50}	respiratory depression, 50%
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SC	simulated concentration
SD	standard deviation
STEL	short-term exposure limit
T	toluene
TLV	threshold limit value
TTD	target-organ toxicity dose
TWA	time-weighted average
UDP	uridine-5'-diphosphate
µg	micrograms
µL	microliters
µmol	micromole
U.S.	United States
VMA	vanillylmanelic acid
X	xylenes
〉	greater than
≥	greater than or equal to
=	equal to
〈	less than
≤	less than or equal to

1. Introduction

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

Benzene, toluene, ethylbenzene, and xylenes frequently co-occur at hazardous waste sites. Various combinations of these chemicals are among the most frequently found binary mixtures in completed exposure pathways at hazardous waste sites. Media contaminated with these chemicals include air, water, and soil. Contamination of groundwater can result in volatilization into indoor air when the groundwater is used as household water. In addition, contamination of groundwater and subsurface soil can result in migration of these chemicals into basements as soil gas. The chemicals are used as solvents in products such as paints and coatings, and are constituents of petroleum products, particularly gasoline, jet fuels, and kerosene. The BTEX chemicals are discussed in the Toxicological Profile on Total Petroleum Hydrocarbons (ATSDR 1999a), but more recent information, including a physiologically-based pharmacokinetic (PBPK) model for the whole mixture, has triggered this reassessment of the joint toxic action of these chemicals.

Each of the chemicals in the mixture of concern is volatile, well absorbed, extensively metabolized, and does not persist in the body for long periods of time. All of the BTEX chemicals can produce neurological impairment, and exposure to benzene can additionally cause hematological effects including aplastic anemia and acute myelogenous leukemia. The critical nature of the neurotoxicity (i.e., the noncancer effect expected to occur at the lowest exposure levels) is reflected by the use of neurological impairment as the basis for 9 of 13 MRLs for BTEX chemicals, including 6 of 8 inhalation MRLs (ATSDR 1995, 1997, 1999b, 2000). The carcinogenic (leukemogenic) potential of benzene is well established as indicated by its consensus classification as a human carcinogen by the National Toxicology Program (NTP 2001), U.S. Environmental Protection Agency (EPA) (IRIS 2001), and International Agency for Research on Cancer (IARC 1987). Ethylbenzene is possibly carcinogenic to humans based on a recent assessment by IARC (2000). Toluene and xylenes have been categorized as not classifiable as to human carcinogenicity by both EPA (IRIS 2001) and IARC (1999a, 1999b), reflecting the lack of evidence for the carcinogenicity of these two chemicals.

2. Joint Toxic Action Data for the Mixture of Concern and Component Mixtures

2.1 Mixture of Concern

No data were located regarding health effects in humans or animals exposed to mixtures exclusively containing benzene, toluene, ethylbenzene, and xylene. Some toxicokinetic and mechanistic information is available from an interaction-based PBPK model of this mixture, as discussed below (Haddad et al. 1999a).

A PBPK model has been developed for mixtures of BTEX in the rat (Haddad et al. 1999a). This model predicts toxicokinetic interactions in the quaternary mixture, as indicated by venous blood levels of chemicals, by using information on binary interactions among the component chemicals. Development of the model initially involved: (1) refining and verifying the validity of existing PBPK models for the four individual chemicals; (2) linking (interconnecting) pairs of the individual chemical PBPK models at the level of hepatic metabolism by introducing binary interaction terms for potential mechanisms of action (competitive, noncompetitive, and uncompetitive metabolic inhibitions[1]); and (3) characterizing the mechanism of interactions in the binary mixtures by optimally fitting model simulations to experimental data on venous blood concentrations of parent chemicals in rats exposed by inhalation to all binary combinations of the four components. For the characterization of the interaction mechanism for each binary mixture, groups of five rats were simultaneously exposed for 4 hours to 100 ppm of benzene and 50, 100, or 200 ppm of toluene, ethylbenzene, or xylene. The blood concentration data for the mixtures of toluene/xylene, xylene/ethylbenzene, and ethylbenzene/toluene were obtained from a PBPK modeling study of the ternary mixture of toluene, ethylbenzene, and xylene (Tardif et al. 1997, Section 2.2.1), and the data for the benzene mixtures (benzene/toluene, benzene/ethylbenzene, and benzene/xylene) were obtained as part of the quaternary mixture study. The PBPK analyses of the blood kinetic data from all binary exposure studies suggested competitive inhibition of hepatic metabolism as the most plausible mechanism of interaction (Haddad et al. 1999a). This mechanism was chosen among the others because the shape of the simulation curves of the venous blood concentrations of benzene, toluene, ethylbenzene,

[1]Simultaneous exposure to a mixture can cause two or more chemicals to compete for enzyme-mediated biotransformation, resulting in an inhibition of at least one of the chemicals. The inhibition is termed ‘competitive’ when the chemicals compete as substrates for the same site on an enzyme. In ‘noncompetitive inhibition’, the inhibitor binds to the enzyme, causing a change in the stereochemical arrangement of the enzyme such that the substrate cannot bind. If the chemicals have different enzymatic binding sites, and the substrate must first bind to the enzyme before the inhibitor can, the interaction is termed ‘uncompetitive inhibition’ (Purcell et al. 1990).

or xylene during binary exposures was closer to that of the experimental data, and it is conceptually the most logical choice, as all four chemicals are known substrates for the same cytochrome P450 isozyme (CYP2E1) at low exposure concentrations.

The metabolic inhibition constant (K_i) for each binary interaction was estimated from blood concentration data collected during exposure to the binary mixtures and incorporated into the quaternary mixture PBPK model (Haddad et al. 1999a). The quaternary model adequately simulated the inhalation kinetics (blood concentrations) of all four components in rats following the 4-hour exposure to quaternary mixtures of: (1) 50 ppm of each BTEX component; (2) 100 ppm of each BTEX component; and (3) 100 ppm of benzene and 50 ppm each of toluene, ethylbenzene, and xylene, thereby providing support for the *a priori* mechanism of competitive inhibition. The model indicates that the competitive inhibitory effect (leading to increased hepatic venous concentrations) tends to increase with increasing number and concentration of inhibitors, as illustrated by the blood concentration data in Table 2-1. The predicted blood level of benzene is about 40% higher from the binary mixture benzene/toluene compared to benzene alone. The addition of the third chemical, ethylbenzene, to the binary mixture resulted in levels of toluene and benzene that were increased approximately 26 and 16%, respectively, compared to the binary concentrations. Similarly, addition of xylene to the ternary mixture affected the kinetics of all three chemicals by increasing levels of benzene, toluene, and ethylbenzene by approximately 7, 6, and 9%, respectively, compared to the ternary concentrations. The magnitude of the modulation of binary interactions invoked by the addition of another chemical to an existing "network" of binary interactions (i.e., at the binary, ternary, or quaternary level) depends on its inhibition potency and blood concentration (Haddad et al. 1999a). With increasing mixture complexity, the blood level of a chemical is increased according to the potency and number of inhibitors, rather than by modification of the K_i for binary interactions.

Table 2-1. Quaternary PBPK Model Predictions of Hepatic Venous Blood Concentrations of Benzene (B), Toluene (T), Ethylbenzene (E), and Xylene (X) in Rats After a 4-hour Inhalation Exposure to 100 ppm B Alone or Combined With 100 ppm of T, E, and/or X

	Hepatic venous concentration							
	Benzene		Toluene		Ethylbenzene		Xylene	
Mixture components	mg/L	%	mg/L	%	mg/L	%	mg/L	%
B	1.847	—	—	—	—	—	—	—
B + T	2.576	39.4[a]	2.499	—	—	—	—	—
B + T + E	2.981	15.7[b]	3.150	26.0[b]	5.830	—	—	—
B + T + E + X	3.192	7.1[c]	3.328	5.7[c]	6.377	9.4[c]	6.244	—

[a]Percent increase compared to benzene alone
[b]Percent increase compared to binary mixture (B+T)
[c]Percent increase compared to ternary mixture (B+T+E)

Source: Haddad et al. 1999a

The PBPK model for BTEX (Haddad et al. 1999a) was linked to a PBPK model for dichloromethane to construct a quinary model for dichloromethane/benzene/toluene/ethylbenzene/xylenes (DBTEX) in rats (Haddad et al. 2000). The dichloromethane model was connected to the BTEX model by linking it to each component of the mixture via the binary interaction terms for metabolic inhibition. Analysis of blood kinetic data in rats that were exposed for 4 hours to 100 ppm of dichloromethane and 50, 100, or 200 ppm of benzene, toluene, ethylbenzene, or *m*-xylene was consistent with competitive metabolic inhibition as the interaction mechanism. This mechanism is plausible because all five chemicals are metabolized primarily by the same cytochrome P450 isozyme (CYP2E1). The DBTEX model adequately predicted the blood kinetics of the five chemicals in rats that were exposed to mixtures of various concentrations for 4 hours (100 ppm of dichloromethane and 50 or 100 ppm of toluene, ethylbenzene, or *m*-xylene, or 100 ppm of dichloromethane and 50 or 100 ppm of benzene, toluene, ethylbenzene, or *m*-xylene). The model for DBTEX in rats was scaled to humans by changing the rat physiological and physiochemical parameters to human values and keeping the biochemical parameters species-invariant (except for the K_m of dichloromethane) (Haddad et al. 2001). The assumption that the metabolic interaction constants are species-invariant was based on previous findings in a PBPK study of the ternary mixture toluene/ethylbenzene/xylene, in which the rat to human model extrapolation was validated with human experimental data (Tardif et al. 1997) (Section 2.2.2). Iterative use of the human DBTEX model

to estimate blood levels of the five chemicals, following 8-hour exposures to mixtures of 0.5 ppm benzene and varying concentrations of dichloromethane (10–50 ppm), toluene (5–50 ppm), ethylbenzene (10–100 ppm), and *m*-xylene (10–100 ppm), predicted that component interactions are negligible at exposure concentrations equal to and lower than 16 ppm dichloromethane, 0.5 ppm benzene, 16 ppm toluene, 33 ppm ethylbenzene, and 33 ppm xylene (Haddad et al. 2001). Simulations of BTEX in humans, which could be conducted by using the DBTEX model with the exposure concentration of dichloromethane set to zero, were not performed.

As discussed above, the Haddad et al. (1999a) study developed and validated a binary interaction-based PBPK model for simulating the blood concentrations of BTEX in rats following inhalation exposure to the quaternary mixture. The approach used to develop the BTEX model is an extension of that used to develop PBPK models for ternary mixtures of toluene, ethylbenzene, and xylene in rats and humans (Tardif et al. 1997). As discussed in Section 2.2.1, the model for the ternary mixture has been validated in humans exposed to low inhalation concentrations of the components. PBPK models for the quinary mixture DBTEX were similarly developed in rats and humans, although they were only validated in rats (Haddad et al. 2000, 2001). Because the models for the ternary, quaternary, and quinary mixtures are complementary due to their common basis (i.e., they use linked data on binary interactions to predict effects on component blood levels resulting from higher-order interactions), they are useful for assessing the relevance of joint toxic action of BTEX mixtures to public health as discussed in Section 2.3.

2.2 Component Mixtures

The following subsections present discussions of PBPK models and mechanistic information, as well as evaluations of pharmacokinetic and toxicity data, pertinent to the joint toxic action of combinations of benzene, toluene, xylene, and ethylbenzene. Information was found for two ternary and all six binary mixtures of these chemicals.

2.2.1 Benzene, Toluene, and Xylenes

PBPK models for mixtures containing benzene, toluene, and xylene have not been specifically reported. The PBPK model for BTEX (Section 2.1) could be used to model benzene/toluene/xylene by setting the exposure concentration of ethylbenzene to zero.

Blood cell counts (erythrocytes, leukocytes, and platelets) and levels of hemoglobin, leukocyte alkaline phosphatase, and serum immunoglobulins (IgG, IgA, and IgM) were evaluated in 35 painters who were "constantly" exposed to mixtures of benzene, toluene, and xylene during their work for an unspecified duration (Lange et al. 1973). Estimated mean workplace air concentrations ranges were 0.011–0.158 mg/L (3.4–49.0 ppm) for benzene, 0.08–0.27 mg/L (21.6–72.9 ppm) for toluene, and 0.12–0.63 mg/L (27.6–144.9 ppm) for xylene. A control group was comprised of 42 unexposed adults. Effects that were attributed to exposure included slight macrocytic anemia, thrombocytopenia, reduced leukocyte alkaline phosphatase, reduced serum IgG and IgA, and increased serum IgM, which are typical of benzene toxicity.

Possible interactions among the components of a benzene/toluene/xylene mixture were studied using an *in vitro* rat embryonic development assay (Brown-Woodman et al. 1994). The embryos were explanted on gestation day 10 and cultured with the chemicals in the medium for approximately 40 hours. Embryonic development was assessed by presence of a heart beat, yolk sac indexes, morphology and crown-rump length, number of somite pairs, and protein content of the embryos. The minimum concentration causing retardation of embryonic development and a no-effect level were determined for each of the component chemicals. The ternary mixture was tested at a total molar concentration in culture medium (2.37 µmol/mL serum) and compared with effects from a minimally toxic concentration of each component (2.87, 2.71, and 2.75 µmol/mL for benzene, toluene, and xylene, respectively). The average percentage of each chemical contributing to the total concentration in the ternary mixture was not reported, although an equal amount of each chemical (0.2 µL/mL serum) was added to the medium (i.e., before losses due to volatilization). The embryotoxic responses to the tertiary mixture (as indicated by the degree of retardation of embryonic growth and development) were similar to responses to each of the equimolar doses of the individual components, suggesting that benzene, toluene, and xylene jointly acted in an additive manner. For example, the effect of the ternary mixture on yolk sac diameter (5.44±0.76 mm) was not significantly different from that produced by benzene alone (4.95±0.66 mm), toluene alone (4.84±0.83 mm), or xylene alone (4.90±0.64 mm). While the results are suggestive of additive joint action of benzene, toluene, and xylene on these endpoints, a full characterization of the joint toxic action is precluded by several design limitations, including the lack of information on how dose and dose proportion of the components may influence joint action.

2.2.2 Toluene, Ethylbenzene, and Xylene

No data were located regarding health endpoints in humans or animals exposed to mixtures containing toluene, ethylbenzene, and xylene.

A PBPK model is available for the ternary mixture of toluene/ethylbenzene/*m*-xylene in rats and humans (Tardif et al. 1997). The approach used to develop this model is essentially the same as for the quaternary mixture (BTEX) summarized in Section 2.1.1 in that it is based on binary interactions in the component chemicals and accounts for all plausible binary chemical interactions. Existing individual PBPK models for toluene, xylene, and ethylbenzene were linked in pairs via the hepatic metabolism term (mechanism of metabolic interaction). The Michaelis-Menten equation for each binary mixture was modified to test four possible mechanisms of metabolic interaction: no inhibition, competitive inhibition, uncompetitive inhibition, and noncompetitive inhibition (see Footnote 1 in Section 2.1 for an explanation of the differences in these mechanisms of metabolic interaction). The metabolic inhibition constant (K_i) for each pair of chemicals was estimated from the best fit of the binary model simulations to previously determined experimental data on the blood concentrations of toluene, ethylbenzene, and xylene in rats exposed by inhalation to binary combinations of 100 or 200 ppm of each chemical for 4 hours (Tardif et al. 1993a, 1996). Competitive metabolic inhibition was considered to be the most plausible mechanism of interaction for all binary combinations of the mixture components (at relevant exposure concentrations), and incorporation of the corresponding K_i values into the ternary mixture model adequately simulated the time course of the blood concentrations of toluene, ethylbenzene, and xylene in rats exposed to a mixture containing 100 ppm of each of these chemicals for 4 hours in a previous study (Tardif et al. 1996).

Following validation of the ternary mixture model in the rat, it was scaled to predict the kinetics of toluene, ethylbenzene, and xylene in the blood and alveolar air of humans exposed to a combination of 17, 33, and 33 ppm, respectively, for 7 hours (Tardif et al. 1997). The scaling of the model from rats to humans involved: (1) substituting rat physiological parameters and blood:air partition coefficients with those of humans; (2) scaling the maximal velocity for hepatic metabolism (V_{max}) on the basis of body weight$^{0.75}$; and (3) keeping all other model parameters species-invariant. Assuming that the metabolic inhibition constants (K_i values) are species-invariant implies that the nature and magnitude of the competition involving toluene, ethylbenzene, and xylene for binding to CYP2E1 does not change between species. This assumption was accepted as the default because the cytochrome P-450 isozyme (CYP2E1) for metabolism of all three substrates (toluene, xylene, and ethylbenzene) is the same in rats

and humans, and it was previously used to successfully predict the kinetics of binary mixtures of toluene/xylene in humans based on competitive inhibition mechanism as elucidated in the rat (Tardif et al. 1995; see Section 2.2.7). The ternary model predictions were validated by comparison with experimental data on time-course of blood and alveolar air concentrations from four volunteers exposed for 7 hours to toluene (17 ppm), ethylbenzene (33 ppm), or xylene (33 ppm), alone or in combination. Each of these exposure levels is one-third of the respective American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (TLVs) for these chemicals, apparently selected with the expectation that the subjects would not experience toxic effects. This expectation was based on the standard ACGIH hazard index (HI) approach in which additive joint action is assumed for mixtures of chemicals producing similar effects (i.e., 17 ppm toluene/50 ppm toluene + 33 ppm ethylbenzene/ 100 ppm ethylbenzene + 33 ppm xylene/100 ppm xylene = 1, where the denominator is the TLV for each chemical) (ACGIH 2001).

The human PBPK model, based on the mechanism of competitive metabolic inhibition as elucidated in the rat, adequately simulated both the blood and alveolar air concentrations of toluene, xylene, or ethyl-benzene observed in the exposed humans (Tardif et al. 1997). Overall, both the human model predictions and experimental data indicated that blood and alveolar air concentrations during and after exposure to the ternary mixture were similar to those measured during and after exposure to the individual chemicals alone. A statistically significant increase was found for blood concentrations of xylene during the mixture exposure, but this change was not reflected by the alveolar air concentration data obtained during the same experiment. Experimental measurements of the major urinary metabolites of toluene (hippuric acid and *o*-cresol), xylene (methyl hippuric acid), and ethylbenzene (mandelic acid and phenyl glyoxylic acid), performed 3, 7, and 24 hours after the start of the 7-hour exposure period, similarly indicated that the ternary mixture did not significantly modify the metabolism of the individual components compared with exposure to the individual agents alone. With the exception of *o*-cresol and phenyl glyoxylic acid during the first 3 hours of exposure, the amounts of urinary metabolites were not significantly different between the individual and combined chemical exposures. The PBPK model was not used to simulate metabolite concentrations. These observations suggest that, at the exposure levels examined, competitive metabolic interactions did not occur among the three components.

The human ternary mixture PBPK model (Tardif et al. 1997) enabled calculation of biological hazard indexes (BHIs) for 8-hour exposures to varying simulated mixtures of the three chemicals (5–40 ppm toluene, 10–50 ppm *m*-xylene, and 10–50 ppm ethylbenzene) (Haddad et al. 1999b). The BHIs were calculated using the following equation:

$$BHI = \sum_{i-1}^{n} \frac{SC_i}{BEI_i}$$

SC refers to the simulated venous blood concentration of the component chemical and biological exposure index (BEI) refers to the blood level of that chemical in a healthy individual (i.e., at the TLV for toluene [50 ppm], *m*-xylene [100 ppm], and ethylbenzene [100 ppm]). SC values were predicted for the mixture components using the ternary mixture PBPK model, and individual chemical PBPK models were used to calculate the TLV-based BEIs. The BHIs were subsequently compared to exposure concentration-based HI values for each mixture, calculated as follows, where E is the exposure level of the chemical:

$$HI = \sum_{i-1}^{n} \frac{E_i}{TLV_i}$$

In principle because the PBPK model used to predict the SC values is interactions-based, the BHI values (based on blood levels) were expected to be the same as the HI values (based on exposure levels) if the toxicokinetic interactions among the mixture components are negligible. As shown in Table 2-2, the BHI values approach the HI values as the exposure concentrations of the components are reduced. The BHI and HI values were the same (i.e., 0.80) with mixtures of 10–20 ppm toluene, 20–30 ppm xylene, and 20–30 ppm ethylbenzene, indicating that competitive metabolic inhibitions are negligible at these concentrations.

Table 2-2. Comparison of Exposure-based Biological Hazard Indexes Calculated for Various Mixtures of Toluene (T), Ethylbenzene (E), and *m*-Xylene (X)

Exposure concentration (ppm)			HI	Venous blood concentration (mg/L)			BHI
T	E	X		T	E	X	
5	40	50	1	0.08	0.87	0.94	1.04
40	10	10	1	0.55	0.20	0.15	1.05
20	45	15	1	0.34	0.98	0.27	1.11
16.5	33	33	0.99	0.27	0.70	0.59	1.06
8	50	30	0.96	0.14	0.11	0.55	1.00
10	30	30	0.80	0.15	0.60	0.48	0.80
20	20	20	0.80	0.28	0.40	0.31	0.80

HI = hazard index; BHI = biological hazard index

Source: Haddad et al. 1999b

The effects of ternary and binary mixtures of toluene, xylene, and ethylbenzene on the kinetics of these chemicals in the blood were compared in rats (Tardif et al. 1996). Groups of four rats were exposed by inhalation for 4 hours to 100 or 200 ppm of each chemical singly, in six binary mixtures, and in one ternary mixture, such that the total concentration in all of the mixtures was 300 ppm (Table 2-3). Concentrations of unchanged toluene, xylene, and ethylbenzene in venous blood were measured postexposure (5–120 minutes) and areas under the blood concentration curves (AUC) were calculated. Exposures to both the ternary and binary mixtures resulted in significantly ($p<0.05$) higher AUCs of toluene, xylene, and ethylbenzene compared to the individual chemical exposures. For the ternary mixture exposure (100 ppm of each chemical), the AUCs for toluene, xylene, and ethylbenzene were increased by factors of 3.9, 3.6, and 2.4, respectively, compared to 100 ppm of the individual chemicals. There was generally no difference in the effect exerted by the ternary mixture and the binary mixtures when the comparison was based on AUCs for individual chemicals (the only exception was the AUC for xylene, which was increased more by coexposure to 200 ppm ethylbenzene in the binary mixture than by coexposure to 100 ppm ethylbenzene and 100 ppm toluene in the ternary mixture). When the total AUC (i.e., concentration) of a mixture was compared to the sum of the AUCs for its individual chemicals, four of the six binary mixtures produced higher total AUCs compared to the ternary mixture, although the

magnitude of increase in total AUC was greater for the ternary mixture (3.17-fold) than all of the binary mixtures (mean, 1.97-fold) (Table 2-3). The investigators surmised that the greater interactive effects in the ternary mixture compared to the binary mixtures of same total concentration (300 ppm) may be related to the concentration of one component in each binary mixture being near or above its metabolic saturation level (about 200 ppm).

Table 2-3. Comparison of Increases in Total Concentration (Total AUC) of Parent Chemicals in the Blood Following 4-hour Inhalation Exposures to Ternary and Binary Mixtures of Toluene (T), Ethylbenzene (E), and *m*-Xylene (X) in Rats

Mixture	Total AUC (mmol.min/L)		Ratio of mixed to single exposures[c]
	Mixed exposure[a]	Sum of single chemical exposures[b]	
T 100 ppm + E 200 ppm	11.4	6.3	1.81
T 200 ppm + E 100 ppm	9.3	4.5	2.07
T 100 ppm + X 200 ppm	11.2	5.1	2.19
T 200 ppm + X 100 ppm	7.0	4.1	1.70
E 100 ppm + X 200 ppm	11.5	5.7	2.02
E 200 ppm + X 100 ppm	13.4	6.5	2.06
mean ± SD of binary mixtures	10.6±2.2	5.4±1.0	1.97±0.18
T 100 ppm + E 100 ppm + X 100 ppm	9.2	2.9	3.17

[a]Total AUC (mixed exposure) calculated according to the following equation/example:
Total AUC = AUC_T (mixture) + AUC_X (mixture)
[b]Total AUC (sum of single chemical exposures) calculated according to the following equation/example: Total AUC = AUC_T (individual exposure) + AUC_X (individual exposure)
[c]Ratio of AUCs for mixed and single chemical exposures calculated according to the following equation/example: R = AUC_T (mixture)/AUC_T (single) + AUC_X (mixture)/AUC_X (single)

AUC = areas under the blood concentration curves; SD = standard deviation

Source: Tardif et al. 1996

The human PBPK model predictions and experimental data indicate that exposure to ternary mixtures of approximately 20 ppm each of toluene, ethylbenzene, and xylene will not result in significant increases in blood levels of these chemicals compared to individual chemical exposure (Haddad et al. 1999b; Tardif et al. 1997), indicating that metabolic interactions are negligible at these exposure concentrations. This information, when evaluated with similar predictions from the PBPK models of the quaternary (BTEX) mixture in rats and quinary (DBTEX) mixture in humans (Haddad et al. 1999a, 2000, 2001) (Section 2.1), provides a basis for assessing the joint neurotoxic action of BTEX in humans as discussed in Section 2.3.

2.2.3 Benzene and Toluene

PBPK models have been developed to characterize the nature of the metabolic interaction between inhaled benzene and toluene in rats. For a model by Purcell et al. (1990), inhalation uptake (disappearance of gas from closed chamber) curves were obtained for 6-hour exposures to 200 ppm benzene, 200 ppm toluene, 200 ppm benzene with 1,000 ppm toluene, and 1,000 ppm benzene with 200 ppm toluene in rats. The model simulated the inhalation uptake process, and an optimal fit to the uptake curves for simultaneous exposure was obtained by adjusting the metabolic interaction terms (competitive, noncompetitive, or uncompetitive inhibition) for each chemical. The simulations resulting from the noncompetitive inhibition model adequately fit the experimental data, whereas the competitive and uncompetitive models did not fit the data (see Footnote 1 in Section 2.1 for an explanation of the differences in these mechanisms of metabolic interaction). Comparison of the chamber concentration difference between a single chemical exposure and mixed exposure indicated that toluene more effectively inhibits benzene's metabolism than does the reverse. Comparison of the metabolic inhibition constants for the noncompetitive models of the two mixtures (i.e., benzene in the presence of toluene, toluene in the presence of benzene) similarly indicated that toluene is a better inhibitor of benzene's metabolism than benzene is of toluene's metabolism.

A PBPK model for inhalation of toluene and benzene in rats was also developed as part of the model for the quaternary mixture discussed in Section 2.2.1 (Haddad et al. 1999a). The binary mixture model is based on individual PBPK models for toluene and benzene that were linked via the saturable metabolism term in the liver compartment by modifying the metabolism term to accommodate alternative mechanisms of interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). Comparisons of binary model simulations of blood levels of the chemicals in rats that were exposed to 100 ppm benzene plus 50, 100, or 200 ppm toluene for 4 hours showed that the competitive metabolic interaction model best described the experimental data (Haddad et al. 1999a). This finding

differs from that of Purcell et al. (1990), summarized above, who concluded that a PBPK model based on noncompetitive inhibition best fit the exposure data for mixtures of benzene and toluene.

The predictions from the PBPK modeling study of Haddad et al. (1999a) (i.e., that interactions between benzene and toluene from 50 to 200 ppm are consistent with competitive inhibition) do not conflict with those of Purcell et al. (1990) (i.e., that interactions from 200 to 1,000 ppm are consistent with noncompetitive inhibition) because the noncompetitive and competitive descriptions result in the same low-concentration behavior and only diverge at higher concentrations. Characterization of the interaction between benzene and toluene (and other BTEX components) is complicated by the metabolic involvement of multiple P-450 isozymes. In particular, although CYP1A1 is the main isozyme involved in the metabolism of these chemicals (see Appendices A–D), the contribution of other isozymes may result in a behavior quantitatively similar to noncompetitive inhibition at high exposure concentrations.

Metabolic interactions between benzene and toluene have been investigated in a number of other studies, including *in vitro* metabolism assays in rat and mouse liver microsomes (Andrews et al. 1977; Sato and Nakajima 1979) and *in vivo* (inhalation, subcutaneous, and intraperitoneal) metabolism studies in rats, mice, and humans (Andrews et al. 1977; Brondeau et al. 1992; Ikeda et al. 1972; Inoue et al. 1988; Sato and Nakajima 1979). Most of these studies examined the effects of toluene on the metabolism of benzene. The findings are consistent with the PBPK studies in indicating that toluene and benzene are inhibitors of each other's metabolism, the magnitude of inhibition is dose-dependent (i.e., may not occur at low levels of exposure), and toluene may have a greater suppressive effect on benzene metabolism than benzene on toluene metabolism. Although the dose-dependency of the metabolic interactions is well-documented, only limited empirical information is available regarding the threshold of these interactions for inhalation exposures (Brondeau et al. 1992; Inoue et al. 1988; Sato and Nakajima 1979).

Levels of urinary metabolites were measured at the end of a workshift in 65, 35, and 55 Chinese men who were occupationally exposed to arithmetic mean time-weighted average (TWA) concentrations of 31.9±24.8 ppm benzene, 44.7±21.3 ppm toluene, and 17.9±29.3 ppm benzene + 20.5±25.8 ppm toluene, respectively (Inoue et al. 1988). The respective geometric mean exposure concentrations (geometric standard deviations) were 20.4 (3.086) ppm, 38.2 (1.920) ppm, and 6.2 (4.187) ppm+11.9 (3.670) ppm. A control group was comprised of 35 unexposed workers. A linear relationship was established between the exposure concentrations of each chemical and levels of urinary metabolites when the control group was combined with each exposed group for calculation. Comparisons of regression lines for exposure concentration vs. urinary metabolite level showed that the mixture suppressed the metabolism of benzene

to a greater extent than the metabolism of toluene. In particular, the slopes of the regression lines for excretion of two of three benzene metabolites (phenol and quinol, but not catechol) in the mixture group were less than half of that in the benzene group, whereas the slopes of the regression lines for both toluene metabolites (hippuric acid and *o*-cresol) in the mixture group were more than half of the slopes for the toluene group (although still smaller than those of the toluene group). Although the findings are consistent with mutual metabolic suppression between benzene and toluene in humans at relatively low average levels of inhalation exposure, insufficient data were reported for estimation of thresholds. Regression analysis also indicated that urinary excretion of the benzene metabolite, 1,2,4-benzenetriol, was reduced by the coexposure to toluene (Inoue et al. 1989a).

Rates of disappearance of benzene and toluene from blood and alveolar air were not affected in three volunteers following inhalation exposure to 25 ppm benzene + 100 ppm toluene for 2 hours compared to 25 ppm benzene alone or 100 ppm toluene alone (Sato and Nakajima 1979), indicating that there was no metabolic interaction at this combination of exposure levels.

Urinary excretion of the benzene metabolite, *t*,*t*-muconic acid, was measured in groups of 8 rats that were simultaneously exposed to 5 or 20 ppm benzene and 50, 100, 200 or 1,000 ppm toluene by inhalation for 4 hours (Brondeau et al. 1992). Toluene reduced muconic acid excretion in a concentration-dependent manner, especially in the 20-ppm benzene groups, whereby coexposure to 100, 200, and 1,000 ppm toluene caused statistically significant ($p<0.05$) decreases of 28, 44, and 85%, respectively, compared to the rats exposed to benzene alone. The decreases in urinary muconic acid in the 5-ppm benzene groups were statistically significant only with coexposure to 1,000 ppm toluene (85% reduced compared to benzene alone).

Studies investigating the influence of toluene on benzene toxicity have shown that exposure to toluene inhibits benzene-induced hematologic and immunologic effects in animals (Andrews et al. 1977; Gad-El-Karim et al. 1984; Gut et al. 1980; Hsieh et al. 1990a; Plappert et al. 1994; Tunek et al. 1981, 1982). The inhibition of the hematologic effects of benzene by toluene is well-established and independent of exposure route (inhalation, oral, and injection), and the magnitude of toxicity inhibition appears to be dose-dependent (Hsieh et al. 1990a; Plappert et al. 1994). The findings are consistent with competitive metabolic interaction between toluene and benzene resulting in toluene inhibition of benzene metabolism and subsequent inhibition of hematotoxicity.

Benzene-induced hematotoxic effects that have been shown to be influenced by simultaneous exposure to toluene (generally at toluene dose levels greater than benzene) include reduced (compared with exposure to benzene alone) red blood cell ^{59}Fe uptake (a common measure of erythropoiesis) and concentrations of benzene metabolites in bone marrow of mice given single subcutaneous doses (Andrews et al. 1977), and clastogenic effects in bone marrow and/or peripheral blood cells of mice treated by subcutaneous injection for 6 days (Tunek et al. 1981, 1982), inhalation for 8 weeks (Plappert et al. 1994), or gavage for 2 days (Gad-El-Karim et al. 1984). In the inhalation study, exposure of mice to either 300 or 900 ppm benzene combined with either 250 or 500 ppm toluene for 6 hours/day, 5 days/week for up to 8 weeks, significantly reduced benzene-induced anemia and deoxyribonucleic acid (DNA) damage in peripheral blood, bone marrow, and liver cells (Plappert et al. 1994). The protective effect of toluene was most pronounced on the genetic toxicity of benzene, where the coexposures reduced the extent of DNA damage, as assessed by amount of cellular DNA, to about 50% of benzene alone in all three types of cells. Toluene exposure alone did not significantly increase DNA damage compared to controls. In the oral study, mice that were administered two gavage doses of mixed benzene (440 mg/kg) and toluene (860 or 1,720 mg/kg), 24 hours apart, had significantly reduced numbers of micronuclei and chromosomal aberrations per metaphase and percentage of damaged cells compared with exposure to 440 mg/kg benzene alone (Gad-El-Karim et al. 1984). Exposure to toluene alone induced no clastogenic activity. Similarly, simultaneous exposure of mice to a mixture of 166 mg/L benzene and 325 mg/L toluene in drinking water for 28 days completely inhibited immunotoxic effects (decreased thymus mass, increased B- and T-cell mitogenesis, and decreased antibody response to sheep red blood cells) produced by exposure to 166 mg/L benzene alone (Hsieh et al. 1990a). Coexposure to a lower level of toluene (80 mg/L+166 mg/L benzene) did not inhibit the benzene-induced immunotoxic effects, indicating that this amount of toluene was not sufficient for an inhibitory antagonistic effect on benzene immunotoxicity.

Information is available on hematological, biochemical (blood and urine), and subjective neurological endpoints in Chinese workers who were primarily exposed to benzene, toluene, or mixtures of these two chemicals (Yin et al. 1987). The benzene group (majority engaged in shoemaking, some in printing) included 146 workers (62 men, 84 women) who were exposed to mean TWA concentrations of 47.9 ppm benzene and 6.4 ppm toluene for a mean duration of 61 months. The toluene group (engaged in several occupations including shoemaking and printing) included 94 workers (38 men, 56 women) who were exposed to mean TWA concentrations of 42.8 ppm toluene and 1.3 ppm benzene for a mean duration of 82 months, and the mixed exposure group (engaged in automobile spray painting) included 75 workers (55 men, 20 women) who were exposed to approximately equal mean TWA concentrations of 14.0 ppm benzene and 17.5 ppm toluene for a mean duration of 149 months. A control group was comprised of

138 workers (48 men, 90 women) with no direct exposure to solvent vapors in the factories where the exposed workers were employed. There were no clear hematological changes among any of the groups as indicated by results of differential blood cell counts and hemoglobin/hematocrit measurements. Effects included a slight but statistically significant ($p<0.01$) increase in mean leukocyte numbers in the mixed benzene/toluene group ($6.62\pm1.53x10^3$/mm) compared to the control, benzene, and toluene groups (5.78 ± 1.55, 5.81 ± 1.53, and $6.20\pm1.69x10^3$/mm, respectively), but no statistical comparisons between exposed groups were performed and there was no corresponding or consistent change in the percentage of cases with abnormal ($<4.00x10^3$/mm) leukocyte values (8.9, 10.3, 3.2, and 2.7% in the control, benzene, toluene, and benzene/toluene groups, respectively). Additionally, there was no indication of pancytopenia (a reduction in all three blood cell types characteristic of benzene toxicity). The blood biochemistry assays and urinalyses showed no alterations indicative of liver or kidney damage, including changes in levels of liver enzymes and bilirubin in serum, blood urea nitrogen, and protein in urine.

The subjective symptom survey showed that the prevalence of total symptoms was higher in the three exposed groups than in the unexposed controls (Yin et al. 1987). The percentages of workers with any symptoms in the control, benzene, toluene, and benzene/toluene groups were 7.1, 80.1, 89.1, and 74.4%, respectively, at the time of the study, and 73.2, 93.0, 94.1, and 86.0%, respectively, during the 6 months preceding the study. An explanation for the high prevalence of symptoms in the control group before the study is not provided by the investigators. The prevalence of the three most common symptoms during the study (i.e., dizziness, headache, and throat irritation) were generally dose-related in the benzene and toluene groups when these groups were subdivided on the basis of low and high levels of exposure. For example, among the women workers during the study, dizziness was reported by 6.9% of the controls, 55.0 and 63.8% of the low and high benzene groups (<40 and ≥ 40 ppm, respectively), and 67.9 and 65.5% of the low and high toluene groups (<41 and ≥ 41 ppm, respectively). Headache among women workers was reported by 0% of the controls, 17.5 and 21.3% of the low and high benzene groups, respectively, and 14.3 and 31.0% of the low and high toluene groups, respectively. Results were essentially the same in the men exposed to benzene or toluene (data not reported). Although two of the three most common symptoms are characteristic of benzene and toluene neurotoxicity, data on the prevalence of these symptoms in the benzene/toluene group were not reported, precluding comparisons with the other exposed groups. Group comparisons are further complicated by the differences in mean TWA exposure concentrations among the benzene, toluene, and mixed exposure groups.

The only other information regarding interactive effects of benzene and toluene on neurologic endpoints is provided by a 4-week oral study in mice (Hsieh et al. 1990b). Brain levels of various biogenic amines

and their metabolites were investigated in groups of five male CD-1 mice that were exposed to drinking water containing neither chemical (untreated controls), benzene alone (166 mg/L), toluene alone (80 or 325 mg/L), or mixed benzene (166 mg/L) and toluene (80 or 325 mg/L). Concentrations of the catecholamines norepinephrine (NE) and dopamine (DA); the catecholamine metabolites vanillylmanelic acid (VMA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA); and the indoleamine serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured in six discrete regions of the brain (hypothalamus, medulla oblongata, cerebellum, corpus striatum, cerebral cortex, and midbrain). Compared to untreated controls, exposure to benzene alone produced significant ($p<0.05$) increases in NE in the hypothalamus, cortex, midbrain, and medulla oblongata; DA in the hypothalamus and corpus striatum; and 5-HT in all brain regions except cerebellum. Exposure to toluene alone significantly increased the concentrations of NE in the hypothalamus and medulla oblongata; DA in the hypothalamus and cortex; and 5-HT in the corpus striatum, cortex, midbrain, and medulla oblongata. Benzene alone and toluene alone also caused increased levels of various monoamine metabolites in these brain areas. Exposure to combined benzene/toluene also caused increased concentrations of NE, DA, and 5-HT in some brain regions in comparison to unexposed controls, but none of the levels induced by mixed exposure were significantly different than those produced by exposure to either chemical alone. Concurrent exposure to benzene/toluene significantly ($p<0.05$) raised and lowered brain concentrations of monoamine metabolites in three and two instances, respectively, when compared to benzene and/or toluene alone. Due to the statistically similar brain levels of NE, DA, and 5-HT following mixed and single chemical exposure and lack of a clear pattern in effect of combined exposure on brain levels of monoamine metabolites, there were no clear interactive effects on levels of neurochemicals. Exposure to benzene and toluene, alone or combined, did not cause any treatment-related gross behavioral alterations, other overt clinical signs of toxicity, or adverse changes in growth, body weight, or food and water consumption.

Possible interactions between the components of a benzene/toluene mixture were studied using the *in vitro* rat embryonic development assay (Brown-Woodman et al. 1994) described in Section 2.2.1. The mixture was tested at a total concentration that was approximately the same as a minimally toxic level of each individual component and at a lower concentration that was similar to a no-effect level of each component. The average percentage of benzene and toluene contributing to the total concentration in the mixtures was 41.6 and 58.4%, respectively. Embryotoxic effects of the mixture were similar to those produced by similar equimolar concentrations of benzene (2.87 μmol/mL) and toluene (2.71 μmol/mL) alone. The no-effect level of the mixture (1.61 μmol/mL) was reported to be similar to equimolar concentrations of the individual chemicals that were without effects. The results are suggestive of

additive joint action on these endpoints but, study design limitations, as discussed in Section 2.2.1, preclude full characterization of the joint toxic action.

Table 2-4 provides a summary of the interaction data regarding the effects of toluene on the metabolism of benzene and effects of benzene on the metabolism of toluene. Table 2-5 summarizes the data regarding the effects of toluene on the toxicity of benzene and effects of benzene on the toxicity of toluene. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

Table 2-4. Summary of Available Data on the Influence of Toluene on Metabolism of Benzene and the Influence of Benzene on Metabolism of Toluene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/No effect	Less than additive		
Toluene Influence on Metabolism of Benzene						
Inhalation Exposure (ppm)[a]						
Acute	Blood levels of toluene and benzene			50 + 100 (r)[b] 100 + 100 200 + 100	< additive	Haddad et al. 1999a
Acute	Closed chamber air levels of toluene and benzene			200 + 1000 (r)	< additive	Purcell et al. 1990
Acute	Levels of benzene and toluene in blood and alveolar air		100 + 25 (h)[b]		no interaction	Sato and Nakajima 1979
Acute	Urinary level of benzene metabolite (*t*,*t*-muconic acid)		50 + 5 (r) 50 + 20 100 + 5 200 + 5	100 + 20 (r) 200 + 20 1,000 + 5 1,000 + 20	no interaction (low dose mixtures) < additive (high dose mixtures)	Brondeau et al. 1992
Repeated exposure	Urinary levels of benzene metabolites (phenol, quinol, catechol) and toluene metabolites (hippuric acid and *o*-cresol)			20.5 + 17.9 (h)	< additive	Inoue et al. 1988

Table 2-4. Summary of Available Data on the Influence of Toluene on Metabolism of Benzene and the Influence of Benzene on Metabolism of Toluene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/No effect	Less than additive		
Benzene Influence on Metabolism of Toluene						
Inhalation Exposure (ppm)[a]						
Acute	Blood levels of toluene and benzene			100 + 50 (r) 100 + 100 100 + 200	< additive	Haddad et al. 1999a
Acute	Closed chamber air levels of toluene and benzene			1,000 + 200 (r)	< additive	Purcell et al. 1990
Acute	Levels of benzene and toluene in blood and alveolar air		25 + 100 (h)		no interaction	Sato and Nakajima 1979
Repeated exposure	Urinary levels of benzene metabolites (phenol, quinol, catechol) and toluene metabolites (hippuric acid and *o*-cresol)			17.9 + 20.5 (h)	< additive	Inoue et al. 1988

[a] First dose listed is for the chemical influencing the other chemical's metabolism.
[b] Species code: r = rat; h = human

Table 2-5. Summary of Available Data on the Influence of Toluene on Toxicity of Benzene and the Influence of Benzene on Toxicity of Toluene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/No effect	Less than additive		
Toluene Influence on Toxicity of Benzene						
Inhalation Exposure (ppm)[a]						
Repeated exposure	Anemia and DNA damage in blood, bone marrow, and liver cells			250 + 300 (m)[b] 250 + 900 500 + 300 500 + 900	< additive	Plappert et al. 1994
Repeated exposure	Hematological, urinary, and serum chemistry indices		17.5 + 14.0 (h)[b]		no interaction	Yin et al. 1987
Oral Exposure (mg/kg)[a]						
Acute	Micronuclei, chromosomal aberrations and cell damage in bone marrow cells			860 + 440 (m) 1,720 + 440	< additive	Gad-El-Karim et al. 1984
Repeated exposure	Thymus mass, B- and T-cell mitogenesis, antibody response to SRBC		80 + 166[c] (m)	325 + 166[c] (m)	no interaction (low dose mixture) < additive (high dose mixture)	Hsieh et al. 1990a
Repeated exposure	Brain levels of biogenic amines and their metabolites		80 + 166[c] (m) 325 + 166[c]		no interaction	Hsieh et al. 1990b

Table 2-5. Summary of Available Data on the Influence of Toluene on Toxicity of Benzene and the Influence of Benzene on Toxicity of Toluene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/No effect	Less than additive		
Benzene Influence on Toxicity of Toluene						
Inhalation Exposure (ppm)[a]						
Repeated exposure	Anemia and DNA damage in blood, bone marrow, and liver cells			300 + 250 (m) 300 + 500 900 + 250 900 + 500	< additive	Plappert et al. 1994
Repeated exposure	Hematology, urine, and serum chemistry indices		14.0 + 17.5 (h)		no interaction	Yin et al. 1987
Oral Exposure (mg/kg)[a]						
Acute	Micronuclei, chromosomal aberrations and cell damage in bone marrow cells		440 + 1,720 (m)		no interaction	Gad-El-Karim et al. 1984
Repeated exposure	Thymus mass, B- and T-cell mitogenesis, antibody response to SRBC		166 + 80[c] (m) 166 + 325		no interaction	Hsieh et al. 1990a
Repeated exposure	Brain levels of biogenic amines and their metabolites		166 + 80[c] (m) 166 + 325		no interaction	Hsieh et al. 1990b

[a] First dose listed is for the chemical influencing the other chemical's toxicity.
[b] Species code: m = mouse; h = human
[c] Concentrations in drinking water (mg/L)

2.2.4 Benzene and Ethylbenzene

A PBPK model for inhalation of benzene and ethylbenzene in rats was developed as part of the model for the quaternary mixture discussed in Section 2.2.1 (Haddad et al. 1999a). The binary mixture model is based on individual PBPK models for benzene and ethylbenzene that were linked via the saturable metabolism term in the liver compartment by modifying the metabolism term to accommodate alternative mechanisms of interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). Comparisons of binary model simulations of blood levels of the chemicals in rats that were exposed to 100 ppm benzene plus 50, 100, or 200 ppm ethylbenzene for 4 hours showed that the competitive metabolic interaction model best described the experimental data (Haddad et al. 1999a).

No data were located regarding health effects in humans or animals exposed to mixtures exclusively containing benzene and ethylbenzene.

2.2.5 Benzene and Xylenes

A PBPK model for inhalation of benzene and xylene in rats was developed as part of the model for the quaternary mixture discussed in Section 2.2.1 (Haddad et al. 1999a). The binary mixture model is based on individual PBPK models for benzene and xylene that were linked via the saturable metabolism term in the liver compartment by modifying the metabolism term to accommodate alternative mechanisms of interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). Comparisons of binary model simulations of blood levels of the chemicals in rats that were exposed to 100 ppm benzene plus 50, 100, or 200 ppm xylene for 4 hours showed that the competitive metabolic interaction model best described the experimental data (Haddad et al. 1999a).

Possible interactions between the components of a benzene/xylene mixture were studied using an *in vitro* rat embryonic development assay (Brown-Woodman et al. 1994) described in Section 2.2.1. The mixture was tested at a total concentration that was approximately the same as a minimally toxic level of each individual component and at a lower concentration that was similar to a no-effect level of each component. The average percentage of benzene and xylene contributing to the total concentration in the mixtures was 35.9 and 64.1%, respectively. Embryotoxic effects of the mixture were similar to those produced by similar equimolar concentrations of benzene (2.87 μmol/mL) and xylene (2.75 μmol/mL) alone. The no-effect level of the mixture (1.58 μmol/mL) was reported to be similar to equimolar

concentrations of the individual chemicals that were without effects. The results are suggestive of additive joint action on these endpoints but study design limitations, as discussed in Section 2.2.1, preclude full characterization of the joint toxic action.

2.2.6 Toluene and Ethylbenzene

A PBPK model was developed for binary mixtures of toluene and ethylbenzene in rats as part of the development of the model for the ternary mixture discussed in Section 2.2.2 (Tardif et al. 1997). The binary mixture model is based on individual PBPK models for toluene and ethylbenzene that were linked via the saturable metabolism term in the liver compartment by modifying the metabolism term to accommodate alternative mechanisms of interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). Comparisons of binary model simulations with observed blood levels of the chemicals in rats that were exposed to 100 ppm toluene + 200 ppm ethylbenzene or 200 ppm toluene + 100 ppm ethylbenzene for 4 hours showed that the competitive metabolic interaction model best described the experimental data (Tardif et al. 1996). The blood concentrations of unchanged toluene and ethylbenzene were measured postexposure (5–120 minutes) and AUC were calculated. As summarized in Table 2-3 in Section 2.2.2, exposure to the binary mixture resulted in significantly higher AUCs of toluene and ethylbenzene compared with the individual chemical exposures. These findings are consistent with the occurrence of mutual competitive metabolic inhibition at these exposure levels.

2.2.7 Toluene and Xylenes

PBPK models have been developed for mixtures of inhaled toluene and xylene in rats and humans (Tardif et al. 1993a, 1993b, 1995). The rat binary mixture model (Tardif et al. 1993a, 1993b) is based on individual PBPK models for toluene and xylene that were linked via the saturable metabolism term in the liver compartment by modifying the metabolism term to accommodate alternative mechanisms of interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). The adequacy of the single chemical models was assessed by comparing simulations of the time-course blood concentrations of toluene and xylene to previously determined concentrations in blood from rats exposed to 75, 150, or 225 ppm for 5 hours (Tardif et al. 1992). Comparisons of binary model simulations of inhalation uptake (time course of decline in closed chamber gas concentrations) showed that the competitive metabolic interaction model best described the experimental data in rats that were exposed to mixtures of 500 ppm toluene + 1,000 ppm xylene, 1,000 ppm toluene + 500 ppm xylene, or

1,000 ppm toluene + 1,000 ppm xylene (Tardif et al. 1993a, 1993b). The adequacy of the binary chemical PBPK model with the competitive metabolic interaction term was verified by comparing model simulations with previously obtained experimental data on blood levels of the chemicals in rats that were exposed to 75 ppm toluene + 225 ppm xylene, 150 ppm toluene + 150 ppm xylene, or 225 ppm toluene + 75 ppm xylene for 5 hours (Tardif et al. 1992). The validated rat binary chemical model was used to predict the magnitude of inhibitory interactions between toluene and xylene at untested exposure concentrations. Simulations of the percent metabolized (amount metabolized/amount inhaled) of one chemical in the presence of the second chemical showed that, during 5-hour exposure to equiconcentration mixtures, interaction between toluene and xylene became apparent when the exposure concentrations exceeded approximately 50 ppm of each chemical (Tardif et al. 1993a, 1993b).

Comparisons of binary model simulations with observed blood levels of the chemicals in rats that were exposed to 100 ppm toluene + 200 ppm xylene or 200 ppm toluene + 100 ppm xylene for 4 hours also showed that the competitive metabolic interaction model best described the experimental data (Tardif et al. 1996). The blood concentrations of unchanged toluene and xylene were measured postexposure (5–120 minutes) and AUCs were calculated. As summarized in Table 2-3 in Section 2.2.2, exposure to the binary mixture resulted in significantly higher AUCs of toluene and xylene compared with the individual chemical exposures at these concentrations.

The validated PBPK model of toluene/xylene in rats was used as the basis of the human model (Tardif et al. 1995). The human PBPK model was developed by substituting the rat physiological parameters and blood:air partition coefficients with those of humans, scaling the V_{max} on the basis of body weight$^{0.75}$, and assuming that the other metabolic constants (K_m and K_i) are species-invariant on the basis that the same cytochrome P-450 isozyme (CYP2E1) is involved in the metabolism of toluene and xylene in both rats and humans. The model was validated by determining that simulated venous blood and alveolar air concentrations of toluene and xylene adequately compared with those previously observed in volunteers exposed to toluene alone (50 ppm for 7 hours or 95 ppm for 4 hours), xylene alone (40 ppm for 7 hours or 80 ppm for 4 hours), or two mixtures of the chemicals (50 ppm toluene + 40 ppm xylene for 7 hours; 95 ppm toluene + 80 ppm xylene for 4 hours) (Tardif et al. 1991). As detailed in the summary of the Tardif et al. (1991) study below, the 7-hour mixed exposure to 50 ppm toluene and 40 ppm xylene resulted in no apparent change in predicted or observed blood and alveolar air concentrations of the chemicals compared to the single chemical exposures, and the 4-hour exposure to 95 ppm toluene and 80 ppm xylene resulted in increases in the predicted and observed levels compared to the individual exposures. Based on model simulations of anticipated changes in blood levels of toluene following

8-hour exposure to 50 ppm of toluene alone (TLV concentration) or in combination with xylene (50, 100, 200, or 400 ppm), or changes in blood levels of xylene following 8-hour exposure to 100 ppm of xylene alone (TLV concentration) or in combination with toluene (50, 100, 200, or 400 ppm), it was concluded that mixed exposure to air concentrations of toluene and xylene that remain within the TLVs would not result in significant (>10%) changes in blood levels of the chemicals. In other words, the human model predicted that mutual competitive metabolic inhibition does not occur at exposure levels below 50 ppm toluene and 100 ppm xylene.

The xylene used in the human experimental study was a mixture of three isomers (15% *ortho*, 25% *para*, and 60% *meta*), whereas the PBPK simulations were obtained with parameter estimates for *m*-xylene. The acceptability of using *m*-xylene as a surrogate for the xylene mixture was verified during the modeling study in rats (Tardif et al. 1992, 1993a), in which the single chemical PBPK model of xylene adequately simulated the kinetics of a similar mixture of xylene isomers.

As indicated above, experimental data from the Tardif et al. (1991) study were used to validate the human binary PBPK model for toluene/xylene. In this study, five male volunteers were exposed to 50 ppm toluene and 40 ppm xylene either separately or in combination for 7 hours/day for 3 consecutive days. The exposures were repeated 3 times at intervals of 2 weeks. Three other men were exposed to 95 ppm toluene and 80 ppm xylene separately or in combination for 4 hours with no replicate sessions. Urinary excretion of the metabolites hippuric acid (toluene) and methylhippuric acid (xylene), as well as concentrations of unchanged chemicals in venous blood and alveolar air, were evaluated. Values for these indexes in subjects exposed to the lower-level (50/40 ppm) mixture were not significantly different from averages of the single chemical exposures. Exposure to the higher-level (95/80 ppm) mixture caused significantly ($p<0.05$) increased mean blood levels of toluene and xylene (58.9 and 11.9%, respectively) and alveolar air levels of xylene (24.4%) near the end of the exposure period, as well as significantly delayed urinary excretion of hippuric acid without altering the amount excreted. Changes of $\leq$10% in the kinetics of toluene and xylene during combined exposures (compared to the chemicals individually) were considered to be in the range of normal interindividual variations. The results of this study are consistent with the hypothesis that the 50/40 ppm exposure was below the metabolic interaction threshold.

Results of other human studies also indicate the lack of competitive metabolic interaction between toluene and xylene at lower exposure concentrations. Coexposure to 2.2 mmol/m^3 (54 ppm) toluene and 0.9 mmol/m^3 (22 ppm) *p*-xylene for 4 hours had no significant effect on the level of either chemical in the

blood or exhaled air of four male volunteers, compared with exposure to the chemicals individually (Wallen et al. 1985). The amount of methylbenzoic acid (derived from *m*-methylhippuric acid during analysis) excreted in the urine after exposure to 70 ppm *m*-xylene for 4 hours was not affected by simultaneous exposure to toluene at either 45 or 70 ppm in groups of 4 male volunteers (Jakubowski and Kostrzewski 1989). There was no indication of increased urinary concentrations of hippuric acid or methylhippuric acid in 233 workers (122 men, 111 women) who were concurrently exposed to toluene and mixed xylenes for unreported durations in factories engaged in printing, painting, and production of plastic coated wire, compared with unexposed workers from the same factories (Huang et al. 1994). The whole group was exposed to geometric mean personal TWA concentrations of 2.7 ppm toluene and 3.1 ppm total xylenes (0.2, 1.6, and 0.5 ppm *o*-, *m*-, and *p*-xylene, respectively). Although the mean air levels of toluene and xylene were approximately 3 ppm each, peak exposures exceeded 200 ppm toluene and 100 ppm xylenes. Regression analysis of metabolite concentrations in urine collected near the end of an 8-hour shift showed a linear correlation between air concentrations of toluene or xylene and urinary levels of hippuric acid or methylhippuric acid, respectively. The slopes of the regression lines were essentially the same for the three xylene isomers. There was no significant difference in the slopes of the regression lines for toluene and xylenes, suggesting the absence of metabolic interaction.

Neurobehavioral effects of toluene (100 ppm), xylene (100 ppm), and their mixture (50 ppm toluene and 50 ppm xylene) were evaluated in 10 male volunteers who were exposed during 4-hour inhalation sessions (Dudek et al. 1990). A battery of nine tests was conducted that assessed memory (Sperling's Test), cognitive processes (Stroop's Test, Sternberg's Test), motor-visual coordination (Flanagan's Test), speed and precision of hand movements (Aiming), psychomotor efficiency (Simple Reaction Time, Choice Reaction Time, Santa Ana Test), and mood (Profile of Mood State). The tests were performed pre-exposure and after 1 and 2 hours of exposure in a total of three sessions conducted at 1-week intervals. Blood concentrations of toluene and xylene were determined in each session from samples collected at or near the time of the neurobehavioral tests as well as at 15 minutes postexposure. The maximum (4-hour) blood levels of toluene and xylene in the mixed exposure group were similar to and ≈28% lower than, respectively, the levels produced by exposure to the chemicals alone. The only observable exposure-related neurobehavioral effects (compared with pre-exposure performances) were increased simple and/or choice reaction time in the xylene-only and mixed chemical groups in the second (last) tests of the sessions (i.e., after 3 hours of exposure). Exposure to toluene alone did not cause any significant impairment in either test. The only clear effect on simple reaction time was in the xylene-only group, which had ≈22% reduced mean performance ($p<0.001$) versus ≈3% ($p>0.05$) and ≈7% ($p>0.05$) impairment in the toluene-only and mixed exposure groups, respectively. Mean performance in the

choice reaction time test was significantly reduced in the groups exposed to xylene alone (≈13.5%, $p<0.01$) and mixed toluene/xylene (≈9.5%, $p<0.05$), but not to toluene alone (≈5.5%, $p>0.05$). These results showed that combined exposure to 50 ppm toluene and 50 ppm xylene produced effects on neurobehavioral performance that were not different from effects from 100 ppm of either alone, indicating that joint action was additive.

There were no effects on reaction time and short-term memory in another human inhalation study of coexposure to toluene and xylene (Olson et al. 1985). Sixteen men were exposed to control air, toluene (300 mg/m^3 [80 ppm]), *p*-xylene (300 mg/m^3 [69 ppm]), or mixed toluene (200 mg/m^3 [53 ppm]) and xylene (100 mg/m^3 [23 ppm]) for 4 hours on successive days (i.e., one session per day). Each session included three tests (simple reaction time, memory-reproduction, and (choice reaction time) conducted when the subjects entered the chamber and after 2 and 4 hours of exposure. Exposure to the chemicals individually or in mixture did not cause observable performance decreases in any of the tests.

Effects on the olfactory perception of toluene were investigated in five volunteers who were exposed to 50 ppm toluene, 40 ppm xylene, or an "additive mixture of the two" (not otherwise specified) for 7 hours (Mergler and Beauvais 1992). The exposures were conducted on 3 consecutive days with an 11-day interval between each 3-day session. Olfactory perception thresholds (OPTs, the concentrations at which the chemical could be detected) were determined for toluene before exposure, immediately following cessation of exposure, and 60 and 90 minutes postexposure. The OPT for toluene was significantly increased to a similar extent (approximately 6-fold) immediately following exposure to toluene, xylene, or the mixture, suggesting a lack of interactions between the chemicals. Recovery from the olfactory threshold shift to toluene had not attained pre-exposure levels by the end of the 1.5-hour postexposure period. The investigators suggested that the observed effect on OPT for toluene is due to direct chemical contact with olfactory receptor neuronal membranes, and consequent disruption of sensory transduction and succeeding electrical events.

Rotarod performance (an indicator of neuromuscular function) was evaluated in groups of 10 male Wistar rats that were exposed by inhalation to very high concentrations of toluene alone (1,030, 1,980, 2,950, 2,970, 4,120, or 4,850 ppm), xylenes alone (1,030, 2,010, 2,870, 2,930, 4,150, or 4,970 ppm), or their mixture (1,050, 2,030, 2,610, 2,710, 4,130, or 4,700 ppm) for 4 hours (Korsak et al. 1988). The mixture was 50 volume percent toluene and 50 volume percent xylenes, indicating that the concentrations of toluene and xylenes in the mixed exposure groups were 525+525, 1,015+1,015, 1,305+1,305, 1,355+1,355, 2,065+2,065, and 2,350+2,350 ppm. Comparison with pre-exposure test values showed

concentration-related performance disturbances following exposure to toluene and xylene individually and in combination. Probit analysis of exposure vs. response yielded EC_{50} values of 4,050 ppm (95% confidence interval [CI] 3,580–4,580 ppm), 4,520 ppm (95% CI 3,580–4,580 ppm), and 2,770 ppm (95% CI 2,560–2,990 ppm) for toluene, xylene, and mixed toluene/xylene, respectively, showing that the mixture was more potent than the individual chemicals. Statistical comparison of relative potency values, apparently determined from the slopes of the exposure-response curves, indicated that the mixture was about 1.5-fold more potent than the individual agents (see Table 2-6).

Table 2-6. Relative Potency of Toluene, Xylene, and Their 1:1 Mixture on Rotarod Performance in Rats

Reference compound	Reference potency	Compound compared	Relative potency
Toluene	1	Xylene	0.974
Toluene	1	Toluene + Xylene	1.489[a]
Xylene	1	Toluene	1.027
Xylene	1	Toluene + Xylene	1.529[a]

[a]Significantly ($p<0.01$) different than toluene and xylene alone

Source: Korsak et al. 1988

These data are not consistent with additive joint action of toluene and xylene on the neurobehavioral endpoint and suggest that, at these very high exposure levels, greater-than-additive joint action occurred as a consequence of metabolic saturation and resulted in increased blood concentrations of both parent compounds.

Neurotoxic effects of mixed inhalation exposure to toluene and xylene were further assessed by sub-chronic evaluations of rotarod performance and spontaneous motor activity in rats (Korsak et al. 1992). Groups of 12 male Wistar rats were intermittently exposed (6 hours/day, 5 days/week) to control air (sham-exposed), 100 ppm toluene, 100 ppm *m*-xylene, or 50 ppm toluene + 50 ppm *m*-xylene for 6 months, or to control air, 1,000 ppm toluene, 1,000 ppm *m*-xylene, or 500 ppm toluene + 500 ppm *m*-xylene for 3 months. Rotarod performance was tested at monthly intervals throughout the study, and spontaneous motor activity was measured at the end of the 3- and 6-month exposure periods. Additional

study endpoints, evaluated at the end of the exposure periods, included clinical chemistry and hematology indexes, body and organ weights, and liver ultrastructure (Korsak et al. 1992; Rydzynski et al. 1992). Rotarod performance was significantly ($p \leq 0.01$) reduced in all individual chemical and mixed exposure groups compared to the controls; the approximate percentages of control, toluene, xylene, and toluene/xylene groups with abnormal performance as compared to pre-exposure values were 0, 40, 60, and 72%, respectively, in the 1,000 ppm (3-month) study, and 0, 35, 33, and 43%, respectively, in the 100 ppm (6-month) study. Although performance reduction was more pronounced in the groups exposed to the mixtures than in those exposed to the individual chemicals, none of these group differences were statistically significant. A similar pattern was observed for spontaneous motor activity (i.e., significant decreases in single and mixed chemical groups in comparison to unexposed controls, but not between the individual and mixed chemical groups). The approximate percent reductions in spontaneous movements (number per hour) compared to controls in the toluene, xylene and mixed toluene/xylene groups were 37.5, 47, and 62.5%, respectively, in the 100 ppm study (data were not reported for the 1,000 ppm study). Based on these neurotoxicity data, there are no clear indications of greater-than-additive joint action as found in the previously summarized high concentration acute exposure (4-hour) rotarod study (Korsak et al. 1988). Other effects included some hematological changes in the 1,000 ppm study, including reduced red blood cell counts (2.5, 3.4, and 11.8% compared to controls in the toluene, xylene, and, toluene/xylene groups, respectively; $p \leq 0.05$ in the mixed group only), reduced lymphocyte counts (16.8, 25.2, and 21.9%, $p \leq 0.05$ in all groups), and increased monocyte counts (75.9, 96.4, and 97.6%, $p \leq 0.05$ in all groups) (Korsak et al. 1992). There were no changes in serum levels of liver enzymes or other clinical chemistry endpoints (Korsak et al. 1992), although hepatic ultrastructural changes indicative of adaptation (e.g., proliferation of smooth endoplasmic reticulum and increased number of lysosomes) were found in all exposed groups in the 100 and 1,000 ppm studies (Rydzynski et al. 1992). Based on a semiquantitative evaluation of the lesions (not changed, slight/focal or moderate/multifocal), the investigators concluded that proliferation of the smooth endoplasmic reticulum in the mixed exposure group was consistent with an additive effect of the individual chemicals.

Effects of acute inhalation exposure to toluene, xylene, and their half-concentration mixture on behavior and induced seizure characteristics were investigated in rats and mice (Frantik et al. 1988). In one series of experiments, groups of 17 male albino rats were exposed to control air, 540 ppm toluene alone, 460 ppm mixed xylenes alone, or 270 ppm toluene + 230 ppm xylenes for 6 hours and evaluated for changes in spontaneous motor activity and latency, duration and intensity of audiogenic (sound-induced) seizures. Spontaneous motor activity, assessed as meters traveled during the second 30 minutes of exposure, was increased 123, 196, and 117% compared with controls in the toluene, xylene, and toluene/

xylene groups, respectively. The latency of the of audiogenic seizures was increased 529, 750, and 434% compared with controls in the toluene, xylene, and toluene/xylene groups, respectively. A composite score reflecting the latency, duration, and intensity of the audiogenic seizures (maximum value=3) was reduced 60, 91, and 52% in the toluene, xylene, and toluene/xylene groups, respectively. Experiments were also conducted in which effects of exposure to 540 ppm toluene alone, 460 ppm mixed xylenes alone, or 270 toluene + 230 ppm xylenes on electric shock-induced seizures were studied in rats and mice that were exposed for 4 and 2 hours, respectively (Frantik et al. 1988). Evaluation of the latency of hindlimb extension (both species) and duration of maximum tonic extension (rats only) showed no significant differences ($p>0.05$) between the three exposed groups (no unexposed animals tested). The results from this study indicate that toluene alone and xylene alone caused similar neurological changes of increased spontaneous activity and depressed generation, propagation, and maintenance of induced seizures. The effects of combined exposure were not significantly different from the effects from exposure to comparable levels of toluene or xylene alone.

The inhibition of electric shock-induced seizures was further investigated in male rats that were exposed to 270 or 540 ppm toluene alone for 4 hours, 230 or 460 ppm *o*-xylene alone for 4 hours, or 270 ppm toluene + 230 ppm *o*-xylene for 2 hours, as well as in female mice that were similarly exposed to 380 or 760 ppm toluene alone, 320 or 640 ppm *o*-xylene alone, or 380 ppm toluene + 320 ppm *o*-xylene (Vodickova et al. 1995). Seizure inhibition was evaluated by measuring duration of tonic extension of hindlimbs in the rats and velocity of tonic extension in the mice. There were no statistically significant ($p>0.05$) differences in mean duration of hindlimb extension in the rats exposed to the mixture and those exposed to either concentration of toluene alone or xylene alone. Comparisons in the mice showed that seizure propagation was significantly ($p<0.05$) slower in the mixture group (34% reduced compared to pre-exposure) than in the groups exposed to 760 ppm toluene alone or 640 ppm xylene alone (44 and 41% reduced, respectively), indicating that there was a less-than-additive joint effect in this species. Linear regression analysis of effect on air or blood concentrations of the individual chemicals also predicted a less-than-additive effect of the mixture in both species.

Korsak et al. (1988) evaluated effects of 4-hour high-level exposure to toluene (3,030, 3,850, or 4,690 ppm), mixed xylenes (2,600, 4,000, 4,600, or 7,000 ppm), and their mixture (1,060, 2,400, and 4,400 ppm) on respiratory rate in groups of 2–4 Balb C mice. The mixture was 50 volume percent toluene and 50 volume percent xylenes, indicating that the concentrations of toluene and xylenes in the mixed exposure groups were 530+530, 1,200+1,200, and 2,200+2,200 ppm. The RD_{50} concentration (i.e., level that depresses respiratory rate by 50%) was calculated to be 4,750, 2,440 and 1,990 ppm for toluene,

xylene, and mixed toluene/xylene, respectively. These data are consistent with a greater-than-additive joint irritative effect of toluene and xylene on the upper respiratory tract at these relatively high exposure levels.

The cytogenic potential of 50 ppm toluene and 40 ppm mixed xylenes (15, 25, and 60% *o*-, *m*-, and *p*-isomers, respectively) was investigated in five adult male volunteers who were exposed to the chemicals either separately or in combination (50 ppm toluene + 40 ppm xylenes) for 7 hours/day for 3 consecutive days (Richer et al. 1993). The experiment was repeated three times at biweekly intervals. Analysis of peripheral blood cells showed that cell mortality was significantly ($p<0.05$) increased in all exposed groups compared to unexposed controls, but not different in the mixed exposed group compared to the individual chemical exposures. There were no exposure-related changes in cell cycle delay and sister chromatid exchanges in any of the groups. Similar results were found in human blood lymphocytes exposed to either toluene, xylene, or their mixture (i.e., no significant cytogenic effects at lower concentrations, with only cell mortality significantly affected at higher concentrations) (Richer et al. 1993).

Possible interactions between the components of a toluene/xylene mixture were also studied using the *in vitro* rat embryonic development assay (Brown-Woodman et al. 1994) described in Section 2.2.1. The mixture was tested at a total concentration that was approximately the same as a minimally toxic level of each individual component or at a lower concentration that was similar to a no-effect level of each component. The average percentages of toluene and xylene contributing to the total concentration in the mixtures were 44.1 and 55.9%, respectively. Embryotoxic effects of the mixture were similar to those produced by similar equimolar concentrations of toluene (2.71 μmol/mL) and xylene (2.75 μmol/mL) alone. The no-effect level of the mixture (1.90 μmol/mL) was reported to be similar to equimolar concentrations of the individual chemicals that were without effects. The results are suggestive of additive joint action on these endpoints, but study design limitations, as discussed in Section 2.2.1, preclude full characterization of the joint toxic action.

Table 2-7 provides a summary of the interaction data regarding the effects of toluene on the metabolism of xylenes and effects of xylenes on the metabolism of toluene. Table 2-8 summarizes the data regarding the effects of toluene on the toxicity of xylenes and effects of xylenes on the toxicity of toluene. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

Table 2-7. Summary of Available Data on the Influence of Toluene on Metabolism of Xylene and the Influence of Xylene on Metabolism of Toluene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Toluene Influence on Metabolism of Xylene						
Inhalation Exposure (ppm)[a]						
Acute	Blood levels of toluene and xylene			75 + 225 (r)[b] 150 + 150 225 + 75	< additive	Tardif et al. 1992
Acute	Blood levels of toluene and xylene			100 + 200 (r) 200 + 100	< additive	Tardif et al. 1996
Acute	Levels of toluene and xylene in blood and alveolar air and their metabolites (hippuric acid and methylhippuric acid) in urine		50 + 40 (h)[b]	95 + 80	< additive (high dose mixture)	Tardif et al. 1991
Acute	Levels of toluene and xylene in blood and exhaled air		54 + 22 (h)		no interaction	Wallen et al. 1985
Acute	Urinary level of xylene metabolite methylhippuric acid		45 + 70 (h) 70 + 70		no interaction	Jakubowski and Kostrzewski 1989
Repeated	Urinary levels of metabolites of toluene (hippuric acid) and xylene (methylhippuric acid).		2.7 + 3.1 (h)		no interaction	Huang et al. 1994

Table 2-7. Summary of Available Data on the Influence of Toluene on Metabolism of Xylene and the Influence of Xylene on Metabolism of Toluene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Xylene Influence on Metabolism of Toluene						
Inhalation Exposure (ppm)[a]						
Acute	Blood levels of toluene and xylene			75 + 225 (r) 150 + 150 225 + 75	< additive	Tardif et al. 1992
Acute	Blood levels of toluene and xylene			100 + 200 (r) 200 + 100	< additive	Tardif et al. 1996
Acute	Levels of toluene and xylene in blood and alveolar air and hippuric acid and methylhippuric acid metabolites in urine		40 + 50 (h)	80 + 95	< additive in high dose mixture	Tardif et al. 1991
Acute	Levels of toluene and xylene in blood and exhaled air		22 + 54 (h)		no interaction	Wallen et al. 1985
Acute	Urinary excretion of xylene metabolite methylhippuric acid		70 + 45 (h) 70 + 70		no interaction	Jakubowski and Kostrzewski 1989
Repeated	Urinary excretion of metabolites of toluene (hippuric acid) and xylene (methylhippuric acid)		3.1 + 2.7 (h)		no interaction	Huang et al. 1994

[a] First dose listed is for the chemical influencing the other chemical's toxicity.
[b] Species code: r= rat; h = human

Table 2-8. Summary of Available Data on the Influence of Toluene on Toxicity of Xylene and the Influence of Xylene on Toxicity of Toluene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Toluene Influence on Toxicity of Xylene						
Inhalation Exposure (ppm)[a]						
Acute	Neurobehavioral test battery[c]		50 + 50 (h)[b]		additive	Dudek et al. 1990
Acute	Reaction time (simple and choice) and short-term memory		53 + 23 (h)		no interaction	Olson et al. 1985
Acute	Rotarod performance	525 + 525 (r)[b] 1,015 + 1,015 1,305 + 1,305 1,355 + 1,355 2,065 + 2,065 2,350 + 2,350			> additive	Korsak et al. 1988
Repeated	Rotarod performance, spontaneous motor activity, clinical chemistry and hematology indices, liver ultrastructure		50 + 50 (r) 500 + 500		additive	Korsak et al. 1992

Table 2-8. Summary of Available Data on the Influence of Toluene on Toxicity of Xylene and the Influence of Xylene on Toxicity of Toluene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Acute	Spontaneous motor activity and inhibition of sound-induced seizures		270 + 230 (r)		additive	Frantik et al. 1988
Acute	Inhibition of electric shock-induced seizures		270 + 230 (r)		additive	Frantik et al. 1988
Acute	Inhibition of electric shock-induced seizures		270 + 230 (m)[b]	380 + 320 (m)	additive (low dose mixture) < additive (high dose mixture)	Frantik et al. 1988; Vodickova et al. 1995
Acute	Depression of respiratory rate	530 + 530 (r) 1,200 + 1,200 2,200 + 2,200			> additive	Korsak et al. 1988
Acute	Sister-chromatid exchanges and cell mortality in peripheral lymphocytes		50 + 40 (h)		no interaction	Richer et al. 1993
Xylene Influence on Toxicity of Toluene						
Inhalation Exposure (ppm)[a]						
Acute	Neurobehavioral test battery[c]		50 + 50 (h)		additive	Dudek et al. 1990
Acute	Reaction time (simple and choice) and short-term memory		23 + 53 (h)		no interaction	Olson et al. 1985

Table 2-8. Summary of Available Data on the Influence of Toluene on Toxicity of Xylene and the Influence of Xylene on Toxicity of Toluene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Acute	Rotarod performance	525 + 525 (r)[b] 1,015 + 1,015 1,305 + 1,305 1,355 + 1,355 2,065 + 2,065 2,350 + 2,350			> additive	Korsak et al. 1988
Repeated	Rotarod performance, spontaneous motor activity, clinical chemistry and hematology indices, liver ultrastructure		50 + 50 (r) 500 + 500		additive	Korsak et al. 1992
Acute	Spontaneous motor activity and inhibition of sound-induced seizures		230 + 270 (r)		additive	Frantik et al. 1988
Acute	Inhibition of electric shock-induced seizures		230 + 270 (r)		additive	Frantik et al. 1988
Acute	Inhibition of electric shock-induced seizures		230 + 270 (m)	320 + 380 (m)	additive (low dose mixture) < additive (high dose mixture)	Vodickova et al. 1995; Frantik et al. 1988
Acute	Depression of respiratory rate	530 + 530 (r) 1,200 + 1,200 2,200 + 2,200			> additive	Korsak et al. 1988

Table 2-8. Summary of Available Data on the Influence of Toluene on Toxicity of Xylene and the Influence of Xylene on Toxicity of Toluene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Acute	Sister-chromatid exchanges and cell mortality in peripheral lymphocytes		40 + 50 (h)		no interaction	Richer et al. 1993

[a] First dose listed is for the chemical influencing the other chemical's toxicity.
[b] Species code: h = human; r= rat; m = mouse
[c] Nine tests were conducted that assessed memory, cognitive processes, motor-visual coordination, speed and precision of hand movements, psychomotor efficiency, and mood.

2.2.8 Ethylbenzene and Xylenes

A PBPK model was developed for binary mixtures of xylene and ethylbenzene in rats as part of the development of the model for the ternary mixture discussed in Section 2.2.2 (Tardif et al. 1997). The binary mixture model is based on individual PBPK models for toluene and ethylbenzene that were linked via the saturable metabolism term in the liver compartment by modifying the metabolism term to accommodate alternative mechanisms of interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). Comparisons of binary model simulations with observed blood levels of the chemicals in rats that were exposed to 100 ppm xylene + 200 ppm ethylbenzene or 200 ppm xylene + 100 ppm ethylbenzene for 4 hours showed that the competitive metabolic interaction model best described the experimental data (Tardif et al. 1996). The blood concentrations of unchanged toluene and ethylbenzene were measured postexposure (5–120 minutes) and AUC were calculated. As summarized in Table 2-3 in Section 2.2.2, exposure to the binary mixture resulted in significantly higher AUCs of xylene and ethylbenzene compared with the individual chemical exposures. These results are consistent with the occurrence of mutual competitive metabolic inhibition at these exposure levels.

Metabolic interaction studies that monitored urinary metabolites have not consistently found that combined exposure to xylene and ethylbenzene produces a mutual inhibition of metabolism. In a study in which volunteers were exposed to 150 ppm ethylbenzene, 150 ppm *m*-xylene, or both compounds together (150 ppm + 150 ppm) for 4 hours, there was a general mutual inhibition of metabolism manifested by delayed excretion and reduced amounts of metabolites excreted for both compounds (Engstrom et al. 1984). In contrast to these results in humans, ethylbenzene did not appear to affect *m*-xylene metabolism in rats as indicated by urinary excretion of major metabolites, although *m*-xylene did delay the metabolism and excretion of ethylbenzene in this species (Elovaara et al. 1982, 1984). Groups of three rats were exposed to *m*-xylene + ethylbenzene air concentrations of 0+0, 75+25, 300+100, or 600+200 ppm for 6 hours/day for 5 days. The observed delay in ethylbenzene metabolism increased with repetitive dosing and as the combined dose of the two chemicals increased to 600+200 ppm. Additionally, comparison of the mixture-exposed rats after the first 6-hour exposure with rats that were exposed to ethylbenzene alone (100 or 200 ppm) or *m*-xylene alone (300 or 600 ppm) for 6 hours showed that more total metabolites of ethylbenzene were recovered following exposure to 300+100 ppm and 600+200 ppm as compared to the single chemical exposures. Possible explanations offered by the investigators included enhanced excretion of endogenous metabolites, formation of the same metabolite from both compounds, and metabolic saturation.

Limited information is available regarding interactions between ethylbenzene and xylene using toxicity endpoints. Induction of microsomal enzymes in the liver, kidneys and lungs was investigated in rats exposed to 2,000 ppm of either ethylbenzene, *m*-xylene, *o*-xylene, *p*-xylene, or their mixture (23% ethylbenzene, 64.5% *m*-xylene, 10% *p*-xylene, 2% *o*-xylene, and 0.5% toluene) for 6 hours/day for 3 days (Toftgard and Nilsen 1981, 1982). All of these chemicals induced microsomal enzymes to varying degrees, but the effects of the mixture generally paralleled those of *m*-xylene, suggesting that the presence of ethylbenzene did not alter *m*-xylene activity at this high level of exposure. The same experimental protocol was used to study effects on dopamine and noradrenaline levels and turnover in various parts of the brain (Andersson et al. 1981). Mixture-induced changes in the hypothalamus generally reflected the activity of *m*-xylene, but effects of the mixture in the forebrain (increased dopamine levels and turnover compared with controls) were not produced by any of the single chemical exposures. The findings in the forebrain suggest that greater-than-additive joint action occurred, probably between *m*-xylene and ethylbenzene, which were present in the highest concentrations, although no overt behavioral signs occurred in any of the groups.

The inhibition of electroconvulsive shock-induced seizures was investigated in groups of 10 male Wistar rats that were exposed to ethylbenzene (160 or 320 ppm), *m*-xylene (280 or 560 ppm), or mixed ethyl-benzene + *m*-xylene combination in half concentrations (80+140 or 160+280 ppm) by inhalation for 4 hours (Frantik and Vodickova 1995). Blood and brain levels of the chemicals were determined in the rats exposed to the higher of the two concentrations of ethylbenzene, xylene, or their mixture, as well as in additional groups exposed to 640 ppm ethylbenzene, 1,120 ppm xylene, or 320 ppm ethylbenzene + 560 ppm xylene, respectively. Seizure discharge resulting from a short electrical shock applied through ear electrodes, as assessed by measurements of latency and duration of tonic extension of the hindlimbs, was considered to be a reliable indicator of subnarcotic neurotoxicity based on previous findings. Chemicals that depress seizure discharge also inhibit various behavioral activities in higher doses, as well as induce sleep and narcosis at even higher levels. Neither ethylbenzene + xylene mixture (80+140 or 160+280 ppm) was significantly ($p<0.05$) more effective than their double-concentration components in altering the latency or duration of seizure discharge, indicating that there were no nonadditive interactive effects of combined exposure. Exposure to 160 ppm ethylbenzene + 200 ppm xylene had no effect on the blood and brain concentrations of either chemical, although the higher concentration mixture (320+560 ppm) caused significantly ($p<0.05$) increased xylene in the blood and brain (52 and 40%, respectively) with no changes in levels of toluene. At the concentrations tested, latency and duration of seizure discharge induced by the mixture were not significantly different from latency and duration of seizure discharge from the individual chemicals.

Table 2-9 provides a summary of the interaction data regarding the effects of ethylbenzene on the metabolism of xylenes and effects of xylenes on the metabolism of ethylbenzene. Table 2-10 summarizes the data regarding the effects of ethylbenzene on the toxicity of xylenes and effects of xylenes on the toxicity of ethylbenzene. These studies were evaluated in detail in the text. Further evaluation of the relevance of these data is provided in Section 2.3.

Table 2-9. Summary of Available Data on the Influence of Ethylbenzene on Metabolism of Xylene and the Influence of Xylene on Metabolism of Ethylbenzene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Ethylbenzene Influence on Xylene Metabolism						
Inhalation Exposure (ppm)[a]						
Acute	Blood levels of ethylbenzene and xylene			100 + 200 (r)[b] 200 + 100	< additive	Tardif et al. 1996
Acute	Urinary excretion of metabolites of xylene (methylhippuric acid) and ethylbenzene (mandelic and phenylglyoxylic acids)			150 + 150 (h)[b]	< additive	Engstrom et al. 1984
Repeated	Urinary excretion of major metabolites		25 + 75 (r) 100 + 300 200 + 600		no interaction	Elovaara et al. 1982, 1984

Table 2-9. Summary of Available Data on the Influence of Ethylbenzene on Metabolism of Xylene and the Influence of Xylene on Metabolism of Ethylbenzene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Xylene Influence on Ethylbenzene Metabolism						
Inhalation Exposure (ppm)[a]						
Acute	Blood levels of xylene and ethylbenzene			100 + 200 (r) 200 + 100	< additive	Tardif et al. 1996
Acute	Urinary excretion of metabolites of xylene (methylhippuric acid) and ethylbenzene (mandelic and phenylglyoxylic acids)			150 + 150 (h)	< additive	Engstrom et al. 1984
Repeated	Urinary excretion of major metabolites			75 + 25 (r)[b] 300 + 100 600 + 200	< additive	Elovaara et al. 1982, 1984

[a] First dose listed is for the chemical influencing the other chemical's toxicity.
[b] Species code: r = rat; h = human

Table 2-10. Summary of Available Data on the Influence of Ethylbenzene on Toxicity of Xylene and the Influence of Xylene on Toxicity of Ethylbenzene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Inhalation Exposure (ppm)[a]						
Ethylbenzene Influence on Xylene Toxicity						
Acute	Inhibition of electric shock-induced seizures		80 + 140 (r)[b] 160 + 280		additive	Frantik and Vodickova 1995
Repeated	Brain levels of dopamine and noradrenaline	2,000[c] (r)			> additive (dopamine)	Andersson et al. 1981
Repeated	Microsomal enzyme activity in liver, kidneys, and lungs		2,000[c] (r)		no interaction	Toftgard and Nilsen 1981, 1982
Xylene Influence on Ethylbenzene Toxicity						
Acute	Inhibition of electric shock-induced seizures		140 + 80 (r) 280 + 160		additive	Frantik and Vodickova 1995
Repeated	Brain levels of dopamine and noradrenaline	2,000[c] (r)			> additive (dopamine)	Andersson et al. 1981
Repeated	Microsomal enzyme activity in liver, kidneys, and lungs		2,000[c] (r)		no interaction	Toftgard and Nilsen 1981, 1982

[a] First dose listed is for the chemical influencing the other chemical's toxicity.
[b] Species code: r = rat
[c] 2,000 ppm of either ethylbenzene, *m*-xylene, *o*-xylene, *p*-xylene, or their mixture (23% ethylbenzene, 64.5% *m*-xylene, 10% *p*-xylene, 2% *o*-xylene, and 0.5% toluene).

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

Benzene, toluene, ethylbenzene, and xylenes frequently occur together at hazardous waste sites as indicated in Section 1. Public health risks from exposure to BTEX may best be assessed by an approach that considers both the mechanism and toxic consequences of the joint action of the whole mixture, particularly the presence or absence of interactions affecting the responses of the critical target organs. Data on the whole BTEX mixture are lacking; however, a PBPK model was developed that predicts blood levels of the four chemicals in rats. Similar PBPK models have also been developed for binary, ternary, and quinary mixtures of BTEX components in humans as well as rats. No toxicity studies are available for whole BTEX, although health effects of the individual chemicals are generally well characterized (Appendices A–D). Information pertaining to toxic interactions among the BTEX components is essentially limited to data on a few binary mixtures of the chemicals. As discussed below, predictions from the PBPK modeling studies, when used in conjunction with mechanistic, interaction, and toxicity information on the components, provide a sufficient basis for assessing the joint toxic action of the whole mixture in humans.

PBPK models have been developed for inhalation exposure of rats to all binary mixtures of BTEX components (Haddad et al. 1999a; Purcell et al. 1990; Tardif et al. 1993a, 1993b, 1995, 1997), the ternary mixture toluene/ethylbenzene/*m*-xylene (Tardif et al. 1997), the quaternary mixture BTEX (Haddad et al. 1999a), and the quinary mixture DBTEX (Haddad et al. 2000). The PBPK models for the ternary, quaternary, and quinary mixtures were constructed by linking binary models for the constituent chemical pairs in each mixture via a mechanism of metabolic interaction. Competitive metabolic inhibition at CYP2E1 was determined to be the most plausible mechanism of binary interaction for all of the component pairs by optimal fitting of model simulations to blood concentrations of each chemical during actual binary exposures. The PBPK models of the ternary, quaternary, and quinary mixtures therefore are complementary because they share a common framework based on competitive metabolic inhibition and the network of binary interactions present in each mixture.

The PBPK model for the ternary mixture adequately simulated the blood concentrations of each component in rats exposed to a mixture of 100 ppm each of toluene, ethylbenzene, and xylene for 4 hours (Tardif et al. 1997). Similarly, the quaternary mixture PBPK model adequately predicted the blood levels of all four components in rats exposed for 4 hours to BTEX mixtures comprised of 50 ppm of each component, 100 ppm of each BTEX component, or 100 ppm of benzene and 50 ppm each of toluene, ethylbenzene, and xylene (Haddad et al. 1999a). The model simulations and experimental data also

showed that blood concentrations of each component chemical were higher in rats exposed to the mixtures than to the chemicals alone, indicating that metabolic interactions (competitive inhibition) were occurring at both the 50 ppm per component (quaternary mixture) and 100 ppm per component (ternary and quaternary mixtures) exposure levels. The BTEX study also showed that the inhibitory effect of the quaternary mixture was greater (i.e., blood concentrations of the component chemicals were increased to a larger extent) following exposure to 100 ppm of each component (400 ppm total mixture exposure) than to 50 ppm of each component (200 ppm total exposure). Additionally, simulations in rats exposed to 100 ppm benzene alone or in combination with 100 ppm of toluene, ethylbenzene, and/or xylene (Table 2-1) indicate that the inhibitory effect of BTEX mixtures increases with increasing number of components as well as with increasing concentration of components (Haddad et al. 1999a).

The PBPK model for the toluene/ethylbenzene/*m*-xylene ternary mixture in rats was scaled to humans by replacing species-specific model parameters with those for humans, and assuming that the competitive metabolic inhibition constants are species-invariant on the basis that the same isozyme (CYP2E1) is involved in the metabolism of toluene, xylene, and ethylbenzene in both rats and humans (Tardif et al. 1997). The human model adequately simulated the blood and alveolar air concentrations of all three components in volunteers following individual or mixed exposure to 17 ppm toluene, 33 ppm ethylbenzene, and 33 ppm xylene for 7 hours. The model simulations and experimental data showed that the blood and alveolar air levels of each chemical were similar following exposure alone or in combination, thereby indicating that there were no significant metabolic interactions at these lower exposure levels. Measurements of major urinary metabolites of toluene, ethylbenzene, and xylene further indicated that exposure to the ternary mixture did not significantly modify the metabolism of the individual components in humans (Tardif et al. 1997). Iterative use of the human PBPK model to estimate biological hazard indexes for varying exposure concentrations and proportions of the ternary mixture, calculated on the basis of additivity using blood levels of the components as discussed in Section 2.2.2, similarly predicted that component interactions are negligible at concentrations of 10–20 ppm toluene, 20–30 ppm ethylbenzene and 20–30 ppm *m*-xylene (Table 2-2) (Haddad et al. 1999b). Similar results were obtained from iterative simulations with the human PBPK model for DBTEX, which predicted negligible component interactions at exposure concentrations of 16 ppm dichloromethane, 0.5 ppm benzene, 16 ppm toluene, 33 ppm ethylbenzene, and 33 ppm xylene (Haddad et al. 2001).

The ability of the PBPK models to adequately simulate the blood concentrations of BTEX components in rats and humans is based on the assumption that competitive metabolic inhibition is the mechanism of interaction. This assumption is supported by *in vitro* and *in vivo* metabolism and toxicity studies

providing evidence of competitive metabolic interactions for some of the binary mixtures, particularly benzene and toluene (Section 2.2.3) and toluene and xylene (Section 2.2.7). However, even though there is reasonable basis to assume that competitive metabolic inhibition is the likely mechanism of interaction for the ternary, quaternary, and quinary mixtures, it is not necessary that the actual mechanism be conclusively established to use the findings from the PBPK studies in assessing the impact of interactions in health assessments of BTEX-exposed humans. In other words, because there is a good fit between model simulations and experimental data at relevant exposure levels, the "real" mechanism does not necessarily have to be conclusively known in order for the models to have practical usefulness.

The predictions from the ternary mixture PBPK studies are particularly relevant to the public health assessment of BTEX because the model has been validated at pertinent exposure concentrations in humans. Both the human model simulations and experimental data indicate that exposure to mixtures of approximately 20 ppm each of toluene, ethylbenzene, and xylene will not result in significant increases in blood levels of these chemicals compared to individual chemical exposure (Haddad et al. 1999b; Tardif et al. 1997), indicating that competitive inhibition is negligible at these concentrations. Considering that these sub-interaction threshold exposure concentrations are much higher than expected environmental exposure levels of the three chemicals, and correspond to the occupational TLVs for the chemicals in ternary mixture (i.e., 17 ppm toluene, 33 ppm ethylbenzene, and 33 ppm *m*-xylene), thereby indicating a low potential for toxicologically significant interactions, the ternary mixture human data suggest that component interactions will not impact public health assessments of BTEX for hazardous waste sites.

The proximity of the metabolic interaction thresholds for the ternary and quaternary mixtures in humans is unclear due to the lack of PBPK simulations of BTEX in humans. A comparison based on results of the studies of the ternary mixture in humans (i.e., no interactions at 20 ppm for each of three components) and quaternary mixture in rats (i.e., interactions at 50 ppm each of four components) suggests that metabolic interactions are unlikely to be a concern in humans exposed to whole BTEX at the ternary component concentrations. Assuming similar responses between species, the total exposure to 20 ppm each of the four components (80 ppm) would be 2.5-fold lower than the total exposure to 50 ppm of each component (200 ppm total), which exceeded the threshold for interactions. Although the interactive (inhibitory) effects of BTEX mixtures tend to increase with increasing number of components as well as with concentration of components (Haddad et al. 1999a), the comparison suggests that quaternary exposure to 20 ppm of each component would still be below the threshold for interactions. The predictions of the human PBPK model for DBTEX (i.e., no interactions at component concentrations as high as 16 ppm dichloromethane, 0.5 ppm benzene, 16 ppm toluene, 33 ppm ethylbenzene, and 33 ppm xylene) (Haddad et al. 2001) provide incomplete support for the conclusion that metabolic interactions are negligible in humans exposed to BTEX at concentrations below approximately 20 ppm of each

component. The simulations with the DBTEX model were constrained by use of a fixed benzene exposure level of 0.5 ppm (the TLV concentration). This exposure level is in the range of maximum estimated concentrations of benzene in air near hazardous waste sites (ATSDR 1997), indicating that interactions are unlikely to occur in people environmentally exposed to BTEX. Because 0.5 ppm was the only concentration of benzene used in the model simulations, the conclusion that the interaction threshold for benzene is approximately 20 ppm is speculative. This speculation could be verified by using the DBTEX model to simulate BTEX, i.e., by setting the exposure concentration of dichloromethane to zero and testing a range of concentrations of benzene and the other components.

As discussed above, the PBPK model for BTEX assumes competitive inhibition for all component interactions on the basis of both plausibility and kinetic data (Haddad et al. 1999a). Plausibility is based on evidence that the principal isozyme responsible for the metabolism of each of the BTEX components at low concentrations is CYP2E1. Competitive inhibition would be expected if the components are all substrates for the same isozyme. This expectation was tested by comparison of model predictions with kinetic data on parent chemical blood concentrations following mixed inhalation exposures at component concentrations in the range of 50–200 ppm, and a competitive inhibition description provided a better fit to the data than non- or uncompetitive inhibition. These results supported the use of a competitive description at inhalation concentrations in the range of the data (50–200 ppm) and below. However, they do not necessarily support the use of a competitive description at higher concentrations.

An indication that the competitive inhibition-based PBPK model for BTEX may not be adequate at high concentrations is provided by the results of PBPK modeling studies of binary mixtures of benzene and toluene. As discussed in Section 2.2.3, Haddad et al. (1999a) found that interactions between benzene and toluene at exposure concentrations of 50–200 ppm are best described by a competitive inhibition-based model, whereas Purcell et al. (1990) found that interactions at higher levels (200–1,000 ppm) are consistent with noncompetitive inhibition. These results do not conflict because noncompetitive and competitive descriptions result in the same low-concentration behavior and differ only at high concentrations. The contribution of isozymes other than CYP2E1 to the metabolism of the BTEX components (see Appendices A–D) also suggests that interactions may not be adequately described by the competitive inhibition-based BTEX model at high concentrations.

Although the adequacy of the competitive inhibition-based PBPK model for BTEX is unlikely to be a concern for most environmental inhalation exposure scenarios, it could be important in the case of acute exposures from spills and occupational exposures. Additionally, it is unclear if the PBPK model description would be appropriate for oral exposures (e.g., from drinking contaminated water). Because ingested BTEX mixtures are subject to presystemic metabolism, whereas inhaled BTEX mixtures are not,

the results obtained with the inhalation model are not necessarily applicable to oral exposures. In particular, although it is reasonable to expect that the nature of the interactions associated with low level oral exposures would also be consistent with competitive (or noncompetitive) inhibition, there is no clear basis for predicting the oral threshold for interactions.

No toxicity endpoints were evaluated in the PBPK studies and no epidemiological and toxicological studies of BTEX have been performed. Health effects data on ternary mixtures of BTEX components are limited to two investigations of benzene/toluene/xylene, a mixture that has not been modeled in PBPK studies. These studies provide an effect level for hematological and immunological effects in exposed workers (Lange et al. 1973), and evidence of dose additivity in an *in vitro* rat embryonic development assay (Brown-Woodman et al. 1994), but are an insufficient basis for hazard assessment. Considering the health effects information on the individual BTEX chemicals summarized in the appendices, as well as the data on binary component mixtures discussed in Section 2.2, neurotoxicity, hematotoxicity, immunotoxicity, and carcinogenicity are the critical effects of concern for human exposure to BTEX.

Based on findings in human and animal studies, acute or repeated exposure to any of the BTEX component chemicals is expected to produce neurological impairment resulting from the parent chemicals acting on components of neuronal membranes (see Appendices A–D). Nervous system toxicity from lipophilic solvents such as the BTEX components is thought to involve reversible intercalation in lipid bilayers of nerve membranes (yielding changes in membrane fluidity), and/or reversible interactions with membrane proteins (yielding conformational changes) leading to altered ion transport, enzymic activities, and neurotransmitter receptor functions necessary for normal nerve impulses and regeneration of action potentials (Balster 1998; Cruz et al. 1998; Engelke et al. 1996; Franks and Lieb 1985, 1987; Mihic et al. 1994; von Euler 1994). Animal data further indicate that repeated exposure to higher levels of toluene, ethylbenzene and xylenes can damage liver and kidney tissues due to the formation of reactive metabolites (see Appendices B, C, and D). The critical nature of the neurotoxicity of benzene, toluene, ethylbenzene, and xylene is reflected by the selection of neurological endpoints as the basis of 9 of the 13 MRLs for these chemicals, including 6 of 8 inhalation MRLs (see Table 2-11). Eight of the nine neurotoxicity-based MRLs are for toluene and xylene. The four non-neurotoxicity-based MRLs are for oral exposure to xylene (intermediate oral MRLs for mixed xylenes and *m*-xylene), inhalation exposure to benzene (acute inhalation MRL), and inhalation exposure to ethylbenzene (intermediate inhalation MRL). These MRLs do not imply a low neurotoxic potential for these chemicals and durations, but rather that there is insufficient information on sensitive neurological effects at low levels of exposure. For example, although the intermediate oral MRLs for mixed xylenes and *m*-xylene are based

on kidney and liver toxicity, respectively, all three inhalation MRLs for mixed xylenes, as well as the acute oral MRL for *p*-xylene, are based on neurotoxicity. Similarly, the immuno/hematotoxicity-based acute inhalation MRL for benzene and developmental toxicity-based intermediate inhalation MRL for ethylbenzene are for exposure categories and chemicals for which sensitive neurological effects are poorly characterized, although the neurotoxicity of these chemicals is recognized as discussed below.

Available exposure-response data for neurological effects of acute inhalation exposure to benzene and intermediate inhalation exposure to ethylbenzene mainly reflect relatively insensitive endpoints such as overt symptoms and signs of central nervous system toxicity (ATSDR 1997, 1999b). Although these data, as well as information summarized in Appendices A and C, clearly show that the nervous system is a target of benzene and ethylbenzene, more studies are needed to identify thresholds for neurotoxicity and better characterize the relative sensitivity of neurological, immunological, and developmental endpoints. Indications that neurotoxicity is likely to be a critical effect for acute exposure to benzene and intermediate exposure to ethylbenzene include the following inhalation data, as summarized by ATSDR (1997, 1999b) in Levels of Significant Exposure (LSE) tables: (1) proximity of the lowest-observed-adverse-affect level (LOAEL) for neurological symptoms (60 ppm in humans) to the LOAEL for immunological effects (10 ppm in mice) used to derive the acute MRL for benzene; (2) use of a neurotoxicity LOAEL (0.78 ppm in mice) as the basis for the intermediate inhalation MRL for benzene; (3) similarity of the no-observed-adverse-affect level (NOAEL) for neurotoxicity (99 ppm in rats) to the NOAEL for developmental toxicity (97 ppm in rats) used to derive the intermediate MRL for ethylbenzene; and (4) a neurotoxicity LOAEL (382 ppm in rats) that is lower than the LOAELs for developmental toxicity (959 ppm in rats) and immunotoxicity (959 ppm in rats) for intermediate exposure to ethylbenzene. Increased concern for neurotoxicity compared to immunological and developmental effects is further indicated by data for toluene and xylenes, including the following (ATSDR 1995, 2000): (1) the acute MRL for toluene is based on a NOAEL for neurotoxicity (40 ppm in humans) that is higher than the LOAEL for immunological effects (2.5 ppm in mice); (2) the chronic

Table 2-11. Health Effects Forming the Basis of ATSDR MRLs for Chemicals of Concern (See Appendices A, B, C, and D for More Details)

Chemical	Exposure route	Exposure duration		
		Acute	Intermediate	Chronic
Benzene	Inhalation	Immunological effects in mice	Neurological effects in mice	—
	Oral	—	—	—
Toluene	Inhalation	Neurological effects in humans	—[a]	Neurological effects in humans
	Oral	Neurological effects in rats	Neurological effects in mice	—
Ethylbenzene	Inhalation	—	Developmental effects in rats	—
	Oral	—	—	—
Xylenes (mixed)	Inhalation	Neurobehavioral effects in humans	Neurodevelopmental effects in rats	Neurotoxicity symptoms and eye and respiratory tract irritation in humans
	Oral	—	Renal toxicity in rats	—
o-Xylene	Inhalation	—	—	—
	Oral	—	—	—
m-Xylene	Inhalation	—	—	—
	Oral	—	Liver toxicity in rats	—
p-Xylene	Inhalation	—	—	—
	Oral	Neurological effects in rats	—	—

[a]No data were considered suitable for use in deriving an intermediate duration MRL for inhalation exposures to toluene. ATSDR concluded, however, that the neurotoxicity-based chronic inhalation MRL would also be protective for intermediate duration exposures (ATSDR 2000).

ATSDR = Agency for Toxic Substances and Disease Registry; MRL = Minimum Risk Level

Source: ATSDR 1995, 1997, 1999b, 2000

MRL for toluene is based on a LOAEL for neurotoxicity (35 ppm in humans) that is similar to chronic LOAELs for immunological effects (41 and 44 ppm in humans); (3) the *de facto* intermediate MRL for toluene (i.e., the chronic MRL for toluene, which is also protective for intermediate exposures) is based on a LOAEL for neurotoxicity (35 ppm in humans) that is higher than the intermediate LOAEL for immunological effects (2.5 ppm in mice); and (4) the acute MRL for mixed xylenes is based on a LOAEL for neurotoxicity (100 ppm in humans) that is higher than LOAELs for developmental toxicity (53 and 58 ppm in rats).

Hematotoxicity and carcinogenicity are additional concerns for exposure to BTEX based on known effects of the benzene component. There is strong evidence that these health effects are benzene-specific and due to the formation of reactive metabolites (see Appendix A). The most characteristic toxic effect of long-term benzene exposure is a decrease in bone marrow cellularity, which appears to ultimately lead to aplastic anemia and development of leukemia. A chronic inhalation MRL for benzene was not derived due to lack of appropriate exposure-response data on noncancer effects (ATSDR 1997). The intermediate inhalation MRL for benzene is based on neurological effects that occurred at a lower exposure level than for hematological changes. The carcinogenic potential of benzene is well established as reflected by its classification as a human carcinogen by NTP (2001), EPA (IRIS 2001), and IARC (1987). There is also a concern for the carcinogenicity of the ethylbenzene component due to its classification by IARC (2000) as possibly carcinogenic to humans based on recent animal bioassay data (see Appendix C). Ethylbenzene is not listed as a known or anticipated human carcinogen by NTP (2001), and EPA determined that ethylbenzene is not classifiable as to human carcinogenicity (IRIS 2001), but both of these assessments predate the data used as the basis of the IARC classification. The mechanism of carcinogenicity of ethylbenzene has not been elucidated, but is likely related to the formation of reactive metabolites (Appendix C). The human and animal evidence does not support a concern for the carcinogenicity of the other BTEX components; reflecting the weights of available evidence for toluene and xylenes, these chemicals are considered not classifiable as to human carcinogenicity by both EPA (IRIS 2001) and IARC (1999a, 1999b).

In the absence of data on toxic or carcinogenic responses to the whole mixture, the health hazards from exposure to BTEX are best assessed by a components-based approach that considers both the shared (neurologic) and unique (hematologic/immunologic/carcinogenic) critical effects of the four mixture components. Available ATSDR methods for assessing hazards of the shared critical effects are based on an assumption that the responses to the mixture components are additive, and therefore require

judgements concerning the presence or absence of chemical interactions affecting the responses (ATSDR 2001a). The unique critical effects are best assessed on a benzene-specific basis.

Although no data are available on the response of the shared critical target organ, the nervous system, to mixtures of all four BTEX chemicals, the PBPK model studies provide a basis for projecting how the whole mixture is likely to act in producing neurological effects. The PBPK studies facilitate the assessment of neurological hazards of BTEX mixtures by providing estimated and observed exposure levels that reflect the net effect of component interactions at the target tissue. In other words, the modeled/observed blood concentrations of benzene, toluene, ethylbenzene, and xylene represent target tissue doses because the neurological effects are likely due to direct actions of the parent chemicals on neuronal membranes. Based on the PBPK model simulations and experimental data discussed above, there is compelling evidence that metabolic interactions (competitive inhibitions) are negligible in humans exposed to BTEX at concentrations of approximately 20 ppm of each component (Haddad et al. 1999a, 1999b, 2001; Tardif et al. 1997). Although the exact threshold for metabolic interactions is unclear due to incomplete information on the interaction threshold for the benzene component, the available data imply that exposure to lower (e.g., environmental) levels of BTEX is unlikely to cause greater-than-additive joint action in the nervous system. This indicates that an additive components-based approach, such as the Hazard Index method, is appropriate for assessing neurotoxic hazards from exposure to BTEX.

As discussed above, the PBPK studies facilitate the use of the Hazard Index method to assess the joint neurotoxic action of BTEX by providing an estimate of the threshold for interactions (i.e., by defining the additive region in which the method is applicable). Another approach that could be used to incorporate information on component interactions in the assessment of BTEX mixtures is the binary weight-of-evidence (BINWOE) modification to the hazard index (ATSDR 2001a, 2001b). Unlike the PBPK model-based approach, the BINWOE method is qualitative and does not provide an integrated view of the potential for non-additive effects over a range of relevant exposures. This method evaluates joint action data for all possible pairs of chemicals in order to determine a BINWOE for the effect of each chemical on the toxicity of every other chemical in the mixture. Each BINWOE is a single qualitative determination of what the direction of interactions is likely to be, when they do occur, and includes confidence ratings for that determination. Thus, if joint toxic action is known to be additive at low levels of exposure and greater than additive at higher exposures, the BINWOE determination will predict greater than additive. The BINWOEs are collectively examined for any patterns that might be evident, and the overall weight of evidence is used to predict whether the whole mixture would increase the hazard

beyond what would be expected based on additivity of the components (i.e., to modify the hazard index for interactions). A BINWOE analysis does not define the exposure region in which its predicted direction of interaction is applicable, and is intended to be used when joint action data are inadequate to support more quantitative methods, such as the PBPK modeling of BTEX, that do provide an integrated assessment of the potential for interactions in the low-exposure region relevant to environmental concerns.

Although a BINWOE analysis is not the most appropriate approach for BTEX given the inherent advantages of PBPK-based assessment, it is relevant that the binary interaction studies generally support the direction of interaction predicted by the PBPK models for higher levels of exposures. Binary interaction matrixes that indicate the plausible direction of interactions when they do occur, as predicted from the interaction studies of metabolic and neurological effects, are presented in Tables 2-12 and 2-13. These matrixes show that joint action was less than additive for metabolic effects in all 12 predictions, greater than additive for neurological effects in 2 of 12 predictions, and additive for neurological effects in 2 of 12 predictions. Because less-than-additive metabolic interaction implies greater-than-additive neurotoxicity (due to increased levels of unmetabolized chemicals that can act on neuronal membranes), the overall assessment is that the mixture components are likely to jointly act on the nervous system in a greater-than-additive manner, which is consistent with the PBPK model predictions for levels of exposure above the interaction threshold.

Studies that directly examined the joint toxic action of BTEX chemicals on the nervous system mainly consist of human and animal investigations of a few binary mixtures of components, particularly benzene/toluene, toluene/xylene, and ethylbenzene/xylene (Sections 2.2.3, 2.2.6, and 2.2.7, respectively). The interpretation of a number of these studies is complicated by experimental designs that preclude conclusively determining if effects of combined exposure were jointly additive because the mixtures were tested at a higher dosage level than the dose levels of the individual components. Considering the better-designed studies in particular, there is no clear evidence that binary component mixtures may jointly act on the nervous system in a less-than-additive or greater-than-additive mode at exposure concentrations below approximately 1,000 ppm. For example, dopamine levels and rate of turnover were higher in the forebrain of rats exposed for 6 hours/day for 3 days to 2,000 ppm of ethylbenzene and xylene in mixture than to either chemical individually at 2,000 ppm (Toftgard and Nilsen 1981, 1982), suggesting that there was a greater-than-additive joint action at this relatively high level of exposure.

Table 2-12. Binary Interaction Matrix for Metabolic Effects from Simultaneous Exposure to Chemicals of Concern

		ON METABOLISM OF			
		Benzene	Toluene	Ethylbenzene	Xylenes
EFFECT OF	Benzene		<	<	<
	Toluene	<		<	<
	Ethylbenzene	<	<		<
	Xylenes	<	<	<	

INTERACTIONS: = additive; > greater than additive; < less than additive; ? indeterminate

Table 2-13. Binary Interaction Matrix for Nervous System Effects from Simultaneous Exposure to Chemicals of Concern

		ON NERVOUS SYSTEM EFFECTS OF[a]			
		Benzene	Toluene	Ethylbenzene	Xylenes
EFFECT OF	Benzene		?	?	?
	Toluene	?		?	=
	Ethylbenzene	?	?		=
	Xylenes	?	=	=	

INTERACTIONS: = additive; > greater than additive; < less than additive; ? indeterminate.

[a]The direction of interaction determinations do not consider metabolic interactions, which are summarized in Table 2-12

Greater-than-additive joint neurotoxic action was also suggested by a comparison of relative potency factors for impaired rotarod performance in rats, which showed that a 4-hour exposure to mixtures of toluene and xylene (1,050–4,700 ppm total) were about 1.5-fold more potent than similar concentrations of toluene alone or xylene alone (Korsak et al. 1988). In contrast, there were no clear indications of greater-than-additive joint action on rotarod performance and spontaneous motor activity in rats exposed for 6 hours/day, 5 days/week to 1000 ppm toluene alone, 1000 ppm xylene alone, or 500 ppm toluene plus 500 ppm xylene for 3 months, or to 100 ppm toluene alone, 100 ppm xylene alone, or 50 ppm toluene plus 50 ppm xylene for 6 months (Korsak et al. 1992). Other lower concentration studies similarly found that combined exposure produced effects that were not significantly different from effects from exposure to comparable levels of either component. Endpoints in these studies included neurobehavioral performance (assessed by a battery of nine tests) in humans exposed to 100 ppm toluene alone, 100 ppm xylene alone, or 50 ppm toluene plus 50 ppm xylene for 4 hours (Dudek et al. 1990); spontaneous motor activity and sound-induced seizures in rats and mice exposed to 540 ppm toluene alone, 460 ppm xylenes alone, or 270 ppm toluene plus 230 ppm xylenes for 2–6 hours (Frantik et al. 1988); and electric shock-induced seizures in rats exposed to $\geq$160 ppm ethylbenzene alone, $\geq$280 ppm xylene alone, or $\geq$80 ppm ethylbenzene plus $\geq$140 ppm xylene for 4 hours (Frantik and Vodickova 1995). The neurotoxicity studies of the binary component mixtures therefore provide no data that are inconsistent with the predictions of the PBPK studies (i.e., that effects from exposures to BTEX at concentrations below approximately 20 ppm of each component are likely to be additive).

The potential hazards due to the benzene-specific hematological and carcinogenic effects of BTEX are best assessed by an approach that considers the mechanistic relationship between these effects. As summarized in Appendix A, it is well established that exposure to benzene can cause damage to the human hematopoietic system, resulting in effects that include aplastic anemia with subsequent manifestation of acute myelogenous leukemia (AML). Additionally, there is general agreement that benzene metabolites, rather than benzene, are the agents involved in the induction of the hematotoxicity and cancer. Assessing the joint toxic action of chemical mixtures usually involves separate strategies for the noncarcinogenic and carcinogenic effects of the components (ATSDR 2001a). However, considering that the only unique critical noncarcinogenic and carcinogenic effects of BTEX chemicals are causally related and induced by the same component, benzene, it is logical to group the hematotoxic and leukemogenic effects together in assessing hazards from exposure to the benzene component. Using cancer risk as the basis for quantifying benzene hazard would be protective of hematotoxicity as well as leukemia.

The PBPK model predictions indicate that toluene, ethylbenzene, and xylene are unlikely to influence the hematotoxic and carcinogenic effects of benzene, and that benzene, toluene, and xylene are unlikely to influence the carcinogenicity of ethylbenzene, at exposure concentrations below approximately 20 ppm of each component (Haddad et al. 1999a, 1999b, 2001; Tardif et al. 1997). Exposure to higher concentrations of BTEX is 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, thereby decreasing the potential for hematotoxicity and carcinogenicity. Studies of binary mixtures of BTEX components are consistent with the PBPK model predictions in indicating less-than-additive joint metabolic action for levels of exposure above the interaction threshold (Table 2-12).

Additionally, as discussed in Section 2.2.3 and indicated in Table 2-14, it is well established that toluene can inhibit the hematological effects of benzene. Although ethylbenzene and xylenes are also expected to reduce the hematotoxic/carcinogenic potential of benzene due to competitive metabolic interactions, available toxicity data for these pairs of chemicals are indeterminate (Table 2-14). Similarly, there are no binary toxicity data supporting the possible reduction in ethylbenzene carcinogenicity due to competitive metabolic interactions with benzene, toluene, and xylenes.

Table 2-14. Binary Interaction Matrix for Hematological and Clastogenic Effects from Simultaneous Exposure to Chemicals of Concern

		ON HEMATOLOGICAL AND CLASTOGENIC EFFECTS OF[a]			
		Benzene	Toluene	Ethylbenzene	Xylenes
EFFECT OF	Benzene		=	?	?
	Toluene	<		?	=
	Ethylbenzene	?	?		?
	Xylenes	?	=	?	

INTERACTIONS: = additive; > greater than additive; < less than additive; ? indeterminate

[a]The direction of interaction determinations do not consider metabolic interactions, which are summarized in Table 2-12.

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).

4. Conclusions

No studies are available that directly characterize health hazards and dose-response relationships for exposures to whole mixtures of benzene, toluene, ethylbenzene, and xylenes. All four components can produce neurological impairment, and benzene can additionally cause hematological effects which may ultimately lead to aplastic anemia and development of acute myelogenous leukemia. Concern for the carcinogenicity of BTEX is also raised by evidence that ethylbenzene is carcinogenic. No studies were located that directly examined joint toxic actions of mixtures of benzene, toluene, ethylbenzene, and xylenes on the nervous system, but additive joint action is plausible. Results of PBPK model simulations and experimental exposures with BTEX and ternary and quinary mixtures of its components (Haddad et al. 1999a, 1999b, 2000, 2001; Tardif et al. 1997) strongly suggest that joint neurotoxic action is expected to be additive at BTEX concentrations below approximately 20 ppm of each component. Neurotoxicity interaction studies of binary component mixtures support the plausibility of additive joint action at environmental levels of BTEX exposure. It is unclear whether the PBPK models are adequate for characterizing interactions from inhalation of BTEX mixtures above approximately 200 ppm of each component, or if the results are applicable to oral exposures. A component-based hazard index approach that assumes additive joint action and uses ATSDR MRLs based on neurological impairment is recommended for exposure-based assessments of possible neurotoxic health hazards from mixtures of BTEX. The possible hematotoxic and leukemogenic hazards of BTEX exposures should be evaluated on a benzene-specific basis because the other mixture components do not induce these effects. Considering the causal relationship between the noncancer hematological effects of benzene and development of leukemia, as well as lack of a cancer risk value for ethylbenzene, it is recommended that the inhalation cancer unit risk value for benzene be used to assess the benzene/ethylbenzene-related hematological/carcinogenic hazards from exposures to BTEX. Exposure to relatively high concentrations of BTEX (above approximately 20 ppm of each chemical) is expected to increase the potential for neurotoxicity and decrease the potential for hematotoxicity/carcinogenicity due to competitive metabolic interactions among the mixture components.

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Appendix A: Background Information for Benzene

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for benzene. The summaries are mainly based on information presented in the ATSDR (1997) toxicological profile for benzene and therefore do not represent reviews of the primary literature. The chemical structure for benzene is included in Appendix E.

A.1 Toxicokinetics

Benzene is rapidly but incompletely absorbed by humans and animals following inhalation exposure (ATSDR 1997). Results of several studies indicate that average respiratory uptake is approximately 50% in humans. For example, in a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours, absorption was 70–80% during the first 5 minutes of exposure, but subsequently decreased to about 50% (range, 20–60%) by 1 hour (Srbova et al. 1950). Respiratory uptake was approximately 47% in six subjects exposed to 52–62 ppm benzene for 4 hours (Nomiyama and Nomiyama 1974), and 52 and 48% in three subjects exposed to 1.6 or 9.4 ppm, respectively, for 4 hours (Pekari et al. 1992). In rodents, the extent of uptake increased linearly with concentration for exposures up to approximately 200 ppm (ATSDR 1997). At concentrations >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15% in rats and from 50 to 10% in mice when the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). Mice and rats have different absorption characteristics in that the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987).

Quantitative oral absorption data are not available for benzene in humans. Oral doses of benzene are extensively absorbed in animals (generally >90%), although benzene was administered in oil in many oral studies to assure predictable solubility and dose concentration control (ATSDR 1997; Cornish and Ryan 1965; Parke and Williams 1953a, 1953b; Sabourin et al. 1987). One of the studies showed that gastrointestinal absorption was >97% in rats and mice when the animals were treated with benzene by gavage at doses 0.5–150 mg/kg (Sabourin et al. 1987). Dermal absorption of benzene is low in humans and animals. Approximately 0.05% of an applied dose (0.0026 mg/cm^2) was determined in one human study (Franz 1984), and another estimated a dermal absorption rate of approximately 0.4 mg/cm^2/hour under conditions of complete saturation (35–43 cm^2 of skin was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours) (Hanke et al. 1961). Animal studies found that dermal absorption was

<1% following a single direct application of liquid benzene to Rhesus monkeys, minipigs, and hairless mice (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). Absorbed benzene is widely distributed to tissues, with the relative uptake dependent on the perfusion rate of the tissue by blood, and the total potential uptake dependent on fat content and metabolism (ATSDR 1997; Sato et al. 1975; Tauber 1970).

The metabolism and elimination of benzene appear to be qualitatively similar in humans and animals (ATSDR 1997; Henderson et al. 1989; Sabourin et al. 1988). Benzene is initially metabolized by cytochrome P-450-dependent mixed function oxidases primarily in the liver via several toxification and detoxification (via conjugation) pathways. Several cytochrome P-450 isozymes are involved in the metabolism of benzene, although the predominant form is CYP2E1 (Gut et al. 1993; Nakajima et al. 1993). The first metabolic step is the formation of the epoxide benzene oxide. This is followed by two toxification pathways, one involving ring hydroxylation and the second involving ring opening, resulting in the formation of putative toxic metabolites (ATSDR 1997; Henderson et al. 1989). In the first pathway involving ring hydroxylation, opening of the epoxide ring is followed by aromatization resulting in formation of phenol. Phenol is further converted into hydroquinone, which is oxidized to benzoquinone. The conjugates formed from hydroquinone (hydroquinone glucuronide and hydroquinone sulfate) are markers for this toxification pathway leading to benzoquinone. Phenol can also be metabolized to catechol and trihydroxy benzene. Metabolism of benzene oxide leads to the formation of benzene dihydrodiol. Catechol can also be formed from benzene dihydrodiol via metabolism by cytosolic dehydrogenases (Henderson et al. 1989). The second pathway involving ring-opening leads to the formation of muconic acid, apparently via the precursor muconic dialdehyde.

There are two detoxification pathways. One detoxification pathway leads to the formation of mercapturic acid via glutathione conjugates of benzene oxide, which are subsequently metabolized to prephenyl mercapturic acid and phenyl mercapturic acid and eliminated by biliary excretion (ATSDR 1997; Henderson et al. 1989; Sabourin et al. 1987; Schrenk et al. 1992). The major portion of benzene oxide is nonenzymatically rearranged to phenol. The second detoxification pathway involves the formation of water-soluble urinary metabolites, which are glucuronide or sulfate conjugates of phenol. Further metabolites of phenol and benzene dihydrodiol (e.g., catechol, hydroquinone, and trihydroxy benzene) are excreted as sulfate or glucuronide conjugates and are also considered detoxification products.

Although the metabolism of benzene occurs primarily in the liver, studies in rats and mice indicate that a small amount is metabolized independently of the liver in the bone marrow, the site of characteristic benzene toxicity (Ganousis et al. 1992; Irons et al. 1980). It is believed that biotransformation is essential

for benzene-induced bone marrow damage, but it is unclear whether benzene is activated in the marrow, activated elsewhere and transported to the marrow, or metabolized in the liver and the metabolites activated in the marrow (ATSDR 1997). Benzene has been found to stimulate its own metabolism (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). Differences in species, routes of exposure, and dosing regimens affect the disposition and metabolic fate of benzene (Sabourin et al. 1987, 1988, 1989a, 1989b, 1992). Benzene metabolism (both total and amounts of individual metabolites) is dose-dependent in all species studies thus far, including humans (ATSDR 1997). At low doses, more of the benzene is converted to putative toxic metabolites than at high doses. At high doses, benzene inhibits phenol metabolism to hydroquinone, apparently through competition for a common site on the CYP2E1 isozyme to which hydroquinone and catechol also bond. The effect that dose and species can have on the metabolism of benzene is illustrated by a study in which rats and mice were administered benzene by gavage at doses of 0.5–150 mg/kg (Sabourin et al. 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of the administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. Total metabolites did not increase at higher doses in mice, suggesting saturation of metabolic pathways. Other studies similarly indicate that mice metabolize benzene more efficiently than rats (Medinsky et al. 1989a, 1989b; Travis et al. 1990).

Human and animal data show that exhalation is the main route for elimination of unmetabolized benzene, metabolites are excreted predominantly in the urine, and only a small amount of the absorbed amount is eliminated in feces (ATSDR 1997). Respiratory uptake (the amount of benzene absorbed from the lungs) and respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 47 and 17%, respectively, in six humans exposed to 52–62 ppm benzene for 4 hours (Nomiyama and Nomiyama 1974). Results from a study of 23 humans who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6 and 0.07–0.2% of the retained benzene was excreted in the breath (within 5–7 hours) and urine, respectively (Srbova et al. 1950). The major route of excretion following a 6-hour nose-only inhalation exposure of rats and mice to ^{14}C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor concentrations of 10–1,000 ppm, the mice received 150–200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5 and <9% of the radioactivity in the rats and mice, respectively. At lower exposure concentrations (11–130 ppm), both rats and mice excreted <6% of the radioactivity in the expired air. At the highest exposure concentrations (870 ppm in rats, 990 ppm in mice), both rats and mice exhaled a significant amount of unmetabolized benzene (48 and 14%, respectively) following termination of exposure. The percentage of total excreted radioactivity that was

not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. Approximately 90% of the radioactivity was excreted as urinary metabolites by rats at ≤260 ppm and by mice at ≤130 ppm. The total urinary metabolite formation was 5–37% higher in the mice than in the rats at all doses, apparently due to the greater amount of benzene inhaled by mice per kg of body weight. The effect of dose on excretion of radioactivity was also studied in rats and mice following oral administration of 0.5–300 mg/kg single doses of ^{14}C-benzene (Sabourin et al. 1987). At doses <15 mg/kg, ≥90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of radioactivity in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. Of a 150 mg/kg dose, 50 and 69% was eliminated in the expired air largely as unmetabolized benzene in rats and mice, respectively.

Four PBPK models have been developed to describe the behavior of benzene in rats, mice, and humans. The Medinsky model addresses species differences in benzene kinetics using rats and mice (Medinsky et al. 1989a, 1989b, 1989c). The Travis model specifically addresses human pharmacokinetics of benzene in comparison to experimental animal data (Travis et al. 1990), whereas the Bois and Paxman model (Bois and Paxman 1992) addresses the effect of exposure rate on benzene metabolism. The Sun model (Sun et al. 1990) addresses the formation of hemoglobin-benzene derived adducts in the blood, as a tool in monitoring benzene exposure.

A.2 Health Effects

The nervous and hematopoietic systems are the main targets of benzene. Acute inhalation or oral exposure to high levels of benzene has caused symptoms and signs of central nervous system toxicity in humans and animals (ATSDR 1997; Carpenter et al. 1944; Cornish and Ryan 1965; Midzenski et al. 1992; Tauber 1970; Thienes and Haley 1972). Effects such as dizziness, vertigo, tremors, narcosis, and cardiac arrhythmias have been observed following both acute nonlethal and lethal exposures. Intermediate-duration inhalation and oral exposures to benzene induced neurological effects in animals that included reduced limb grip strength, behavioral disturbances, and changes in brain levels of monoamine transmitters and acetylcholinesterase (Dempster et al. 1984; Frantik et al. 1994; Hsieh et al. 1988; Li et al. 1992).

Hematotoxicity is the most noted and characteristic systemic effect resulting from intermediate and chronic benzene exposure in humans and animals. All of the major types of blood cells are susceptible

(erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A common clinical finding is cytopenia, which is a decrease in various cellular elements manifested as anemia, leukopenia, or thrombocytopenia in humans (ATSDR 1997). Benzene-associated cytopenias vary and can involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood. Prolonged exposure to benzene can cause severe damage to the bone marrow involving cellular aplasia in humans and animals. This condition, known as aplastic anemia, is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow (ATSDR 1997). Benzene-induced aplastic anemia can progress to AML, which is characterized by the appearance in the peripheral blood of cells morphologically indistinguishable from myeloblasts (Aksoy 1980; Aksoy et al. 1974; Doskin 1971; Rozen et al. 1984). Human studies that provide some estimate of levels of exposure indicate that adverse hematological effects occurred at levels >10 ppm and generally not at levels <1 ppm (ATSDR 1997). Adverse hematological effects begin to appear in animals at benzene concentrations of 10–100 ppm and above. Oral data are essentially limited to findings in intermediate- and chronic-duration animal studies showing that loss of blood elements occurs following exposure to benzene in drinking water or by gavage at doses as low as 8–25 mg/kg/day (ATSDR 1997).

Other health effects of benzene include immunological changes in humans and animals, which appear to be largely related to decreases in circulating leukocytes and the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies (ATSDR 1997). Adequate reproductive and developmental toxicity data on benzene are essentially limited to results of inhalation studies in animals (ATSDR 1997). There is suggestive evidence of benzene-induced testicular effects (e.g., atrophy/ degeneration, decrease in spermatozoa, increase in abnormal sperm forms), particularly in mice following intermediate duration exposure to 300 ppm (ATSDR 1997; Ward et al. 1985; Wolf et al. 1956). Results of developmental toxicity studies indicate that inhalation exposure to high levels of benzene is fetotoxic and maternally toxic in several species as shown by decreased fetal weight and/or minor skeletal variants. Fetotoxic effects in rodents occurred at benzene levels ≥47 ppm (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985), although there was evidence of transient hematopoietic anomalies in fetuses and offspring of mice exposed to 5–20 ppm benzene (Keller and Snyder 1986, 1988). Chromosomal damage in bone marrow cells and lymphocytes are well documented effects of benzene based on findings in human occupational studies and *in vivo* studies in animals (ATSDR 1997). As indicated above, there is a well established causal relationship between occupational exposure to benzene and acute myelogenous leukemia (ATSDR 1997; Aksoy et al. 1971, 1972, 1974). Benzene-induced leukemia has not been clearly demonstrated in laboratory animals, although increased incidences of lymphomas, Zymbal gland carcinomas, and other

neoplasms were found in rats and mice following chronic inhalation or oral exposure (ATSDR 1997; Cronkite et al. 1984, 1985, 1986, 1989; Farris et al. 1993; NTP 1986; Snyder et al. 1980, 1984, 1988).

A.3 Mechanisms of Action

The most characteristic toxic effect of benzene is a decrease in bone marrow cellularity, which appears to ultimately lead to aplastic anemia and development of leukemia. The compensatory proliferative response (regenerative hyperplasia) to the anemia observed in the bone marrow, thymus, and spleen of exposed animals may play a role in the carcinogenic response (Rozen and Snyder 1985; Snyder 1987; Snyder and Koscis 1975; Snyder et al. 1984, 1993; Toft et al. 1982).

Metabolites appear to play key roles in the development of the hematotoxic, clastogenic, and carcinogenic effects of benzene. Studies of benzene metabolism and mechanisms of toxic action have identified or postulated a number of biologically reactive metabolites, including benzene oxide, benzene dihydrodiol, hydroquinone, catechol, benzoquinones, and muconaldehyde (ATSDR 1997). It is generally believed that benzene metabolites covalently bind to cellular macromolecules (including DNA, ribonucleic acid [RNA], and proteins), thereby leading to dysfunction in the bone marrow (including stem, progenitor, and stromal cells) and other tissues. For example, benzene and its metabolites may induce oxidative DNA damage or interfere with the incorporation of iron into bone marrow precursors (Longacre et al. 1981; Snyder and Kalf 1994; Snyder et al. 1989), chelates of iron and hydroquinone or 1,2,4-benzenetriol appear to be potent DNA cleaving agents (Rao 1996; Singh et al. 1994), and hydroquinone may accumulate in bone marrow to act as substrates for myeloperoxidase, forming benzoquinone which is myelotoxic and clastogenic (ATSDR 1997; Medinsky et al. 1994).

The relationship between adduct formation and toxicity is not clear, and multiple metabolic pathways and mechanisms are involved in benzene toxicity and carcinogenicity. The multiple metabolic pathways provide opportunities for modulation of benzene metabolism, either by competition with other chemicals for the available cytochrome P-450 sites, by induction or inhibition of the oxidation or conjugation enzymes, or by direct competition between benzene and its metabolites (Medinsky et al. 1994). There also may be synergism between metabolites (Eastmond et al. 1987; Snyder et al. 1989) or between glutathione-depleting metabolites of benzene and oxygen radicals (generated by futile cycling of cytochrome P-450 or cycling of quinone metabolites) (Parke 1989).

The acute neurological effects of benzene are similar to the general anesthetic effects of other lipophilic solvents, and consequently are presumed to result from a direct effect of the parent compound on central nervous system cell membranes unrelated to its metabolites (Snyder et al. 1993). There is a paucity of mechanistic information on the chronic nervous system toxicity of benzene (ATSDR 1997; Snyder et al. 1993).

A.4 Health Guidelines

ATSDR (1997) derived an acute-duration inhalation MRL of 0.05 ppm for benzene based on a LOAEL of 10 ppm for immunotoxicity (reduced lymphocyte proliferation) following mitogen stimulation in mice (Rozen et al. 1984). The animal LOAEL was converted to a human equivalent concentration ($LOAEL_{HEC}$) of 14.7 ppm and divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) to yield the MRL. The mice were exposed 6 hours/day for 6 days.

ATSDR (1997) derived an intermediate-duration inhalation MRL of 0.004 ppm for benzene based on a LOAEL of 0.78 ppm for neurological effects (increased rapid response time) in mice (Li et al. 1992). The animal LOAEL was converted to a $LOAEL_{HEC}$ of 0.33 ppm and divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) to yield the MRL. The mice were exposed 6 hours/day, 6 days/week for 30 days.

ATSDR (1997) did not derive a chronic-duration inhalation MRL or acute-, intermediate-, or chronic-duration oral MRLs for benzene due to lack of suitable data.

The EPA Integrated Risk Information System (IRIS) database does not list a RfD or RfC for benzene (IRIS 2001). EPA has classified benzene as a "known" human carcinogen (Category A) under the 1986 Risk Assessment Guidelines (IRIS 2001). Under EPA's proposed revised Carcinogen Risk Assessment Guidelines, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2001). Based on leukemia data in humans, an inhalation unit risk range of $2.2x10^{-6}$–$7.8x10^{-6}$ per $\mu g/m^3$ was estimated for benzene (IRIS 2001). The inhalation unit risk range was extrapolated to an oral (drinking water) unit risk range of $4.4x10^{-4}$–$1.6x10^{-3}$ per mg/L (IRIS 2001).

The NTP (2001) concluded that benzene is known to be a human carcinogen. IARC (1987) classified benzene as a Group 1 carcinogen (carcinogenic to humans).

ACGIH (2001) recommends a TLV-TWA of 0.5 ppm and short-term exposure limit/ceiling (STEL/C) of 2.5 ppm for benzene based on cancer as the critical effect. ACGIH has included benzene in the carcinogenicity category A1 (confirmed human carcinogen).

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Appendix B: Background Information for Toluene

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for toluene. The summaries are mainly based on information presented in the ATSDR (2000) toxicological profile for toluene and therefore do not represent reviews of the primary literature. The chemical structure for toluene is included in Appendix E.

B.1 Toxicokinetics

Studies with humans and animals indicate that toluene is readily absorbed from the respiratory and gastrointestinal tracts and, to a lesser extent, through the skin (ATSDR 2000). Respiratory uptake was rapid in humans exposed to 80 ppm toluene, as shown by the appearance of toluene in the blood within 10–15 minutes of exposure (Hjelm et al. 1988). About 50% of deuterium-labeled toluene was absorbed from the lungs in volunteers exposed to 53 ppm for 2 hours during a period of light exercise (Lof et al. 1993). Seven humans exposed to 50 ppm toluene in a closed chamber showed an average retention of 83% of the inspired concentration (Benoit et al. 1985). Complete gastrointestinal absorption in humans was indicated by monitoring exhaled air for toluene and urine for toluene metabolites (hippuric acid and *ortho*-cresol) following oral administration of toluene as a 2 mg/minute infusion for 3 hours through a feeding tube into the stomach (Baelum et al. 1993). Complete absorption of orally administered toluene has also been observed in rats, although the rate of oral absorption was slower than pulmonary absorption (maximum blood levels were observed 1.5–3 hours and 15–30 minutes after administration, respectively) (Pyykko et al. 1977). The rate of dermal absorption of liquid toluene through human forearm skin was found to be slow, ranging from 14 to 23 mg/cm^2/hour (Dutkiewicz and Tyras 1968). Dermal absorption of toluene vapor has been demonstrated in animals (ATSDR 2000). For example, dose and duration-related increases in whole body toluene levels were found in nude mice that were exposed to 300, 1,000, or 3,000 ppm toluene under conditions where there was no respiratory intake of toluene (Tsuruta 1989). The calculated skin absorption coefficient was 1.24 cm/hour. The skin absorption rates were 0.0009, 0.0046, and 0.0144 mg/cm^2/hour for the 300, 1,000 and 3,000 ppm concentrations, respectively.

Distribution of absorbed toluene in humans and laboratory animals is characterized by preferential uptake in lipid-rich and highly vascular tissues such as the brain, bone marrow, and body fat (ATSDR 2000). Toluene is distributed between the plasma and red blood cells at approximately a 1:1 ratio in humans based on *in vitro* data and a 1:2 ratio in rats based on *in vivo* data (Lam et al. 1990). In the red blood cells, toluene appears to be associated with the hemoglobin rather than the cell membrane. The

interaction of toluene with the red blood cell increases the amount of toluene that can be accommodated by the aqueous blood medium and facilitates transport of toluene to all areas of the body (including the brain) at a rate that is greater than if toluene was transported only in the plasma. Within the brain, toluene has a greater affinity for the areas that contain lipid-rich white matter, such as the brain stem and midbrain, rather than the areas with larger amounts of gray matter (Ameno et al. 1992; Bergman 1979).

The first step in toluene metabolism is catalyzed by several cytochrome P-450 species (ATSDR 2000; Gut et al. 1993; Nakajima and Wang 1994; Nakajima et al. 1991, 1992a, 1992b, 1993, 1997; Tassaneeyakul et al. 1996). Most toluene is initially metabolized by side-chain hydroxylation to form benzyl alcohol, followed by oxidation to benzoic acid. The main CYP isozyme involved in the formation of benzyl alcohol is CYP2E1, although CYP2B1, CYP2B6, CYP2C6, CYP1A2, and CYP1A1 (in decreasing order) are also active. Most of the benzoic acid is then conjugated with glycine to form hippuric acid, although some can be conjugated with uridine-5'-diphosphate (UDP)-glucuronate to form the acyl-glucuronide. In both humans and rats, up to about 75–80% of inhaled toluene that is absorbed can be accounted for as hippuric acid in the urine. Much of the remaining toluene is exhaled unchanged. A very small portion (<1–5%) of absorbed toluene undergoes ring hydroxylation by CYP1A2, CYP2B2, or CYP2E1 to form 2,3- and 3,4-epoxide intermediates and subsequently *ortho*- or *para*-cresols, which are conjugated with sulfate or glucuronate (Baelum et al. 1993, Nakajima and Wang 1994; Nakajima et al. 1997; Tassaneeyakul et al. 1996). Other minor metabolites include S-benzyl mercapturic acid and S-*p*-toluylmercapturic acid, which are thought to be formed by a series of steps beginning with benzyl alcohol and 3,4-toluene expoxide, respectively (Angerer et al. 1998). The liver is expected to be the main site of toluene metabolism, based on the concentration of CYP isozymes in the liver relative to other tissues. Studies in rats indicate that the expression of hepatic CYP isozymes is influenced by various factors, including age, sex, and level of toluene exposure (Nakajima and Wang 1994).

As indicated above, studies with humans and laboratory animals indicate that following acute periods of inhalation exposure to toluene, absorbed toluene is excreted predominately in the urine as metabolites and, to a lesser extent, as nonmetabolized toluene in exhaled air (Lof et al. 1993; Ogata 1984; Tardif et al. 1998). For example, following a 2-hour exposure with light physical exercise to deuterium-labeled toluene at a concentration of 200 mg/m^3 (53 ppm), an average 78% of retained label was excreted as urinary hippuric acid within 20 hours by a group of nine volunteers (Lof et al. 1993). A significant portion of absorbed toluene in this and other studies has been estimated to be exhaled as nonmetabolized toluene (7–20% of absorbed toluene) (Carlsson 1982; Leung and Paustenbach 1988; Lof et al. 1993). Analyses of kinetic data for toluene concentrations in blood, exhaled breath, or adipose tissue following

inhalation exposure of humans and rats indicate that most absorbed toluene is rapidly eliminated from the body and that a smaller portion (that which gets into adipose tissues) is slowly eliminated (Leung and Paustenbach 1988; Lof et al. 1993; Pellizzari et al. 1992; Pierce et al. 1996, 1999; Rees et al. 1985). For example, using PBPK models, mean terminal half-lives of about 30–38 hours were calculated for changes in blood toluene concentrations between 50 and 100 hours after cessation of 2-hour inhalation exposures of male subjects to 50 ppm $^{1}H_{8}$-toluene and 50 ppm $^{2}H_{8}$-toluene (Pierce et al. 1996, 1999). During this terminal phase of disposition, >95% of toluene is expected to be in adipose tissue and the release of toluene from adipose tissues has been proposed to be the rate-limiting step (Pierce et al. 1999). Elimination half-lives ranged from about 12 to 65 hours in subcutaneous adipose tissue samples taken from 12 subjects at several times within 8 days of cessation of exposure to about 80 ppm toluene for four consecutive 30-minute periods, and increasing elimination half-lives were significantly correlated with increasing amounts of body fat (Carlsson and Ljungquist 1982).

PBPK models are available that describe the kinetics of toluene after inhalation exposure in humans (Fisher et al. 1997; Lapare et al. 1993; Pierce et al. 1996, 1999) and rats (DeJongh and Blaauboer 1996, 1997; Tardif et al. 1993b).

B.2 Health Effects

The nervous system is the critical target of toluene toxicity following acute, intermediate, or chronic inhalation or oral exposure to toluene (ATSDR 2000). Effects on the human nervous system from inhaled toluene are well documented. Studies with volunteers under controlled acute (6–8 hours) exposure conditions indicate that subtle neurological impairment is detectable in most subjects at concentrations in the 75–150 ppm range (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1991; Guzelian et al. 1988; Iregren 1986; Rahill et al. 1996). Concentrations of 200–800 ppm can produce exhilaration and light-headedness, and, at higher acute exposure concentrations, intellectual, psychomotor, and neuromuscular abilities are obviously impaired followed by development of narcosis (EPA 1985; von Oettingen et al. 1942). Numerous case studies have associated chronic inhalation exposure to toluene at levels inducing narcosis and euphoria (estimated 4,000–12,000 ppm) with residual or permanent neurological damage as evidenced by abnormal electroencephalograms, structural changes in the brain, tremors, paranoid psychosis, recurrent hallucinations, and impaired speech, hearing, and vision (ATSDR 2000). Studies of workers repeatedly exposed to toluene in workplace air at concentrations ranging from about 30 to 150 ppm have found evidence for increased incidence of self-reported neurological symptoms, performance deficits in neurobehavioral tests, hearing loss, changes in visual-evoked

brainstem potentials, and color vision impairment (Abbate et al. 1993; Boey et al. 1997; Foo et al. 1990; Morata et al. 1997; Orbaek and Nise 1989; Vrca et al. 1995, 1997a, 1997b; Yin et al. 1987; Zavalic et al. 1998a, 1998b, 1998c).

Supporting neurological effects data come from inhalation studies of toluene-exposed animals showing changes in behavior, hearing loss, and subtle changes in brain structure, electrophysiology, and levels of neurotransmitters (ATSDR 2000). For example, performance deficits in trained neuromuscular responses occurred in rats exposed concentrations as low as 125 ppm toluene for 4 hours (Kishi et al. 1988; Mullin and Krivanek 1982; Wood et al. 1983), changes in brain biochemical variables (e.g., dopamine levels, dopamine D2 receptor binding, changes in glial fibrillary acidic protein) were induced in rats exposed to concentrations as low as 50–80 ppm for 6–8 hours/day (API 1997; Hillefors-Bergllund et al. 1995; Ikeda et al. 1986; Little et al. 1998; von Euler et al. 1989, 1993, 1994), hearing loss occurred in rats exposed to concentrations as low as 700–1,000 ppm, 6–14 hours/day for 2–9 weeks (Campo et al. 1997, 1998; Johnson et al. 1988; Pryor and Rebert 1992; Pryor et al. 1984a, 1984b, 1991), and decreases in brain weight and phospholipid content occurred in rats continuously exposed to 320 ppm for 30 days (Kyrklund et al. 1987). Neurological effects observed in animals after acute- or intermediate-duration oral exposure include changed flash-evoked potentials in rats given single gavage doses of toluene as low as 250 mg/kg (Dyer et al. 1988), and changes in brain levels of several neurotransmitters (e.g., norepinephrine, dopamine, serotonin) in mice exposed to 5–105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1990).

Toluene has caused respiratory tract irritation in humans following acute-duration inhalation exposure to concentrations above approximately 100 ppm, but there is little evidence for other adverse effects (ATSDR 2000). Studies of chronic toluene abusers or occupationally exposed humans have provided little evidence for serious liver damage due to inhaled toluene. Increases in liver weight (likely associated with microsomal enzyme induction), but no significant hepatic histological changes, were found in rats and mice following acute, intermediate, or chronic exposure to concentrations above 300 ppm (ATSDR 2000). The kidney may be a target of toluene toxicity following exposure to very high levels of toluene. Renal acidosis was observed in solvent abusers exposed to toluene, but the renal dysfunction usually reversed when exposure ceased, and the cases were frequently confounded by probable exposure to multiple solvents (ATSDR 2000). Kidney damage (e.g., renal tubular casts) was induced in rats after intermediate- and chronic-duration exposure to concentrations above 600 ppm (CIIT 1980; NTP 1990).

There is suggestive evidence that toluene may cause some reproductive problems, especially with repeated inhalation exposure during pregnancy to concentrations above 200 ppm (ATSDR 2000). Increased risks of spontaneous abortions were observed in women occupationally exposed to toluene, or wives of men similarly exposed (Lindbohm et al. 1992; Ng et al. 1992; Taskinen et al. 1989). However, interpretation of these results is limited due to small sample size evaluated, an inability to define accurate exposure levels, failure to account for all possible confounding variables, and the difficulty in validating self-reported data. Occupational exposure to increasing concentrations of toluene (8–111 ppm) has been associated with decreased plasma levels of the luteinizing hormone, follicle stimulating hormone and testosterone levels in males (Svensson et al. 1992a, 1992b). Studies in animals found some minor toluene-induced changes in male and female reproductive organs (e.g., decreased sperm count in male rats [Ono et al. 1995, 1996] and ultrastructural changes in antral follicles in ovary of female rats [Tap et al. 1996]), but no histological evidence of structural damage to the reproductive organs in rats and mice exposed orally for intermediate durations or by inhalation for intermediate or chronic durations (NTP 1990). No evidence for impaired reproductive performance was found in several assays (Ono et al. 1995, 1996; Smith 1983; Thiel and Chahoud 1997), including a 2-generation study of rats intermittently exposed to up to 2,000 ppm (API 1985), although gestational exposure to toluene caused increased fetal mortality in rats intermittently exposed to 2,000 ppm (Ono et al. 1995, 1996), and increased abortions in rabbits continuously exposed to 267 ppm but not 133 ppm (Ungvary and Tatrai 1985).

A number of reports of birth defects in children born to women who abused toluene or other organic solvents during pregnancy suggest that high-level (4,000–12,000 ppm) exposure to toluene during pregnancy can be toxic to the developing fetus, causing effects that included microcephaly, central nervous system dysfunction, growth deficiency, cranofacial and limb abnormalities, and reversible renal tubular acidosis (ATSDR 2000). Results from several inhalation exposure studies of animals indicate that exposure to levels of toluene that begin to produce maternal toxicity can cause fetal effects, including reduced fetal survival and retardation of growth and skeletal development (Courtney et al. 1986; Hudak and Ungvary 1978; Huntingdon Research Centre 1992a, 1992b; Ono et al. 1995; Thiel and Chahoud 1997; Ungvary and Tatrai 1985). No-effect levels in animals for toluene effects on standard developmental endpoints ranged from about 133–750 ppm. In animal studies of oral exposure during gestation, no developmental effects were observed in pregnant mice exposed to doses of ≥1,800 mg/kg/day (Seidenberg et al. 1986; Smith 1983), but exposure of pregnant rats to gavage doses of 650 mg/kg/day produced offspring with decreased body weights, delayed ossification, smaller brain volumes, and decreased forebrain myelination per cell compared with controls (Gospe and Zhou 1998; Gospe et al. 1996). Results from studies of neurobehavioral endpoints in rats following gestational

exposure to toluene suggest that intermittent inhalation exposure to concentrations >1,200 ppm can impair offspring behavioral development (Jones and Balster 1997; Ono et al. 1995; Thiel and Chahoud 1997) and that drinking water exposure during gestation and lactation at doses of 106 mg/kg/day changes postweaning open-field locomotor activity in rat offspring (Kostas and Hotchin 1981).

Human and animal studies generally do not support a concern for the carcinogenicity of toluene. Eleven human epidemiology studies assessed toluene exposure as a possible risk factor for cancer (ATSDR 2000). Cancers of most sites were not significantly associated with toluene exposure in any study and there was weak consistency in the findings of those studies that did find association of a particular cancer type with toluene exposure. Three cohort studies involved workers occupationally exposed predominantly to toluene, whereas the remainder of the human studies primarily involved subjects exposed to mixtures of solvents including toluene (Antilla et al. 1998; ATSDR 2000; Svensson et al. 1990; Walker et al. 1993). The information from the human studies is inadequate to assess the carcinogenic potential of toluene, mainly because of the lack of consistent findings across the studies and the likelihood that many of the studied groups were exposed to multiple chemicals. Chronic bioassays in animals found no dose-related increased incidences of neoplastic lesions in rats or mice exposed by inhalation or in orally exposed rats (CIIT 1980; Maltoni et al. 1997; NTP 1990). Toluene was generally nongenotoxic in *in vivo* studies of exposed humans, *in vitro* microbial assays, and other *in vitro* test systems (ATSDR 2000).

B.3 Mechanisms of Action

The mechanism by which acute exposure to toluene brings about neurological effects such as central nervous system depression and narcosis is generally thought to involve, at least in part, reversible interactions between toluene (the parent compound and not its metabolites) and components (lipids or proteins) of nervous system membranes (ATSDR 2000). Support of parent-material involvement comes from the observation that pretreatment of rats with phenobarbital increased the rate of *in vivo* toluene metabolism and shortened the time of recovery from narcosis from single intraperitoneal doses of toluene (Ikeda and Ohtsuji 1971). Other support for this hypothesis includes the transient nature of anesthesia from acute high level exposure to toluene and the rapidity with which toluene-induced changes in brain biochemical variables can be measured (Korpela and Tahti 1988; Lebel and Schatz 1988, 1989, 1990; Rea et al. 1984). On a molecular level, the acute anaesthetic actions of toluene and other agents have been postulated to involve intercalation of toluene into the lipid bilayer of nerve membranes and/or reversible interactions with proteins in the membrane (Franks and Lieb 1985, 1987).

Clinically obvious neurological impairment (e.g., gait and speech abnormalities) and brain atrophy have been observed in several cases of chronic toluene-inhalation abuse. Magnetic resonance imagery (MRI) of the brain of solvent abusers (Filley et al. 1990; Rosenberg et al. 1988a, 1988b) suggest preferential atrophy in lipid-rich regions of the brain (ATSDR 2000). The MRI changes may be related to lipid compositional changes in the white matter, since these regions are more lipid-rich than gray matter (Ameno et al. 1992). The observations are consistent with a hypothesis that chronic exposure to high concentrations of toluene brings about structural changes in the brain related to lipid compositional changes (ATSDR 2000). Supporting evidence for this hypothesis includes altered phospholipid composition of brain synaptosomes, decreased phospholipid concentrations in the cerebral cortex, and decreased number of neurons in the hippocampus in toluene-exposed rats (Korbo et al. 1996; Kyrklund et al. 1987; Lebel and Schatz 1988, 1989, 1990). It is uncertain if toluene-induced changes in membrane phospholipid content may be caused by increased breakdown of phospholipids or inhibition of synthesis.

B.4 Health Guidelines

ATSDR (2000) derived an acute-duration inhalation MRL of 1 ppm for toluene based on a NOAEL of 40 ppm for subjective neurological effects (headaches, dizziness, and feelings of intoxication) and eye and nose irritation in humans (Andersen et al. 1983) and an uncertainty factor of 10 (to account for human variability). Volunteers were experimentally exposed to toluene 6 hours/day for 4 consecutive days.

ATSDR (2000) did not derive an intermediate-duration inhalation MRL for toluene due to lack of suitable data, but the chronic inhalation MRL would also be protective for intermediate-duration exposures.

ATSDR (2000) derived a chronic-duration inhalation MRL of 0.08 ppm for toluene based on a LOAEL of 35 ppm for neurological effects (color vision impairment) in humans (Zavalic et al. 1998b) and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability). Workers were occupationally exposed to benzene for an average of 16 years.

ATSDR (2000) derived an acute-duration oral MRL of 0.8 mg/kg for toluene based on a LOAEL of 250 mg/kg/day for neurological effects (decreased flash-evoked potential) in rats (Dyer et al. 1988) and an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were administered a single dose of toluene by gavage.

ATSDR (2000) derived an intermediate-duration oral MRL of 0.02 mg/kg/day for toluene based on a LOAEL of 5 mg/kg/day for neurological effects (regional brain increases in monoamine neurotransmitters) in mice (Hsieh et al. 1990) and an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were exposed to toluene in drinking water for 28 days.

ATSDR (2000) did not derive a chronic-duration oral MRL for toluene due to lack of suitable data.

The EPA IRIS database lists an RfD and an RfC for toluene (IRIS 2001). The RfD is based on a NOAEL of 312 mg/kg/day (converted to 223 mg/kg/day) for changes in liver and kidney weights in rats (NTP 1990) and an uncertainty factor of 1,000 (applied to account for inter- and intraspecies extrapolations, for subchronic-to-chronic extrapolation, and for limited reproductive and developmental toxicity data). The animals were exposed to toluene in corn oil by gavage 5 days/week for 13 weeks.

The EPA RfC for toluene is based on a LOAEL of 88 for neurological effects in humans (Foo et al. 1990) and an uncertainty factor of 300 (10 for use of a LOAEL, 10 for human variability, and 3 for database deficiencies) (IRIS 2001). Workers were occupationally exposed to toluene for an average of 5.7 years.

ACGIH (2001) recommends a TLV-TWA of 50 ppm for toluene based on central nervous system effects (reports of headache and irritation in humans associated with 4–6 hours of continuous inhalation of toluene).

The NTP (2001) has not listed toluene as a known or anticipated human carcinogen. EPA determined that toluene is not classifiable as to carcinogenicity (Category D) based on no human data and inadequate animal data (IRIS 2001). IARC (1999a) concluded that toluene is not classifiable as to its carcinogenicity (Group 3) to humans based on inadequate evidence in humans and evidence suggesting lack of carcinogenicity in animals.

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Appendix C: Background Information for Ethylbenzene

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for ethylbenzene. The summaries are mainly based on information presented in the ATSDR (1999b) toxicological profile for ethylbenzene and therefore do not represent reviews of the primary literature. The chemical structure for ethylbenzene is included in Appendix E.

C.1 Toxicokinetics

Ethylbenzene is well absorbed in humans via the inhalation and dermal routes of exposure, although oral absorption data in humans are lacking (ATSDR 1999b). For example, volunteers exposed for 8 hours to ethylbenzene at concentrations of 23–85 ppm were shown to retain 64% of the inspired vapor, with only trace amounts detected in expired air (Bardodej and Bardodejova 1970). Another inhalation study that involved humans exposed to similar levels of ethylbenzene demonstrated mean retention rates of 49%, suggesting possible variability of absorption rates among individuals (Gromiec and Piotrowski 1984). Dermal absorption rates of 24–33 and 0.11–0.23 mg/cm^2/hour have been measured for male subjects exposed to liquid ethylbenzene and ethylbenzene from aqueous solutions, respectively (Dutkiewicz and Tyras 1967). The average amounts of ethylbenzene absorbed after volunteers immersed one hand for up to 2 hours in an aqueous solution of 112 or 156 mg/L ethylbenzene were 39.2 and 70.7 mg ethylbenzene, respectively. Animal data support the human inhalation and dermal findings and further indicate that absorption rates are high following oral exposure as well, as indicated by 72–92% urinary recovery of single oral doses in rats and rabbits (Climie et al. 1983; El Masri et al. 1956).

Information on the distribution of ethylbenzene in humans is available from one inhalation study indicating rapid distribution to adipose tissues throughout the body (Engstrom and Bjurstrom 1978). Oral and inhalation studies in animals support these results (Chin et al. 1980a, 1980b; Climie et al.1983). Ethylbenzene is accumulated primarily in the liver, kidney, and fat. In rats, the concentrations of ethylbenzene in perirenal adipose tissue were reported to increase, although not linearly, with increasing concentrations of ethylbenzene and in a mixture of solvent vapors containing ethylbenzene (Elovaara et al. 1982; Engstrom et al. 1985). The less-than-linear increase of ethylbenzene in adipose tissue with increasing dose was partially attributed to the induction of drug-metabolizing enzymes occurring with increasing exposure concentrations, altered blood flow to adipose tissue, changes in lung excretion, and changes in the distribution of ethylbenzene in different tissues.

Ethylbenzene is metabolized in humans mainly through hepatic cytochrome P-450-mediated side chain oxidation (hydroxylation) to initially form 1-phenylethanol, from which several metabolites are produced that are excreted in the urine (ATSDR 1999b). Isozymes involved in the initial oxidation include CYP2E1 and CYP1A2 (Gut et al. 1993). The major urinary metabolites of ethylbenzene in humans exposed via inhalation are mandelic acid (approximately 64–71%) and phenylglyoxylic acid (approximately 19–25%) (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Minor pathways (e.g., ring hydroxylation) in humans yield hydroxylated derivatives (e.g., *p*-hydroxyacetophenone, *m*-hydroxy-acetophenone, 1-phenyl-1,2-ethanediol, acetophenone, 2-hydroxyacetophenone, and 4-ethylphenol) that are conjugated with glucuronide or sulfate. The principal metabolic pathway in rats is believed to begin with hydroxylation of the side chain as in humans, although the major urinary metabolites following inhalation or oral exposure were identified as hippuric and benzoic acids (approximately 38%), 1-phenyl-ethanol (approximately 25%), and mandelic acid (approximately 15–23%), with phenylglyoxylic acid making up only 10% of the metabolites (Climie et al. 1983; Engstrom 1984; Engstrom et al. 1985). Both *in vivo* studies using rats and *in vitro* studies using rat liver microsomes showed that 4-ethylphenol was also produced from ethylbenzene, perhaps by rearrangement of corresponding arene oxides (Bakke and Scheline 1970; Kaubisch et al. 1972). In rabbits, the most important metabolite is hippuric acid, which is probably formed by oxidative decarboxylation of phenylglyoxylic acid, and rabbits have been shown to excrete higher levels of glucuronidated metabolites than humans or rats (ATSDR 1999b).

The elimination of ethylbenzene has been studied in volunteers exposed by inhalation (ATSDR 1999b). The elimination of the major metabolite mandelic acid was reported to be rapid and biphasic, with half-lives of 3.1 hours for the rapid phase and 25 hours for the slow phase (Gromiec and Piotrowski 1984). During an 8-hour inhalation exposure to 4–46 ppm, 23% of the retained ethylbenzene was eliminated in the urine, and 14 hours following termination of exposure, an additional 44% of the retained ethylbenzene was eliminated. The highest excretion rate of urinary metabolites in humans exposed to ethylbenzene by inhalation occurred 6–10 hours after the beginning of exposure and metabolic efficiency was of the exposure dose (Gromiec and Piotrowski 1984; Yamasaki 1984). Data from occupational exposures have generally confirmed these results (Holz et al. 1995; Kawai et al. 1991, 1992; Ogata and Taguchi 1988). In animals, elimination of ethylbenzene metabolites following inhalation or oral exposure is rapid and occurs primarily via urinary metabolites, and to a much lesser degree, via the feces and expired carbon dioxide (ATSDR 1999b). Quantitative and qualitative differences between species were shown to exist in the percentages of metabolites excreted in the urine.

PBPK models have been developed for inhalation exposure to ethylbenzene in rats and humans (Tardif et al. 1997) and for dermal exposure to ethylbenzene in humans (Shatkin and Brown 1991). The dermal model describes the percutaneous absorption of ethylbenzene in dilute aqueous solution.

C.2 Health Effects

Observations in humans and animals indicate that acute high level inhalation exposure to ethylbenzene causes central nervous system effects and irritation of the eyes and respiratory tract that were generally reversible following cessation of exposure (ATSDR 1999b). Information on neurological effects of ethylbenzene in humans is limited to an early report of dizziness accompanied by vertigo following acute exposure to 2,000–5,000 ppm ethylbenzene (Yant et al. 1930). Neurological effects of acute exposure to ≥2,000 ppm in rats, mice, and/or guinea pigs included decreased arousal and rearing, motor disturbances (gait, mobility, and righting reflex), decreased grip strength, increased landing foot splay, impaired psychomotor coordination, and narcotic effects such as prostration and reduced activity (Bio/dynamics 1986; Cragg et al. 1989; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). Increased motor activity and sporadic salivation were observed in rats at concentrations of 382–400 ppm and above (Cragg et al. 1989; Molnar et al. 1986). No signs of neurotoxicity were found in rabbits intermittently exposed to 2,400 ppm for 4 days or 1,610 ppm for 4 weeks (Cragg et al. 1989). Changes in dopamine and other biochemical alterations occurred in rats and rabbits exposed to 2,000 and 750 ppm, respectively, for 3–7 days (Andersson et al. 1981; Mutti et al. 1988; Romanelli et al. 1986). Evoked electrical activity in the brain was depressed in rats and mice acutely exposed to 245 and 342 ppm ethylbenzene, respectively (Frantik et al. 1994). Information on oral exposure is limited to a report in which no overt behavioral changes were observed in rats administered ethylbenzene by gavage for 6 months at concentrations ranging from 13.6 to 680 mg/kg/day (Wolf et al. 1956).

Ocular irritation, burning, and lacrimation occurred in humans acutely exposed to ≥1,000 ppm ethylbenzene vapor, and throat irritation and chest congestion were observed at ≥2,000 ppm, and (Cometto-Muniz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930). Animal studies also showed ocular irritation and respiratory effects (e.g., pulmonary congestion and reduced respiratory rate) following acute exposure to concentrations generally above 1,000 ppm (ATSDR 1999b).

Animal data suggest that the liver and kidneys may be a target of toxicity for ethylbenzene. Acute inhalation exposure to high concentrations of ethylbenzene induced effects in the liver that were generally mild and indicative of adaptation (increased metabolism), including increased microsomal enzyme

activity and other biochemical changes, ultrastructural changes, and increased liver weight (Bio/dynamics 1986; Cragg et al. 1989; Elovarra et al. 1985; Wolf et al. 1956). Hepatic histological effects that included necrosis were observed in mice, but not rats, that were exposed to concentrations of ethylbenzene up to 750 ppm for 2 years (NTP 1992). Renal effects manifested as histopathological changes (e.g., tubular swelling and hyperplasia), enzymatic changes, and increased organ weight were observed in a number of species following inhalation exposure to ethylbenzene following acute and longer-term exposure to concentrations generally ≥600 and ≥1,200 ppm, respectively (Andrew et al. 1981; Bio/dynamics 1986; Cragg et al. 1989; NTP 1992, 1996; Wolf et al. 1956).

Inconclusive information is available on the hematotoxic potential of ethylbenzene (ATSDR 1999b). Two studies involving long-term monitoring of workers occupationally exposed to ethylbenzene showed conflicting results with respect to effects on the hematopoietic system. One study (Angerer and Wulf 1985) reported an increase in the number of lymphocytes and a decrease in hemoglobin levels, whereas no adverse hematological effects were reported in the other study (Bardodej and Cirek 1988). There was likely simultaneous exposure to other chemicals in both of these studies. Platelet and leukocyte counts were increased in rats exposed to 782 ppm ethylbenzene for 4 weeks, although no hematological effects were observed in rats or other species exposed to similar or higher concentrations (Cragg et al. 1989; NTP 1992; Wolf et al. 1956).

The reproductive effects of ethylbenzene are incompletely characterized, although there are some data suggesting that the male and female reproductive systems may be a target of toxicity. No multigeneration studies have been performed. No histopathological changes were induced in the testes of rat, mice, or rabbits following inhalation exposure to ≤2,400 ppm ethylbenzene for 4 days, rats and mice exposed to ≤782 ppm for 4 weeks, or rabbits exposed to ≤1,610 ppm for 4 weeks (Bio/dynamics 1986; Cragg et al. 1989). There were no effects on sperm, length of the estrous cycle, or histopathology of reproductive organs in male or female rats or mice exposed to 975 ppm for 90 days, although chronic exposure to 750 ppm caused increased incidences of testicular tumors in rats (NTP 1992, 1999). Ungvary and Tatrai (1985) reported an increase in postimplantation death in the offspring of rats exposed to ≥138 ppm continuously during organogenesis and an increase in abortions in rabbits similarly exposed to 230 ppm. Fertility was reduced in female rats exposed for 3 weeks pre-gestation and during gestation to 1,000 ppm, but the investigators did not consider the effect to be significant (Andrew et al. 1981). Acute oral exposure to 500 or 1,000 mg/kg ethylbenzene decreased peripheral hormone levels and delayed the estrus cycle in female rats during the diestrus stage (Ungvary 1986). Decreased levels of hormones (e.g.,

luteinizing hormone, progesterone, and 17 β-estradiol) were accompanied by uterine changes (increased stromal tissue with dense collagen bundles and reduced lumen), but no dose response was noted.

Developmental toxicity studies in animals indicate that inhalation exposure to ethylbenzene can produce minimal fetotoxic effects at exposure levels that may or may not induce minimal maternal changes (ATSDR 1999b). Effects included skeletal anomalies and supernumerary ribs in offspring of rats that were intermittently exposed to 959 ppm of ethylbenzene during gestation (Andrew et al. 1981). Extra ribs and anomalies of the urinary tract occurred in fetuses of rats continuously exposed to 552 ppm ethylbenzene during gestation, and urinary tract anomalies were also increased in fetal mice similarly exposed to 115 ppm (Ungvary and Tatrai 1985).

Ethylbenzene was not genotoxic in most studies, although some marginal effects have been reported. Workers exposed to low levels of ethylbenzene in a styrene plant showed no increases in sister chromatid exchanges, DNA adduct formation, micronuclei, or DNA single-strand breaks in the peripheral lymphocytes (Holz et al. 1995). Micronucleated peripheral erythrocytes were not increased in mice that were exposed to 750 ppm ethylbenzene for 13 weeks (NTP 1999). Ethylbenzene was generally not mutagenic in bacteria or yeast cells *in vitro*, and did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster cells (ATSDR 1999b). A weak positive response was observed when ethylbenzene was tested for sister chromatid exchanges in human lymphocytes *in vitro*, but only at a concentration that was toxic to the cells (Norppa and Vainio 1983). A positive response also was seen in mouse lymphoma cells when ethylbenzene was tested at a near lethal concentration (McGregor et al. 1988).

The carcinogenicity of ethylbenzene has been examined in three studies: an epidemiological study of humans occupationally exposed by inhalation (Bardodej and Cirek 1988), a chronic inhalation bioassay in rats and mice (Chan et al. 1998; NTP 1999), and a chronic oral study in rats (Maltoni et al. 1985). The results of both the human epidemiological study and the oral study in rats were inconclusive. The inhalation bioassay intermittently exposed rats and mice to $\leq$750 ppm ethylbenzene for 104 weeks and concluded that there was clear evidence of carcinogenic activity in male rats based on increased incidences of renal tubule neoplasms and testicular adenomas (Chan et al. 1998; NTP 1999). The inhalation study also concluded that there was some evidence of carcinogenic activity in female rats based on increased incidences of renal tubule adenoma, in male mice based on increased incidences of alveolar/bronchiolar neoplasms, and in female mice based on increased incidences of hepatocellular neoplasms.

C.3 Mechanisms of Action

Relatively little information exists regarding the mechanism of ethylbenzene toxicity. Most studies have focused on the possible mechanism of neurotoxicity. *In vitro* studies on the mechanism of toxicity of ethylbenzene have focused on the effect of this chemical on cell membranes, particularly that of the astrocyte (Engelke et al. 1993; Naskali et al. 1993, 1994; Sikkema et al. 1995; Vaalavirta and Tähti 1995a, 1995b). It has been suggested that changes in the structure and integrity of the cell membrane after partitioning of ethylbenzene into the lipid bilayer may be a mechanism of toxicity of ethylbenzene (Sikkema et al. 1995). Changes in the integrity of the cell membrane may subsequently affect the function of membrane, particularly as a barrier and in energy transduction, and in the formation of a matrix for proteins and enzymes. Engelke et al. (1993) showed that incubation of pig liver microsomes with ethylbenzene caused an accumulation of ethylbenzene in the microsomal membrane, which, in turn, increased the fluidity of the membrane. Although incubation of the microsomal membranes with ethylbenzene did not change the content of cytochrome P-450 or cytochrome b_5 content, or the activities of nicotinamide adenine dinucleotide phosphate (oxidized form) (NADPH)-cytochrome P-450 reductase or nicotinamide adenine dinucleotide phosphate (reduced form) (NADH)-cytochrome b_5 reductase, a change in the reduction kinetics of these enzymes was observed. The authors proposed that the observed change in kinetics may be due to a rearrangement of the cytochrome P-450 molecules in the microsomal membrane as a result of the accumulation of ethylbenzene in the membrane.

Vaalavirta and Tähti (1995a, 1995b) and Naskali et al. (1993, 1994) investigated the effect of ethylbenzene on the membrane of the rat astrocyte as an *in vitro* model for the membrane-mediated effects of solvents on the central nervous system. Cultured astrocytes from the cerebellum of neonatal Sprague-Dawley rats were sensitive to the effects of ethylbenzene, as measured by the inhibition of activity of Na^+, K^+-ATPase, and Mg^{++}-ATPase (Vaalavirta and Tähti 1995a, 1995b). This effect was found to be dose-dependent (Naskali et al. 1994). Inhibition of these membrane-bound enzymes that regulate the ion channels of the membrane may disturb the ability of the cells to maintain homeostasis. Experiments with rat synaptosome preparations, similar to those using microsomal preparations by Engelke et al. (1993), showed that membrane fluidity was increased after exposure to ethylbenzene. ATPase and acetylcholinesterase activity were also decreased, as seen in the astrocyte preparations. Results from the *in vitro* studies described above are consistent with what would be expected of the interaction of a lipophilic chemical with cell membranes. However, there is no direct evidence that the interaction of ethylbenzene with astrocytes *in vivo* is the mechanism of neurological effects following acute exposure to high levels of ethylbenzene in humans or in animals.

The mechanism(s) of toxic effects of ethylbenzene on organs or systems other than the nervous system has not been elucidated, but is likely to be related to the formation of reactive metabolites that ultimately bind to cell macromolecules (ATSDR 1999b; NTP 1999).

C.4 Health Guidelines

ATSDR (1999b) derived an intermediate-duration inhalation MRL of 1.0 ppm for ethylbenzene based on a NOAEL of 97 ppm for developmental effects (skeletal anomalies) in rats (Andrew et al. 1981). The animal NOAEL was converted to a $NOAEL_{(HEC)}$ of 97 ppm and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 to account for human variability) to yield the MRL. The animals were exposed for 7 hours/day, 5 days/week for 3 weeks prior to mating and subsequently through gestation day 19.

No acute- or chronic-duration inhalation MRLs were derived for ethylbenzene due to a lack of appropriate data (ATSDR 1999b).

No acute-, intermediate-, or chronic-duration oral MRLs were derived for ethylbenzene due to a lack of appropriate data (ATSDR 1999b).

The EPA IRIS database lists an RfD and an RfC for ethylbenzene (IRIS 2001). The RfD is based on a NOAEL of 136 mg/kg/day (converted to 97 mg/kg/day) for liver and kidney toxicity in rats (Wolf et al. 1956) and an uncertainty factor of 1,000 (10 for subchronic-to-chronic extrapolation, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were exposed to ethylbenzene in olive oil by gavage 5 days/week for 182 days.

The EPA RfC for ethylbenzene is based on a NOAEL of 100 ppm for developmental toxicity in rats and rabbits (Andrew et al. 1981; Hardin et al. 1981) and an uncertainty factor of 300 (3 for extrapolation from animals to humans, 10 for human variability, and 10 for database deficiencies) (IRIS 2001). The animals were exposed 6–7 hours/day during days 1–19 (rats) and days 1–24 (rabbits) of gestation.

ACGIH (2001) recommends a TLV-TWA of 100 ppm and STEL/C of 125 ppm for ethylbenzene based on irritation and central nervous system effects.

The NTP (2001) has not listed ethylbenzene as a known or anticipated human carcinogen. EPA determined that ethylbenzene is not classifiable as to human carcinogenicity (Category D) due to lack of animal bioassay and human data (IRIS 2001). The NTP and EPA assessments predate the positive findings of the NTP (1999) carcinogenesis bioassay in rats and mice. IARC (2000) concluded that ethylbenzene is possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in animals.

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Appendix D: Background Information for Xylenes

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for xylenes. The summaries are mainly based on information presented in the ATSDR (1995) toxicological profile for xylenes and therefore do not represent reviews of the primary literature. The chemical structures for xylenes are included in Appendix E.

D.1 Toxicokinetics

Xylenes are well absorbed by the inhalation and oral routes. Experimental studies with humans found that retention of the various isomers was similar following inhalation of either *o*-, *m*-, or *p*-xylene and averaged 63.6% (Sedivec and Flek 1976). Other investigators have estimated that between 49.8 and 72.8% of inhaled xylene is retained in humans (ATSDR 1995). There appear to be two phases of respiratory absorption; the first is apparently short, occurring within 15 minutes of initiation of exposure, and the second phase is longer (about 1 hour) and represents the establishment of an equilibrium between the inhaled xylene and blood (ATSDR 1995). Oral absorption data are limited, but indicate that almost complete absorption (87–92%) occurred in rabbits following ingestion of a 1.7–1.8 g dose of *o*-, *m*-, or *p*-xylene. Dermal absorption may occur via exposure to xylene vapors as well as through direct dermal contact with the liquid (ATSDR 1995). Xylenes are absorbed dermally to a much lesser extent than by inhalation or oral exposure, especially following dermal exposure to xylene vapor (Riihimaki and Pfaffli 1978). Absorption of *m*-xylene vapor through human skin was determined to be approximately 0.1–2% of that of via inhalation exposure (Riihimaki and Pfaffli 1978). Based on breath sampling and PBPK analysis following vapor exposure, the contribution to *m*-xylene body burden from the dermal route was estimated to be 1.8%. The rate of dermal absorption varied in two human studies of *m*-xylene, ranging from approximately 2 μg/cm^2/minute in one study to 75–160 μg/cm^2/minute in the other (Dutkiewicz and Tyras 1968; Engstrom et al. 1977).

Absorbed xylene is mainly distributed to lipid-rich tissues, particularly adipose and brain (ATSDR 1995). High uptake also occurs in well-perfused organs such as the liver and kidneys. Estimates of the amount of inhaled xylene accumulated in human adipose tissue range from 5 to 10% of the absorbed dose (Astrand 1982; Engstrom and Bjurstrom 1978). The level of xylenes detected in mouse fetal tissues (brain, liver, lung, kidney), which are low in lipids, was only 2% of that found in the maternal brain tissue, which contains large amounts of lipids (Ghantous and Danielsson 1986).

Metabolism of xylenes in humans occurs primarily by hepatic cytochrome P-450-catalyzed oxidation of a side-chain methyl group to yield methylbenzoic acids (*o*-, *m*-, or *p*-toluic acids), which are conjugated with glycine to form methylhippuric acids (ATSDR 1995; Gut et al. 1993; Tassaneeyakul et al. 1996). Important CYP isozymes involved in the methylhydroxylation include CYP2E1 and CYP2B1, and metabolism to methylhippuric acids accounts for almost all (>90%) of the absorbed dose in humans, regardless of the isomer, route of administration, or dose or duration of exposure. Minor metabolic pathways that account for <10% of the absorbed dose in humans include unchanged xylene in the exhaled breath, and methylbenzyl alcohols, *o*-toluic acid glucuronide, xylene mercapturic acid, and xylenols (dimethylphenols) in the urine. CYP1A2 appears to be involved in the formation of the minor phenolic metabolites. The metabolism of xylenes in rats and other laboratory animals is qualitatively similar to that of humans, although glucuronide conjugates make up a larger proportion of the urinary excretion products. A toxic metabolite of xylenes in rats and rabbits that has not been confirmed in humans appears to be methylbenzaldehyde (Carlone and Fouts 1974; Patel et al. 1978; Smith et al. 1982), which is formed by the action of alcohol dehydrogenase on methylbenzyl alcohol in lung and liver tissues. Some studies indicate that the metabolic differences in humans and animals may partly be explained by differences in the size of doses (ATSDR 1995).

In humans, about 95% of absorbed xylene isomers is excreted as urinary metabolites, almost exclusively as methylhippuric acids, with the most of the remaining amount eliminated unchanged in the exhaled air (ATSDR 1995). Less than 0.005 and 2% of the absorbed dose is excreted in the urine unchanged and as xylenols, respectively. There appear to be at least two distinct phases of elimination, a relatively rapid one (1-hour half-life) and a slower one (20-hour half-life, corresponding to elimination from the muscles and adipose tissue). PBPK modeling suggests that the urinary excretion of *m*-methylhippuric acid in humans following inhalation of *m*-xylene is linear at concentrations up to 500 ppm (Kaneko et al. 1991a, 1991b). Humans exposed to 100 or 200 ppm *m*-xylene for 7 hours excreted 54 and 61%, respectively, of the administered dose by 18 hours after exposure ended (Ogata et al. 1970). Following intermittent acute exposure to 23, 69, or 138 ppm *m*-xylene, excretion of *m*-methylhippuric acid peaked 6–8 hours after exposure began and subsequently decreased rapidly so that almost no xylene or methyhippuric acid was detected 24 hours later (Senczuk and Orlowski 1978).

PBPK models have been developed for inhalation exposure to *m*-xylene in rats and humans (Lapare et al. 1993; Tardif et al. 1993b, 1995).

D.2 Health Effects

Health effects of mixed xylenes, *o*-xylene, *m*-xylene, and *p*-xylene appear to be similar, although the individual isomers are not necessarily equal in potency with respect to a particular effect (ATSDR 1995). Studies in humans and animals document that the central nervous system is a major and sensitive target of xylene toxicity by the inhalation and oral routes. Human experimental studies indicate that acute inhalation exposure to 100 ppm mixed xylene or 200 ppm *m*-xylene causes impaired short-term memory, impaired reaction time, performance decrements in numerical ability, and alterations in equilibrium and body balance (Gamberale et al. 1978; Riihimaki and Savolainen 1980; Savolainen and Linnavuo 1979; Savolainen and Riihimaki 1981; Savolainen et al. 1979, 1980, 1984, 1985a, 1985b). Case and occupational studies together provide suggestive supportive evidence that acute and chronic inhalation exposure to xylene or solvent mixtures containing xylene may also be associated with many neurotoxic effects (ATSDR 1995). Neurological effects in orally- or dermally-exposed humans have not been studied. Animal studies provide further evidence that mixed xylenes and individual isomers are neurotoxicants following inhalation exposure at concentrations ranging from 160 to 2,000 ppm. Signs of neurotoxicity observed in rats, mice, and gerbils following acute- and intermediate-duration inhalation exposure to various xylene isomers have included narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, elevated auditory thresholds and hearing loss, and changes in brain enzyme activity and levels of brain proteins (ATSDR 1995). Neurotoxic effects were also induced in the offspring of rats exposed by inhalation during gestation, including impaired performance in tests for neuromotor abilities (Rotarod) and learning and memory (Morris water maze) and delayed ontogeny of the air righting reflex (ATSDR 1995; Hass and Jakobsen 1993). Effects indicative of central nervous system toxicity (e.g., increased latency of visual-evoked potentials, lethargy, tremors, convulsions, respiratory depression, weakness, unsteadiness, and hyperactivity) similarly occurred in animals following acute-, intermediate-, and chronic-duration oral exposure to xylenes, although most of these were serious nonthreshold effects that occurred at high doses (ATSDR 1995).

Xylene is irritating to the to the respiratory tract, eyes, and skin. Adverse respiratory effects that have been observed in humans and animals following acute-, intermediate-, and/or chronic-duration inhalation exposure xylene include nose and throat irritation, labored breathing, and pulmonary congestion, inflammation, and edema (ATSDR 1995). LOAELs for these effects are incompletely characterized, although there are reports of nose and throat irritation following acute-, and chronic-duration inhalation exposures to concentrations of 100–200 and 14 ppm, respectively (Hake et al. 1981; Nelson et al. 1943; Uchida et al. 1993).

Xylene can affect the liver and kidney effects at exposure levels higher than the LOAELs for neurological effects and respiratory irritation. Inhalation or oral exposure to mixed xylenes and/or individual isomers produced hepatic effects in animals that appear to have been generally mild and adaptive in nature, including hepatic cytochrome P-450 content, proliferation of hepatic endoplasmic reticulum, increased liver weight, decreased hepatic glycogen, decreased hexobarbital sleeping time, and/or congestion of liver cells (ATSDR 1995). Occupational studies provide suggestive evidence that humans exposed to high inhalation levels of solvent mixtures containing xylene may be at increased risk of developing renal dysfunction and/or damage (ATSDR 1995). Renal effects such as increased blood urea concentrations, decreased urinary clearance of endogenous creatinine, increased lysozymuria, increased urinary levels of β-glucuronidase, and increased urinary excretion of albumin, erythrocytes, and leukocytes were observed, but the effects are not solely attributable to xylene due to the confounding exposures. Kidney effects found in inhalation and oral studies in animals included increased renal enzyme activity, increased cytochrome P-450 content, and increased kidney weight without histopathological alterations.

Human and animal data provide no indications of adverse hematological effects following inhalation of xylene. In the past, chronic occupational exposure to xylene was thought to be associated with a variety of hematological effects. However, exposure in all cases was to solvent mixtures known or suspected to contain benzene, and because benzene causes leukemia and other blood dyscrasias in humans, these effects cannot be attributed solely to xylene (ATSDR 1995). Hematological effects were not observed in an occupational study of xylene-exposed workers in which no benzene was involved (Uchida et al. 1993), or in rats, dogs, or guinea pigs exposed by inhalation to 810 ppm mixed xylenes or 780 ppm *o*-xylene for intermediate durations (Carpenter et al. 1975; Jenkins et al. 1970).

Information on the developmental toxicity of xylenes in humans is limited to a few occupational studies that are inadequate for assessing the relationship between exposure to xylenes and developmental effects due to concurrent exposure to other solvents and small numbers of subjects (ATSDR 1995). In animals, inhalation exposure to mixed xylenes (500 ppm) or the individual isomers (350–700 ppm) produced fetotoxic effects in rats, mice, and rabbits, including increased incidences of skeletal variations in fetuses, delayed ossification, fetal resorptions, hemorrhages in fetal organs, and decreased fetal body weight (Balogh et al. 1982; Bio/dynamics 1983; Hass and Jakobsen 1993; Hudak and Ungvary 1978; Litton Bionetics 1978; Mirkova et al. 1983; Ungvary 1985; Ungvary and Tatrai 1985; Ungvary et al. 1980, 1981). The levels at which these effects occurred were generally maternotoxic (ATSDR 1995). Milder neurodevelopmental effects occurred in rats at lower gestational inhalation exposure levels (200–500 ppm) that did not induce maternal toxicity, including impaired performance in tests for

neuromotor (Rotarod) and learning and memory (Morris water maze) abilities, and delayed ontogeny of the air righting reflex (ATSDR 1995; Hass and Jakobsen 1993). A LOAEL of 200 ppm for impaired Rotarod performance was identified in 1- and 2-day-old rat pups that were intermittently exposed to mixed xylenes during gestation (Hass and Jakobsen 1993). An oral study found that mixed xylenes-induced cleft plate and decreased fetal weight in mice exposed to maternally toxic doses (Marks et al. 1982), and a dermal study found changes in brain enzymes (cholinesterase, cytochrome) in the brain of fetal rats (Mirkova et al. 1979).

Information on the reproductive toxicity of xylenes in humans is limited to two studies that found an increased incidence of spontaneous abortions following paternal exposure (Taskinen et al. 1989) or maternal exposure (Taskinen et al. 1994). These findings are inconclusive due to study limitations including multiple chemical exposures and small population size. No reproductive effects were found in rats following inhalation of 500 ppm mixed xylenes from before mating through lactation (Bio/dynamics 1983), but only one generation was assessed and this was primarily a developmental toxicity study. No changes in the histology of the testes and accessory glands or circulating male hormone levels occurred in rats following exposure to 1,000 ppm mixed xylenes for 61 days (Nylen et al. 1989). No histopathological changes were found in reproductive tissues of male and female rats and mice following intermediate- or chronic-duration oral exposure to mixed xylene or its isomers at doses as high as 500–2,000 mg/kg/day (NTP 1986; Wolfe 1988a, 1988b).

Human carcinogenicity data consist of limited and inconclusive occupational studies that examined risks of cancer and leukemia among workers exposed to xylenes together with other solvents (Arp et al. 1983; Wilkosky et al. 1984). Animal carcinogenicity data for xylenes are limited to equivocal oral studies with mixed xylenes (Maltoni et al. 1983, 1985; NTP 1986), and dermal initiation/promotion studies suggesting that xylene may be a promoter for skin cancer and might also act as an initiator or cocarcinogen (Berenblum 1941; Pound 1970; Pound and Withers 1963). No animal carcinogenicity data for xylenes are available for inhalation exposure. The insufficiencies of the available data preclude concluding that there is a causal relationship between xylene exposure and cancer. Mixed xylenes and the individual xylene isomers have been tested for genotoxicity in a variety of *in vitro* and *in vivo* assays with predominantly negative results, indicating that xylenes are nongenotoxic (ATSDR 1995).

D.3 Mechanisms of Action

The mechanisms by which xylene exerts its toxic effects are not completely understood, although a number of theories exist pertaining to induction of effects in the nervous system, developing fetus, lung, and kidney. The central nervous system toxicity observed during exposure to high concentrations of xylene has been attributed to the liposolubility of xylene in the neuronal membrane (Desi et al. 1967; EPA 1985; Gerarde 1959; Savolainen and Pfaffli 1980; Tahti 1992). It has been suggested that xylene disturbs the action of proteins essential to normal neuronal function. This is similar to the way general anesthetic agents work, (i.e., either by a disruption of the lipid environment in which membrane proteins function or by direct interaction with the hydrophobic/hydrophilic conformation of proteins in the membranes). Changes in levels of various neurotransmitters and lipid composition have been observed in several brain areas following acute- and intermediate-duration exposure to xylene (Andersson et al. 1981; Honma et al. 1983; Savolainen and Seppalainen 1979). It is unclear whether these represent direct effects of xylene or are secondary changes resulting from nonspecific central nervous system depression. Some authors have also suggested that metabolic intermediates, such as arene oxides or methylbenzaldehyde, may be responsible for the toxic effects of xylene (Savolainen and Pfaffli 1980). Oxidation of xylene to these intermediates by microsomal enzyme systems may occur within brain cells (Savolainen and Pfaffli 1980).

Inhibition of pulmonary microsomal enzymes has been observed by several investigators (Elovaara et al. 1987; Patel et al. 1978; Silverman and Schatz 1991; Smith et al. 1982; Stickney et al. 1989). The exact mechanism of the enzyme inhibition is unknown, but has been attributed to the formation of a toxic reactive metabolite (such as methylbenzaldehyde) that binds directly to microsomal protein and inactivates the microsomal enzymes (Patel et al. 1978; Smith et al. 1982). Direct effects on microsomal membrane fluidity and/or lipid content do not appear to be involved (Stickney et al. 1989).

The mechanism for xylene-related renal toxicity is unknown, but may be related to the formation of reactive metabolites and subsequent irritation or direct membrane fluidization (EPA 1985). In humans exposed to solvent mixtures containing xylene, the increased urinary levels of β-glucuronidase have been proposed to be due to a faster cellular turnover in the renal tubular epithelium because of a mild toxicity (Franchini et al. 1983). The lysozymuria and increase in urinary excretion of albumin may be indicative of potential damage to the renal tubules and renal glomeruli, respectively (Askergren 1982; Franchini et al. 1983).

The exact mechanism by which mixed xylenes produce toxic effects in fetuses has not been fully investigated. The information on mechanisms of neurotoxicity in the preceding paragraph is relevant to exposed offspring as well as adults. Based on results of studies with rats, *p*-xylene-induced delayed fetal development may be related to decreased levels of progesterone and estradiol (Ungvary et al. 1981). The reduced levels of these hormones may have been due to increased microsomal enzyme activity and increased hormone catabolism.

D.4 Health Guidelines

ATSDR (1995) derived an acute-duration inhalation MRL of 1 ppm for mixed xylenes based on a LOAEL of 100 ppm for neurological effects (increased reaction times) in humans (Dudek et al. 1990) and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability). Volunteers were exposed to xylenes for 4 hours.

ATSDR (1995) derived an intermediate-duration inhalation MRL of 0.7 ppm for mixed xylenes based on a LOAEL of 200 ppm for neurodevelopmental effects (reduced rotarod performance in offspring) in rats (Hass and Jakobsen 1993) and an uncertainty factor of 300 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). The animals were exposed 6 hours/day on gestation days 4–20.

ATSDR (1995) derived a chronic-duration inhalation MRL of 0.1 ppm for mixed xylenes based on a LOAEL of 14 ppm for subjective neurological effects (symptoms including anxiety, forgetfulness, and inability to concentrate) and eye and respiratory tract irritation in humans (Uchida et al. 1993) and an uncertainty factor of 100 (10 for use of a LOAEL and 10 human variability). Workers were occupationally exposed to xylenes for an average of 7 years.

ATSDR (1995) derived an acute-duration oral MRL of 1 mg/kg/day for *p*-xylene based on a NOAEL of 125 mg/kg/day for neurological effects (altered visual evoked potentials) in rats (Dyer et al.1988) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). The animals were administered a single dose of *p*-xylene in oil.

ATSDR (1995) derived an intermediate-duration oral MRL of 0.2 mg/kg/day for mixed xylenes based on a LOAEL of 150 mg/kg/day for renal toxicity (early chronic nephropathy) in rats (Condie et al. 1988) and an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and

10 for human variability). The animals were administered mixed xylenes in oil by gavage daily for 90 days.

ATSDR (1995) derived an intermediate-duration oral MRL of 0.6 mg/kg/day for *m*-xylene based on a LOAEL of 800 mg/kg/day for hepatic toxicity (increased plasma alanine aminotransferase and plasma membrane damage) in rats (Elovaara et al. 1989) and an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were administered *m*-xylene in oil by gavage 5 days/week for 3.3 weeks.

ATSDR (1995) did not derive oral MRLs for *o*-xylene for any duration period, or chronic-duration oral MRLs for mixed xylenes or any xylene isomer, due to lack of suitable data.

The EPA IRIS database lists an oral RfD, but no inhalation RfC for xylenes (IRIS 2001). The RfD is based on a NOAEL of 250 mg/kg/day (converted to 179 mg/kg/day) for neurological effects in rats (NTP 1986) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). The animals were administered mixed xylenes by gavage 5 days/week for 103 weeks. ACGIH (2001) recommends a TLV-TWA of 100 ppm and STEL/C of 150 ppm for xylene (*o*-, *m*-, and *p*-isomers) based on irritation in humans.

The NTP (2001) has not listed xylenes as a known or anticipated human carcinogen. EPA determined that mixed xylenes are not classifiable as to human carcinogenicity (Category D) based on no human data and inadequate animal data (IRIS 2001). IARC (1999b) concluded that xylenes are not classifiable as to their carcinogenicity (Group 3) based on inadequate evidence in humans and animals.

D.5 References

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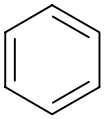
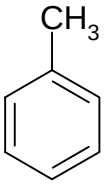
Appendix E: Chemical Structures of Mixture Components

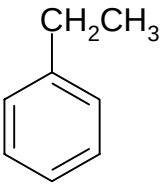
Benzene



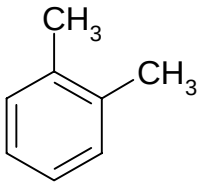
Toluene



Ethylbenzene



Xylenes



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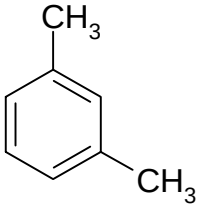

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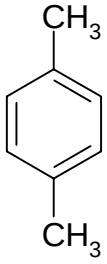

p-xylene