# 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 ($5.78\pm1.55$, $5.81\pm1.53$, 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 $\geq 40$ ppm, respectively), and 67.9 and 65.5% of the low and high toluene groups ($<41$ and $\geq 41$ 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]<br>100 + 100<br>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)<br>50 + 20<br>100 + 5<br>200 + 5 | 100 + 20 (r)<br>200 + 20<br>1,000 + 5<br>1,000 + 20 | no interaction (low dose mixtures)<br>< 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)<br>100 + 100<br>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]<br>250 + 900<br>500 + 300<br>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)<br>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)<br>< additive (high dose mixture) | Hsieh et al. 1990a |
| Repeated exposure | Brain levels of biogenic amines and their metabolites | | 80 + 166[c] (m)<br>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)<br>300 + 500<br>900 + 250<br>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)<br>166 + 325 | | no interaction | Hsieh et al. 1990a |
| Repeated exposure | Brain levels of biogenic amines and their metabolites | | 166 + 80[c] (m)<br>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]<br>150 + 150<br>225 + 75 | < additive | Tardif et al. 1992 |
| Acute | Blood levels of toluene and xylene | | | 100 + 200 (r)<br>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)<br>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)<br>150 + 150<br>225 + 75 | < additive | Tardif et al. 1992 |
| Acute | Blood levels of toluene and xylene | | | 100 + 200 (r)<br>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)<br>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]<br>1,015 + 1,015<br>1,305 + 1,305<br>1,355 + 1,355<br>2,065 + 2,065<br>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)<br>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)<br>1,200 + 1,200<br>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]<br>1,015 + 1,015<br>1,305 + 1,305<br>1,355 + 1,355<br>2,065 + 2,065<br>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)<br>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)<br>< additive (high dose mixture) | Vodickova et al. 1995; Frantik et al. 1988 |
| Acute | Depression of respiratory rate | 530 + 530 (r)<br>1,200 + 1,200<br>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]<br>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)<br>100 + 300<br>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)<br>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]<br>300 + 100<br>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]<br>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)<br>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.