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Physiologically Based Pharmacokinetic Modeling of a Ternary Mixture of Alkyl Benzenes in Rats and Humans

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The objective of the present study was to develop a physiologically based pharmacokinetic (PBPK) model for a ternary mixture of alkyl benzenes [toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ)] in rats and humans. The approach involved the development of the mixture PBPK model in the rat and extrapolation to humans by substituting rat physiological parameters and blood:air partition coefficients in the model with those of humans, scaling maximal velocity for metabolism on the basis of body weight$^{0.75}$ and keeping all other model parameters species-invariant. The development of the PBPK model for the ternary mixture in the rat was accomplished by initially validating or refining the existing PBPK models for TOL, XYL, and EBZ and linking the individual chemical models via the hepatic metabolism term. Accordingly, the Michaelis–Menten equation for each solvent was modified to test four possible mechanisms of metabolic interaction (i.e., no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). The metabolic inhibition constant (K_i) for each binary pair of alkyl benzenes was estimated by fitting the binary chemical PBPK model simulations to previously published data on blood concentrations of TOL, XYL, and EBZ in rats exposed for 4 hr to a binary combination of 100 or 200 ppm of each of these solvents. Competitive metabolic inhibition appeared to be the most plausible mechanism of interaction at relevant exposure concentrations for all binary mixtures of alkyl benzenes in the rat ($K_{i,TOL-XYL}$ = 0.17; $K_{i,TOL-EBZ}$ = 0.79; $K_{i,XYL-TOL}$ = 0.77; $K_{i,XYL-EBZ}$ = 1.50; $K_{i,EBZ-TOL}$ = 0.33; $K_{i,EBZ-XYL}$ = 0.23 mg/L). Incorporating the K_i values obtained with the binary chemical mixtures, the PBPK model for the ternary mixture simulated adequately the time course of the venous blood concentrations of TOL, XYL, and EBZ in rats exposed to a mixture containing 100 ppm each of these solvents. Following the validation of the ternary mixture model in the rat, it was scaled to predict the kinetics of TOL, XYL, and EBZ in blood and alveolar air of human volunteers exposed for 7 hr to a combination of 17, 33, and 33 ppm, respectively, of these solvents. Model simulations and experimental data obtained in humans indicated that exposure to atmospheric concentrations of TOL, XYL, and EBZ that remain within the permissible concentrations *for a mixture* would not result in biologically significant modifications of their pharmacokinetics. Overall, this study demonstrates the utility of PBPK models in the prediction of the kinetics of components of chemical mixtures, by accounting for mechanisms of binary chemical interactions.

For the consideration of data on toxic interactions in health risk assessments, qualitative and quantitative information regarding the nature of interaction mechanisms is required. Even though over 2000 studies on toxic interactions have been published todate, the quantitative aspect of toxicokinetic/toxicodynamic mechanism of interactions has only been elucidated for a few chemical pairs (Krishnan and Brodeur, 1991). Even such well-studied binary chemical interactions are yet to be considered within the context of complex mixture risk assessments, because of our inability to predict the effect of a third or a fourth chemical in the mixture on the interacting binary pairs (Beck *et al.*, 1994). One way of approaching this problem would be to develop biologically based dosimetry and toxicity models, such that multiple interactions can be simultaneously accounted for and systematically analyzed at any level of complexity.

In this regard, physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling represents a potentially useful approach. PBPK models have so far been developed for several binary chemical mixtures: trichloroethylene/1,1-dichloroethylene (Andersen *et al.*, 1987; El-Masri *et al.*, 1996), benzene/toluene (Purcell *et al.*, 1990), trichloroethylene/ethanol (Sato *et al.*, 1990), bromodichloromethane/chlordecone (Thakore *et al.*, 1991), and toluene/xylene (Tardif *et al.*, 1993). These efforts on binary chemical mixture modeling have led the way in the development of PBPK models for multichemical mixtures (Krishnan *et al.*, 1994). Except for a preliminary, theoretical modeling study involving *n*-hexane and two of its metabolites (Andersen and Clewell, 1983), all previous efforts have been limited to the study of binary chemical mixtures.

To achieve the ultimate goal of developing mechanistic

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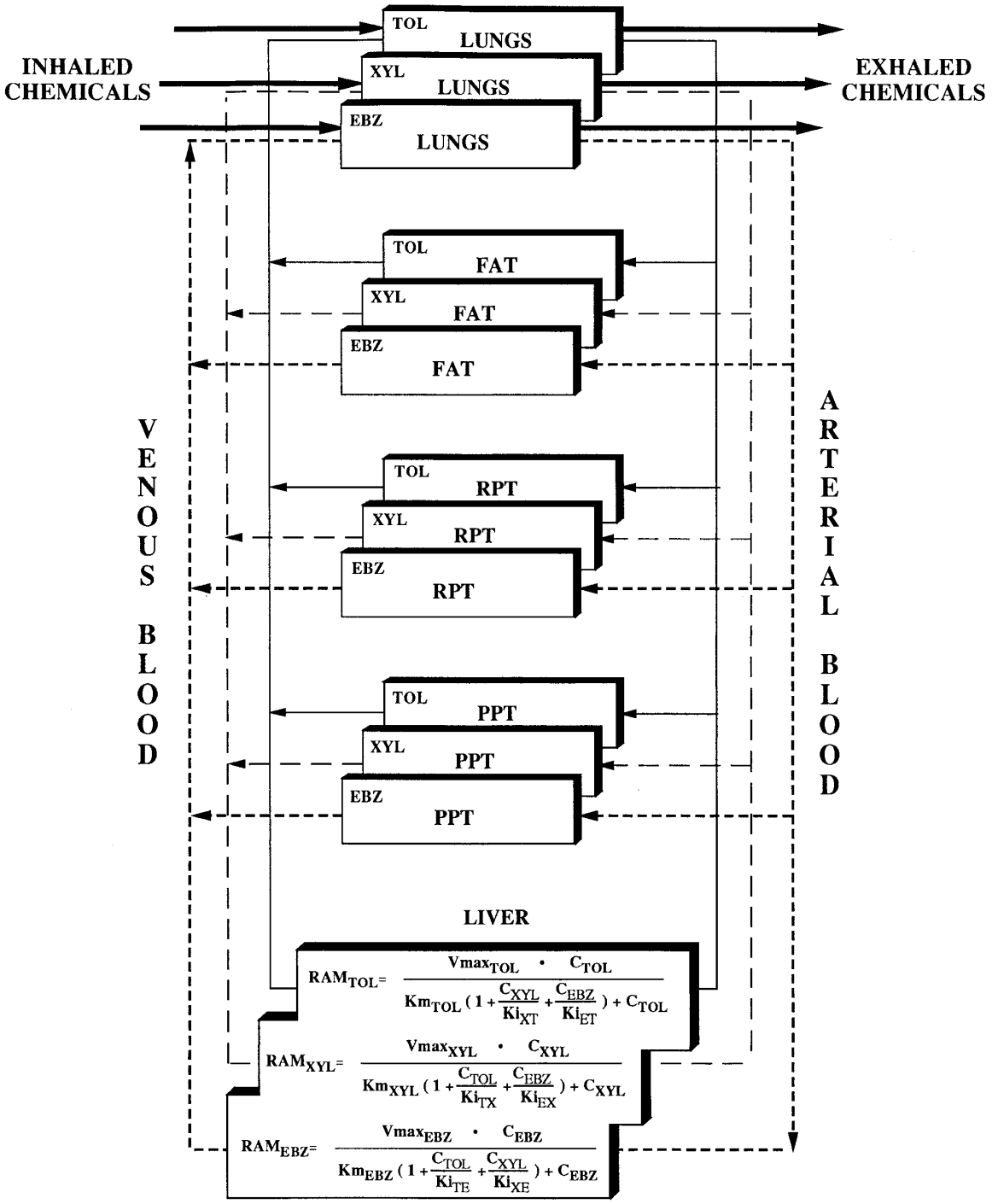

FIG. 1. Structure of the physiologically based pharmacokinetic (PBPK) model for the ternary mixture of alkyl benzenes [toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ)]. It consists of three single chemical PBPK models linked via the metabolism term of the liver compartment. C_{TOL}, C_{XYL}, and C_{EBZ} refer to the venous blood concentration of TOL, XYL, and EBZ leaving the liver compartment; K_{iTX}, K_{iXT}, K_{iEX}, K_{iET}, K_{iXE}, and K_{iTE} refer to the constants describing the competitive inhibition of the metabolism of one mixture constituent by another [TOL (T), XYL (X), and EBZ (E)]. RPT, PPT, and RAM refer, respectively, to the richly perfused tissues, poorly perfused tissues, and to the amount of an alkyl benzene metabolized in the liver. V_{max} and K_m refer to the maximal velocity of metabolism and Michaelis affinity constant.

TABLE 1
Physiological Parameters Used in the PBPK Models

Parameters	Values	
	Rat[a]	Human[b]
Alveolar ventilation rate (liters/hr/kg)	15.0	18.0
Cardiac output (liters/hr/kg)	15.0	18.0
Fraction of cardiac output corresponding to each compartment		
Fat	0.09	0.05
Slowly perfused tissues	0.15	0.25
Richly perfused tissues	0.51	0.44
Liver	0.25	0.26
Fraction of body weight corresponding to each compartment		
Fat	0.09	0.19
Slowly perfused tissues	0.72	0.62
Richly perfused tissues	0.05	0.05
Liver	0.049	0.026

[a] From Tardif *et al.* (1993).
[b] From Arms *et al.* (1988); Kaneko *et al.* (1991).

risk assessment methods for chemical mixtures, we need to develop strategies for PBPK/PD modeling of complex mixtures by accounting for the occurrence of interactions at the binary level. The present study representing our initial effort in this regard involved the investigation of the potential usefulness of PBPK modeling to predict the pharmacokinetics of the components of a ternary mixture, based on knowledge of the mechanisms of binary chemical interactions. For the present modeling study, we chose toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ), which have been reported or postulated to be substrates of cytochrome P-450 2E1 at low exposure concentrations (Toftgard and Nilsen, 1982; Liira *et al.*, 1991; Nakajima *et al.*, 1991; Tardif *et al.*, 1993; Tassanleyakul *et al.*, 1996).

The objective of the present study was to develop a PBPK model for a ternary mixture of alkyl benzenes (TOL, XYL, and EBZ) in rats and humans.

METHODS

The approach involved developing and validating a PBPK model for simulating the kinetics of TOL, XYL, and EBZ in rats exposed to a ternary mixture of these chemicals and then using this model as the basis for simulating the kinetics of these alkyl benzenes in humans.

PBPK Modeling of Alkyl Benzenes in the Rat

PBPK modeling of the alkyl benzene mixtures in the rat was accomplished in four sequential steps, namely, model representation, model parametrization, model simulation, and model validation.

Model representation. Initially, an individual chemical PBPK model was constructed for each of the three alkyl benzenes (TOL, XYL, and EBZ) in the rat. In this model, the rat was represented as a network of four tissue compartments, namely, liver, adipose tissue, slowly perfused tissues, and richly perfused tissues, interconnected with systemic circulation and a gas-exchange lung (Ramsey and Andersen, 1984). The tissue uptake of chemicals was described as a perfusion-limited process, and the rate of change in the amount of alkyl benzenes in each tissue was described with a set of mass balance differential equations (Ramsey and Andersen, 1984). Metabolism was limited to liver, and described as a saturable process characterized by V_{max} (maximal velocity for metabolism; mg/hr) and K_m (Michaelis–Menten affinity constant; mg/L). Subsequently, a PBPK model for the ternary mixture of TOL, XYL, and EBZ was constructed by connecting the three individual chemical models via the hepatic metabolism term (Fig. 1). The mixture PBPK model accounted for all plausible binary chemical interactions (i.e., TOL/XYL, TOL/EBZ, and XYL/EBZ). Based on the hypothesis of metabolic inhibition as the mechanism of interaction for all these binary combinations (i.e., no inhibition, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition) (Andersen *et al.*, 1987), each pair of alkyl benzenes within the mixture model was linked via the metabolism term in the liver compartment.

Model parametrization. The parameters required for the rat PBPK model can be grouped into three types: physiological, physicochemical, and biochemical. The rat physiological parameters required for this model (cardiac output, alveolar ventilation rate, tissue volumes, and tissue blood flow rates) were obtained from the literature (Tardif *et al.*, 1993; Table 1). The physicochemical parameters required for the ternary mixture model refer to rat blood:air and tissue:blood partition coefficients for TOL, XYL, and EBZ.

Whereas the partition coefficients for EBZ were determined by vial-equilibration technique, those for TOL and XYL were obtained from Gargas *et al.* (1989). The rat blood:air and tissue:air partition coefficients of EBZ were estimated by determining its headspace concentration at equilibrium, following the introduction of 0.2 μmol into sealed glass vials (22 ml) containing 1 ml of blood or 0.5 ml of tissue homogenates (1:4 in 0.9% saline). The headspace concentration of EBZ was determined by injecting a 1.0-ml sample with a Tekmar Headspace Autosampler-7000 into a gas chromatograph (Hewlett–Packard 5890A) equipped with a flame ionization

TABLE 2
Partition Coefficients and Metabolic Constants for Toluene, *m*-Xylene, and Ethylbenzene Used in the PBPK Models

Parameters	Toluene[a]	Xylene[a]	Ethylbenzene[d]
Blood:air (rat)	18.0	46.0	42.7
Blood:air (human)	15.6[b]	26.4[c]	28.0
Fat:air	1021.0	1859.0	1556.0
Slowly perfused tissues:air	27.7	41.9	26.0
Richly perfused tissues:air	83.6	90.9	60.3
Liver:air	83.6	90.9	83.8
Metabolic constants			
V_{max} (mg/hr/kg)	4.80[e]	5.50[f]	7.3[f]
K_m (mg/liter)	0.55	0.22	1.39

[a] Gargas *et al.* (1989).
[b] Sato (1991).
[c] Kaneko *et al.* (1991).
[d] Determined experimentally in this study.
[e] Tardif *et al.* (1993).
[f] This study. Based on the best fit of the simulation to the time-course data on the venous blood concentration of solvents following single exposures.

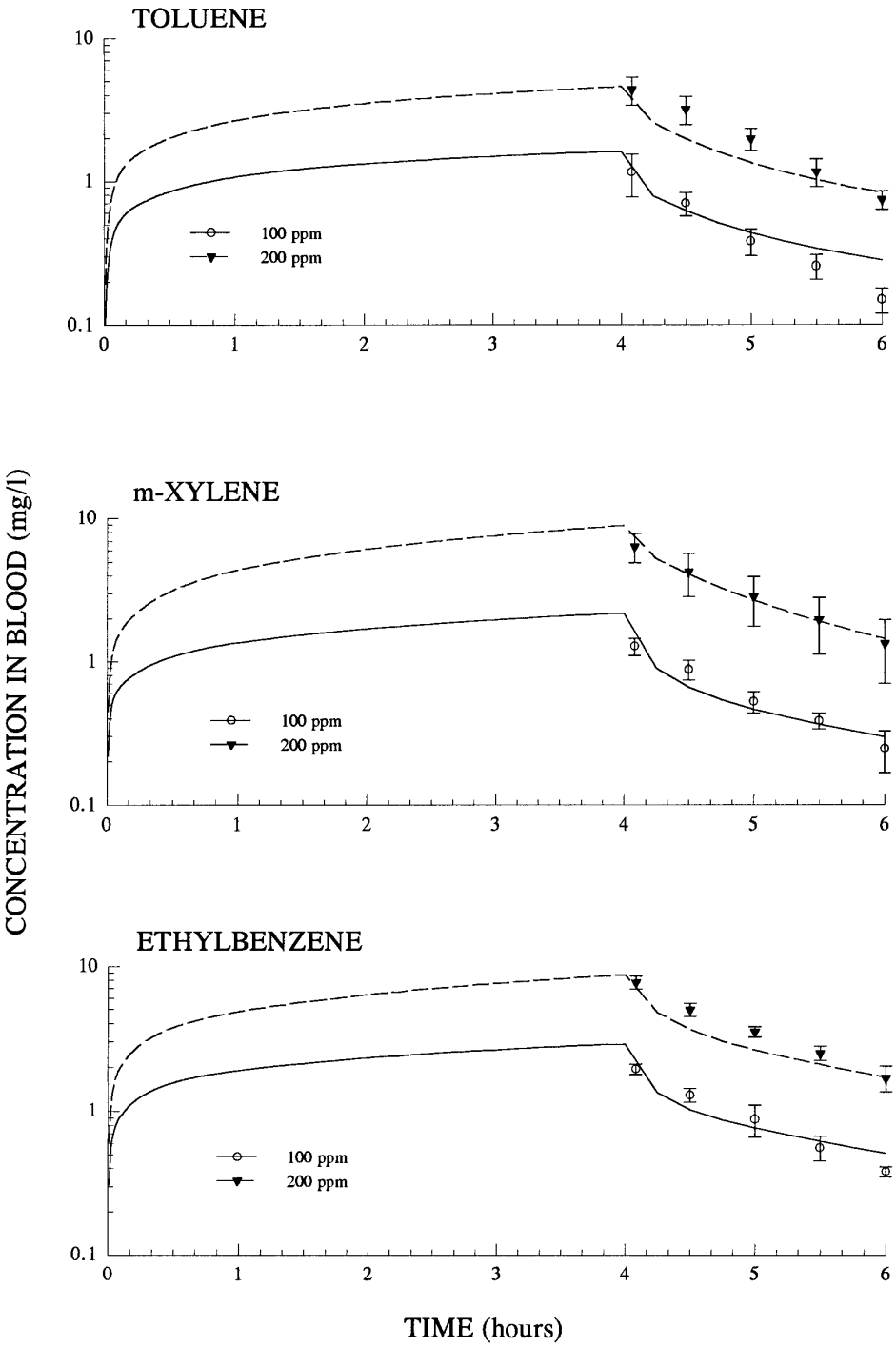

FIG. 2. Comparison of the PBPK model simulations of venous blood concentrations of toluene, *m*-xylene, and ethyl benzene with experimental data (symbols) obtained in rats exposed for 4 hr to 100 and 200 ppm of each of these solvents alone. Experimental data are from Tardif *et al.* (1996), and each data point represents the mean (±SD) of four rats.

detector. The quantitation of EBZ was performed using a glass column (6′ × 1/8″) packed with 10% SP-1000 on Supelcoport 80/100, with nitrogen as the carrier gas (20 ml/min). The oven, injector, and detector temperatures were set at 90, 120, and 200°C, respectively.

The biochemical parameters required for the ternary mixture PBPK model refer to the V_{max} and K_m for each of the three components, and the metabolic inhibition constant (K_i) for each of the binary chemical pair within the ternary mixture. Whereas the V_{max} and K_m for the hepatic metabolism of TOL corresponded to the previously determined values in our laboratory (Tardif *et al.*, 1993), those for XYL and EBZ were estimated by fitting

TABLE 3

Metabolic Inhibition Constants (K_i) According to Various Hypotheses of Metabolic Inhibition among Toluene (TOL), *m*-Xylene (XYL), and Ethylbenzene (EBZ) Tested in the Rat PBPK Model

		K_i (mg/liter)		
Inhibitor	Substrate	C^a	NC	UC
TOL	XYL	0.17	2.25	2.14
TOL	EBZ	0.79	3.04	2.21
XYL	TOL	0.77	3.36	2.33
XYL	EBZ	1.50	5.41	3.49
EBZ	TOL	0.33	1.55	1.14
EBZ	XYL	0.23	3.72	3.65

[a] C, competitive; NC, noncompetitive; UC, uncompetitive. $K_{m_{TOL}}$ = 0.55 mg/liter; $K_{m_{XYL}}$ = 0.22 mg/liter; $K_{m_{EBZ}}$ = 1.39 mg/liter.

model simulations to experimental data on the venous blood concentration in rats exposed to 100 and 200 ppm of these solvents for 4 hr by inhalation (Tardif *et al.*, 1996). The K_i for each mechanism for each solvent pair was estimated from the best fit of the binary chemical PBPK model simulations to the experimental data on the blood concentration of unchanged solvents collected in rats following exposure to each binary mixture (i.e., 100 ppm TOL + 200 ppm XYL; 100 ppm TOL + 200 ppm EBZ; 200 ppm TOL + 100 ppm XYL; 200 ppm TOL + 100 ppm EBZ; 100 ppm XYL + 200 ppm EBZ; and 200 ppm XYL + 100 ppm EBZ) (Tardif *et al.*, 1996).

Model simulation. The algebraic and differential equations describing the pharmacokinetics of the components of the ternary mixture were written as a program and solved with a commercially available software, namely, ACSL (Advanced Continuous Simulation Language; Mitchell and Gauthier Associates, Inc., Concord, MA). All optimizations were performed using OPTDES (Optimal Design of Dynamic Systems, Mitchell and Gauthier Associates Inc.).

Model validation. The ternary mixture PBPK model was validated by comparing the a priori predictions with experimental data on the blood concentrations of TOL, XYL, and EBZ determined in rats following a 4-hr exposure to a mixture containing 100 ppm of each of these solvents (Tardif *et al.*, 1996).

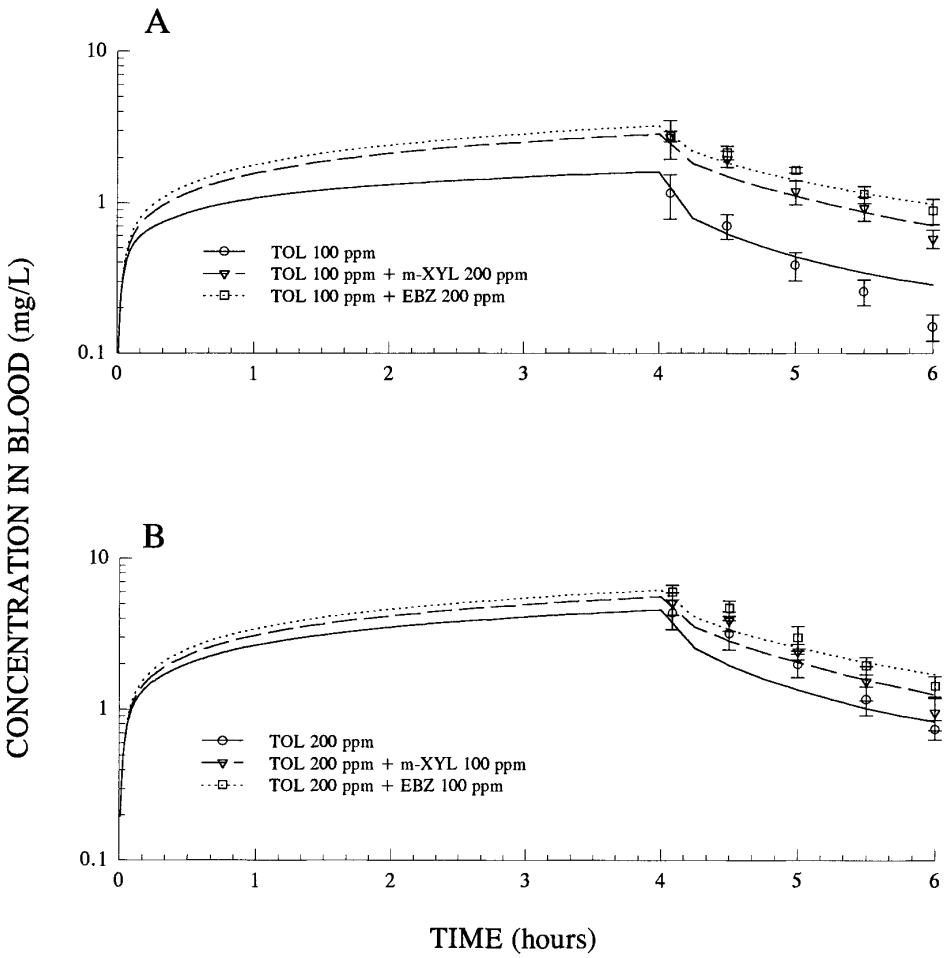

FIG. 3. Comparison of the PBPK model simulations of venous blood concentrations of toluene (TOL) following a 4-hr inhalation exposure to 100 and 200 ppm TOL alone or in combination with ethylbenzene (EBZ) or *m*-xylene (XYL). The competitive metabolic inhibition constants used in binary chemical models were obtained following fitting of model simulations to experimental data. Experimental data are from Tardif *et al.* (1996), and each data point represents the mean (±SD) of four rats.

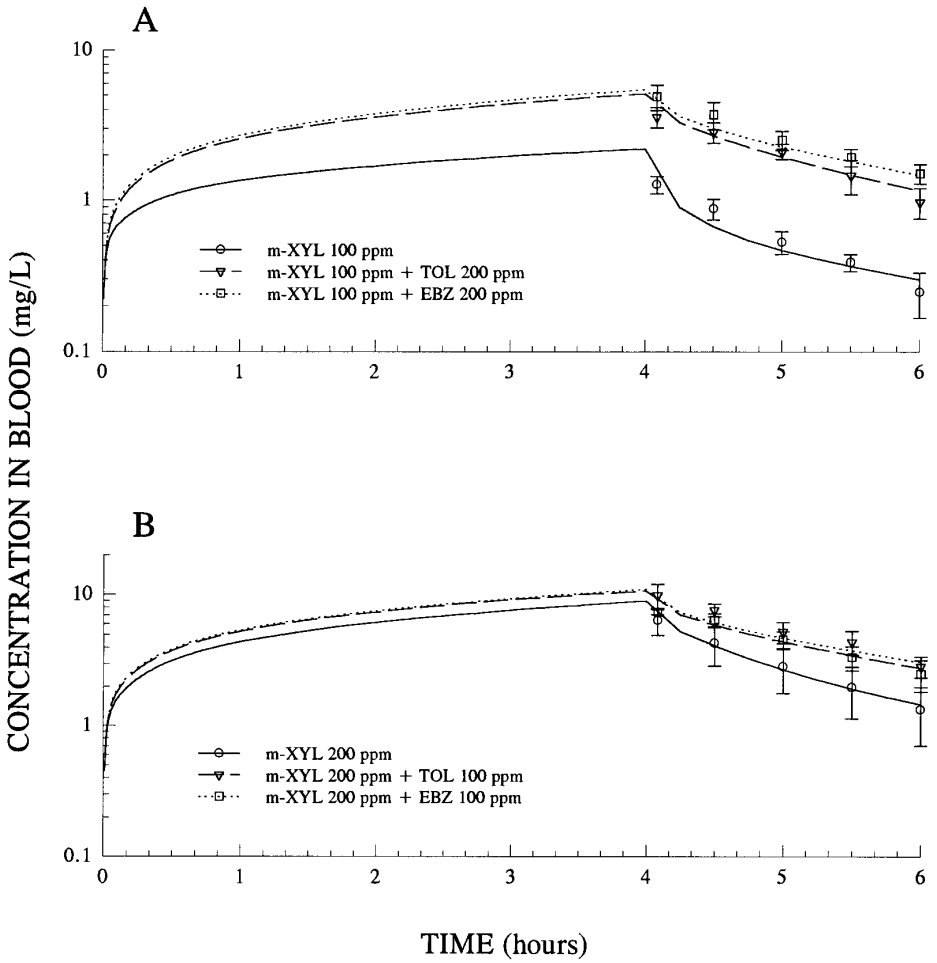

FIG. 4. Comparison of the PBPK model simulations of venous blood concentrations of *m*-xylene (XYL) following a 4-hr inhalation exposure to 100 and 200 ppm XYL alone or in combination with ethylbenzene (EBZ) or toluene (TOL). The competitive metabolic inhibition constants used in binary chemical models were obtained following fitting of model simulations to experimental data. Experimental data are from Tardif *et al.* (1996), and each data point represents the mean (±SD) of four rats.

PBPK Modeling of Alkyl Benzenes in Humans

The structure of the PBPK model used to describe the kinetics of TOL, XYL, and EBZ was identical to that developed for the rat. The numerical values of the species-specific model parameters, however, were replaced with those for humans. The approach involved (i) substituting rat physiological parameters and blood:air partition coefficients with those of humans, (ii) scaling the V_{max} on the basis of body weight$^{0.75}$, and (iii) keeping all other model parameters species-invariant. The assumption of metabolic inhibition constants as being species-invariant implies that the nature and magnitude of the competition involving TOL, XYL, and EBZ for binding to cytochrome P-450 2E1 does not change between species. This assumption can be accepted as the default since the substrates (TOL, XYL, and EBZ) and the isoform (2E1) involved are the same even though the species being considered are different (rat vs human). We have successfully used this approach to predict the kinetics of TOL/XYL mixtures in humans based on competitive inhibition mechanism elucidated in the rat (Tardif *et al.*, 1995).

The human PBPK model for alkyl benzenes was validated with kinetic data collected during a human volunteer exposure study. This study involved the exposure of four adult males (age, 22–47; body weight, 79–90 kg, nonsmokers), with no recent history of occupational exposure to alkyl benzenes. Prior to the exposures, they provided their informed consent, following which they underwent a complete medical examination (Tardif *et al.*, 1991). They were also instructed to avoid alcohol and medication for a period of 48 hr preceding exposures. They were then exposed for 7 hr/day during 4 different days to TOL (17 ppm), XYL (33 ppm), or EBZ (33 ppm), alone or in combination. These exposure concentrations correspond to the allowable occupational exposure concentrations, determined on the basis of the recommendations of ACGIH for solvent mixtures (ACGIH, 1995–1996).

The human exposure chamber, solvent vapor generating system, and monitoring systems used in the present study have previously been described (Tardif *et al.*, 1991). Briefly, the exposures were carried out in a dynamic exposure chamber (18.1 m^3). Solvent atmospheres were generated by the introduction of solvent mixed with purified air (4.3 m^3/min) through 16 diffusers located in the ceiling of the chamber. The concentrations of solvents were monitored by injecting an atmospheric sample every 30 min into a gas chromatograph (Tardif *et al.*, 1991). Additionally, urine samples were collected after the first 3 hr of exposure (0–3 hr), at the end of exposure (3–7 hr), and during 17 hr following the exposure (7–24 hr). The urinary concentrations of hippuric acid (major metabolite of TOL), methyl hippuric acid (metabolite of XYL), phenyl glyoxylic acid, and mandelic

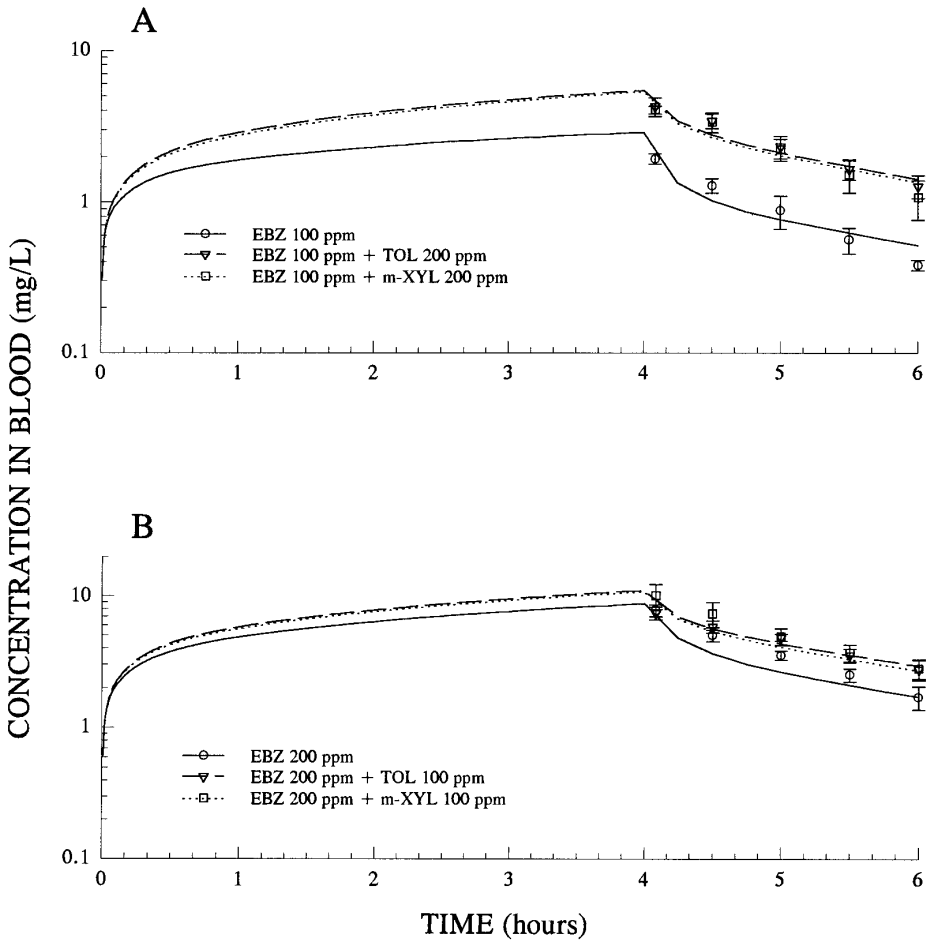

FIG. 5. Comparison of the PBPK model simulations of venous blood concentrations of ethylbenzene (EBZ) following a 4-hr inhalation exposure to 100 and 200 ppm EBZ alone or in combination with toluene (TOL) or *m*-xylene (XYL). The competitive metabolic inhibition constants used in binary chemical models were obtained following fitting of model simulations to experimental data. Experimental data are from Tardif *et al.* (1996), and each data point represents the mean (±SD) of four rats.

acid (metabolites of EBZ) were measured by high-pressure liquid chromatography (Poggi *et al.*, 1982) and corrected for creatinine content. Additionally, ortho-Cresol, a minor metabolite of TOL, was measured by gas chromatography (Truchon *et al.*, 1996).

The human PBPK model for alkyl benzenes was used to simulate the time-course of blood and alveolar air concentrations of TOL, XYL, and EBZ during and following a 7-hr exposure to the TLVs of these substances in mixture (i.e., 17, 33, and 33 ppm, respectively) (ACGIH, 1995–1996). The model predictions were then compared with the experimental data obtained in the human volunteer study. The metabolite concentrations were not simulated in the model; however, the experimental data on the urinary levels of metabolites during individual and combined exposures were useful for providing additional support to the model predictions of the extent of metabolic inhibition of TOL, XYL, and EBZ in humans during combined exposures.

RESULTS

PBPK Modeling of Alkyl Benzenes in the Rat

The blood:air and tissue:air partition coefficients of EBZ determined using vial equilibration technique are presented in Table 2. The V_{max} and K_m for XYL estimated by fitting model simulations to experimental data on its venous blood concentrations were 5.5 mg/hr/kg and 0.22 mg/L (Fig. 2, middle), and for EBZ these values corresponded to 7.3 mg/hr/kg and 1.4 mg/L, respectively (Fig. 2, bottom). For TOL, previously published V_{max} and K_m values adequately predicted the blood concentration profile observed in rats following a four hour inhalation exposure to 100 ppm and 200 ppm of this solvent (Fig. 2, top). The individual chemical rat PBPK models with these parameter estimates were then linked with each other via hypothetical mechanisms of interaction. Accordingly, the K_i for each of the three simple, hypothetical mechanisms of metabolic inhibition (competitive, noncompetitive, and uncompetitive) was obtained for each binary mixture (XYL/TOL, TOL/EBZ, and XYL/EBZ) (Table 3) by fitting the model simulations to experimental data on venous blood concentrations of unchanged solvents (Figs. 3–5). In other words, specifying the K_i (from Table 3) in the PBPK model along with any of the three correspond-

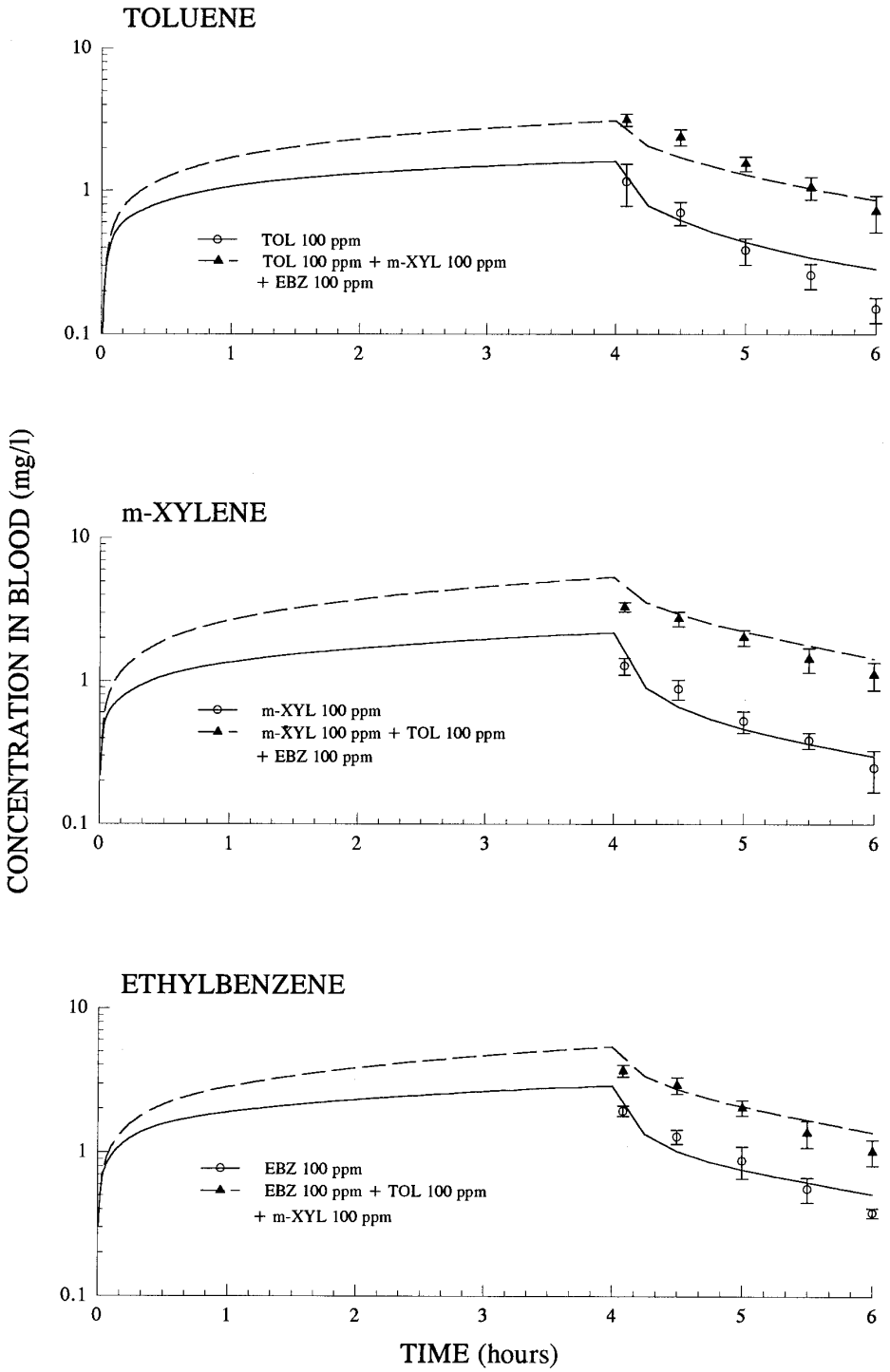

FIG. 6. Comparison of the simulations of venous blood concentrations of toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ) predicted by the individual chemical (solid lines) or a ternary chemical PBPK model (dashed lines) with corresponding experimental data (symbols) obtained in rats exposed for 4 hr to 100 ppm of each of these solvents alone or in combination. Experimental data are from Tardif *et al.* (1996), and each data point represents the mean (±SD) of four rats.

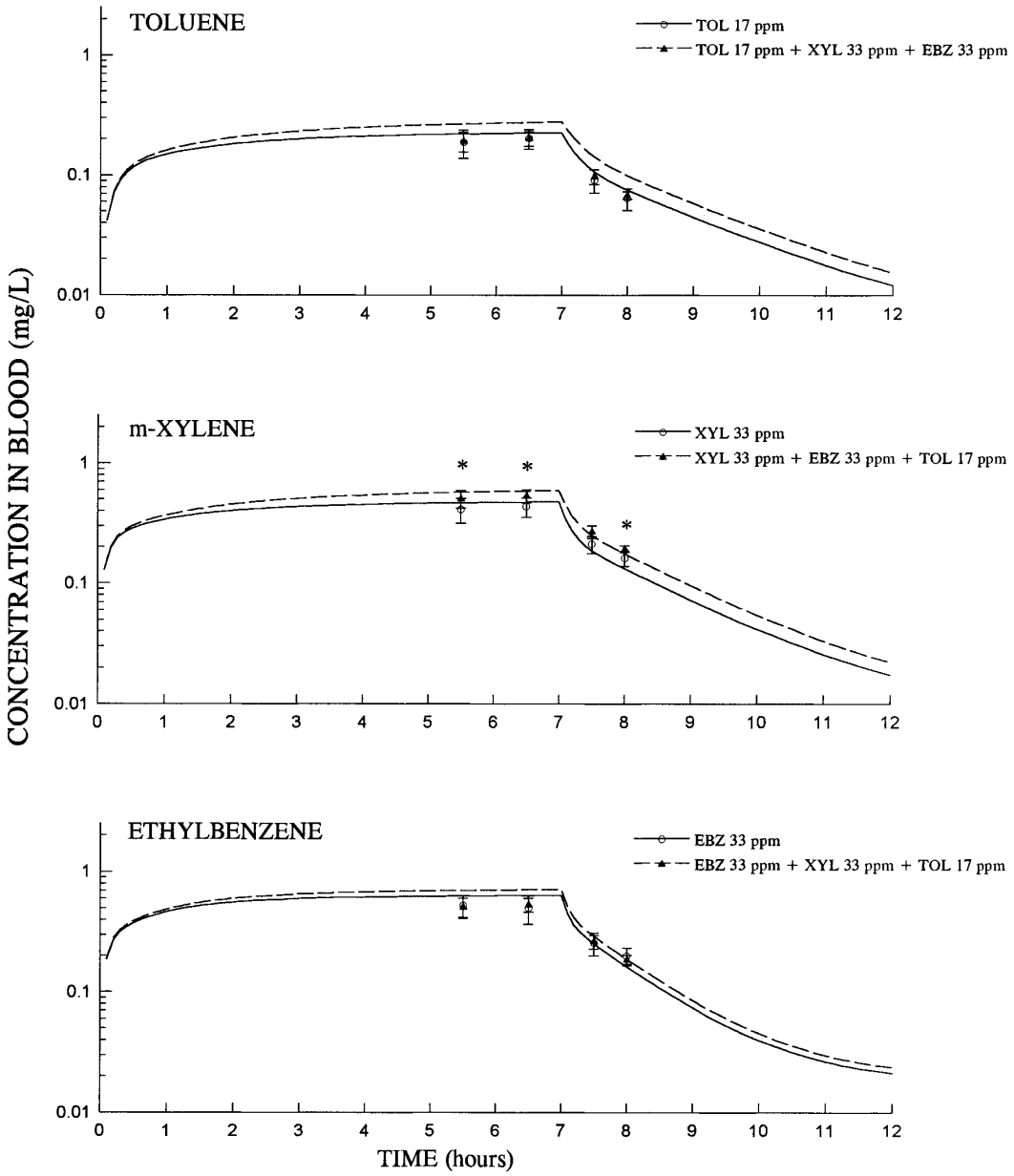

FIG. 7. Comparison of the simulations of venous blood concentrations of toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ) predicted by the individual chemical (solid lines) or a ternary chemical PBPK model (dashed lines) with corresponding experimental data (symbols) obtained in humans exposed for 7 hr to 17, 33, and 33 ppm, respectively, of these solvents alone or in combination. The ternary model simulations were obtained assuming competitive metabolic inhibition as the mechanism of interaction, based on rat data. Each experimental data point represents the mean (±SD) for four adult male volunteers. *Data points for single and mixed alkyl benzene exposures were significantly different ($p < 0.05$).

ing inhibition mechanisms provides simulations that fit the blood kinetic data collected during combined exposures. The value of K_i for competitive inhibition was generally closer to K_m of the respective solvents. Based on this observation, competitive metabolic inhibition was considered to be the plausible mechanism of interaction between the various alkyl benzenes.

The ternary chemical mixture PBPK model, incorporating the competitive metabolic inhibition constants for the various constituent binary chemical mixtures, adequately predicted the venous blood concentrations of TOL, XYL, and EBZ observed after a 4-hr exposure of rats to a mixture containing 100 ppm each of the three solvents (Fig. 6).

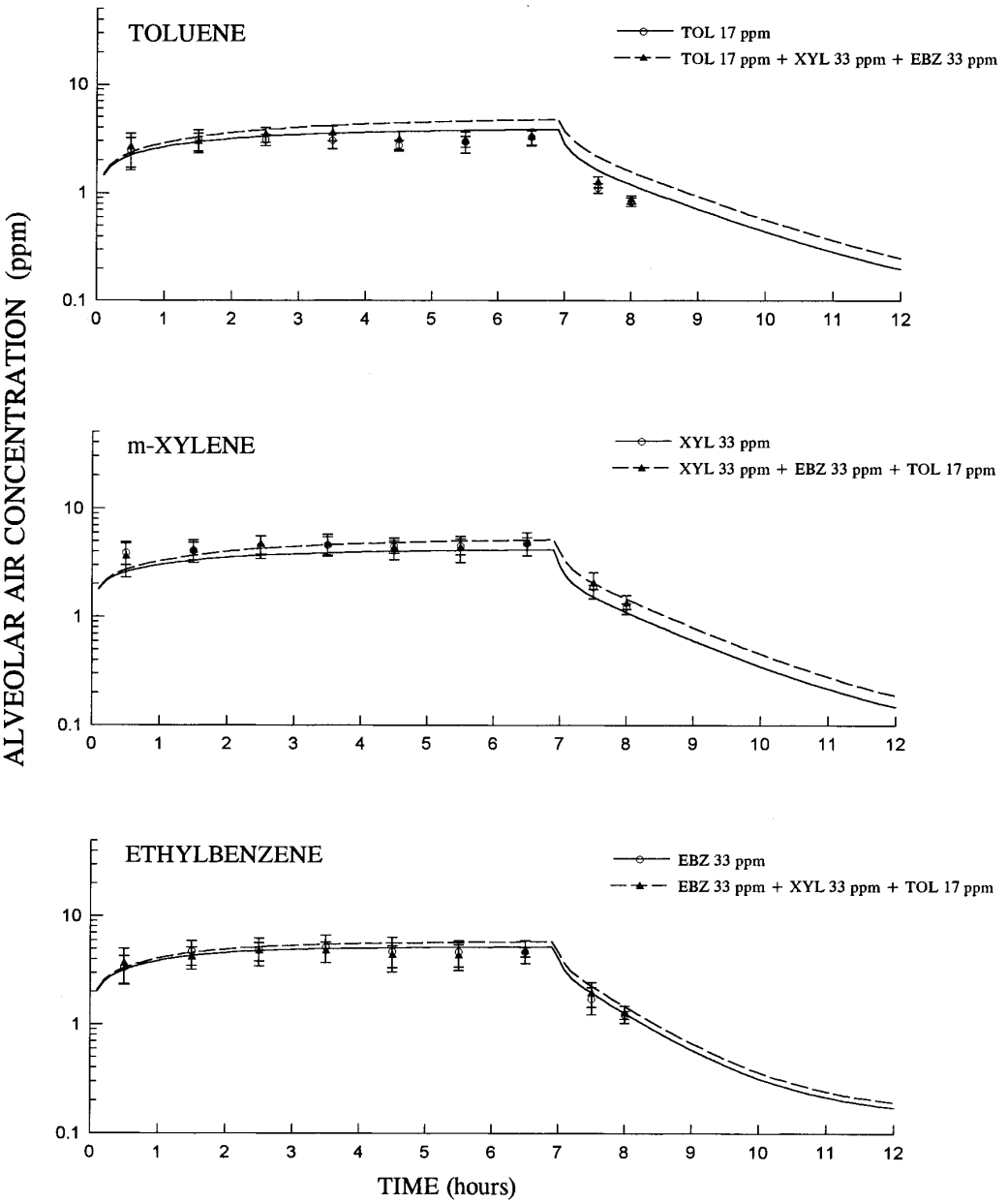

FIG. 8. Comparison of the simulations of alveolar air concentrations of toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ) predicted by the individual chemical (solid lines) or a ternary chemical PBPK model (dashed lines) with corresponding experimental data (symbols) obtained in humans exposed for 7 hr to 17, 33, and 33 ppm, respectively, of these solvents alone or in combination. The ternary model simulations were obtained assuming competitive metabolic inhibition as the mechanism of interaction, based on rat data. Each experimental data point represents the mean (±SD) for four adult male volunteers.

PBPK Modeling of Alkyl Benzenes in Humans

The human PBPK model, based on the quantitative interaction mechanism elucidated in the rat (i.e., competitive metabolic inhibition), simulated adequately the blood and alveolar air concentrations of TOL, XYL, and EBZ observed following individual or combined exposures to 17, 33, and 33 ppm, respectively, of these solvents (Figs. 7 and 8). Overall, the model simulations and experimental data obtained in humans indicated that exposure to atmospheric concentrations of TOL, XYL, and EBZ that remain within the permissible concentrations for a mixture (calculated according to ACGIH (1995–1996)) would not result in biologically significant modifications of their pharmacokinetics. A statistically significant difference (i.e., increase) however, was observed with respect to the blood concentrations of XYL dur-

TABLE 4
Urinary Excretion of Metabolites after a Single 7-hr Human Exposure to Toluene (TOL), *m*-Xylene (XYL), and Ethylbenzene (EBZ) Singly or in Combination

	Amount excreted (μmol)/period							
	0–3 hr		3–7 hr		7–24 hr		0–24 hr	
Urinary metabolite	Single[a]	Mixture[b]	Single	Mixture	Single	Mixture	Single	Mixture
o-Cresol	0.41 ± 0.18	N.D.	0.42 ± 0.13	0.35 ± 0.08	0.98 ± 0.58	0.41 ± 0.81	1.11 ± 1.00	0.75 ± 0.81
Hippuric acid	566 ± 121	677 ± 177	752 ± 83	817 ± 204	2644 ± 690	2350 ± 1303	3962 ± 730	3844 ± 1641
Methylhippuric acid	476 ± 139	406 ± 53	745 ± 188	562 ± 120	405 ± 65	400 ± 40	1626 ± 324	1368 ± 189
Mandelic acid	113 ± 55	117 ± 37	294 ± 111	293 ± 120	520 ± 253	562 ± 69	927 ± 281	973 ± 220
Phenylglyoxilic acid	8.1 ± 16.1	N.D.	83 ± 38	81 ± 35	380 ± 145	373 ± 84	472 ± 169	455 ± 108

Note. N.D., not detected.
[a] Single exposures: TOL, 17 ppm; XYL, 33 ppm; EBZ, 33 ppm.
[b] Mixed exposure: TOL (17 ppm) + XYL (33 ppm) + EBZ (33 ppm).

ing combined exposure (Fig. 7). Such a significant change was not reflected by the alveolar air concentration data collected during the same experiment (Fig. 8).

The data on the excretion of the urinary metabolites of TOL, XYL, and EBZ during human exposure to individual or mixtures of these chemicals are presented in Table 4. With the exception of *o*-cresol and phenyl glyoxylic acid during the first 3 hr of exposure, the amount of metabolites excreted during different time intervals was not significantly different between individual and combined human exposures to alkyl benzenes.

DISCUSSION

Modeling the pharmacokinetics of the components of complex chemical mixtures is a difficult task, since complications arise from the possibility that a third or fourth chemical might alter further the kinetics of interacting chemical pairs in the mixture. The principal reason for not considering data on chemical interactions in risk assessments appears to be related to the state of our knowledge with respect to the predictability of the effects of additional chemicals on each interacting pair within a complex mixture. Such predictability can only be achieved by understanding the mechanism of interaction of each pair, and by interconnecting the interacting chemicals within a biological modeling framework. The present study is an example of how these phenomena in a ternary chemical mixture can, relatively easily, be described within PBPK models.

The complexity of the chemical mixture problem requires the consideration of simple approaches to begin with. The simplest approach to PBPK modeling of mixtures would involve the consideration of the mixture as a single entity. This approach, designated as the ''compound X'' approach, would involve the determination of the changes in the disposition of a chemical of interest after exposure to ''compound X'' (Constan *et al.*, 1993). Accordingly, when enzyme inhibition occurs, a modifying factor (i.e., K_i) is incorporated into the metabolism term of the chemical of interest. This K_i is actually a hybrid constant representing the concentration and the affinity of the true inhibitor(s) which is (are) not known in this instance. Since the potency, identity, and kinetics of individual inhibitors in the mixture are not known, this kind of an empirical PBPK model for chemical mixtures may not be useful for conducting extrapolations (e.g., high dose to low dose extrapolation particularly when the proportions of the mixture constituents change).

The various toxicokinetic extrapolations essential within the context of the dose–response assessment of chemical mixtures may be conducted with a more complex PBPK model, as the one developed in this study. The present approach involves the modeling of each component of the mixture plus the possible interactions among the components. The most important implication of this modeling exercise is that the kinetics of the components of complex mixtures can be predicted only with information on binary chemical interactions.

The ability of PBPK models to extrapolate the extent/magnitude of a metabolic interaction from binary mixtures to more complex mixtures arises from the very nature and basis of these models. This aspect can be explained with a hypothetical example. Let's consider a binary chemical mixture AB in which there is a competition between the constituents A and B for hepatic metabolism. In this case, the binary chemical PBPK model can simulate the kinetics of both A and B with the consideration of the impact of the mutual inhibitory effects on each other's metabolism. What happens when we add another chemical, C? Within the

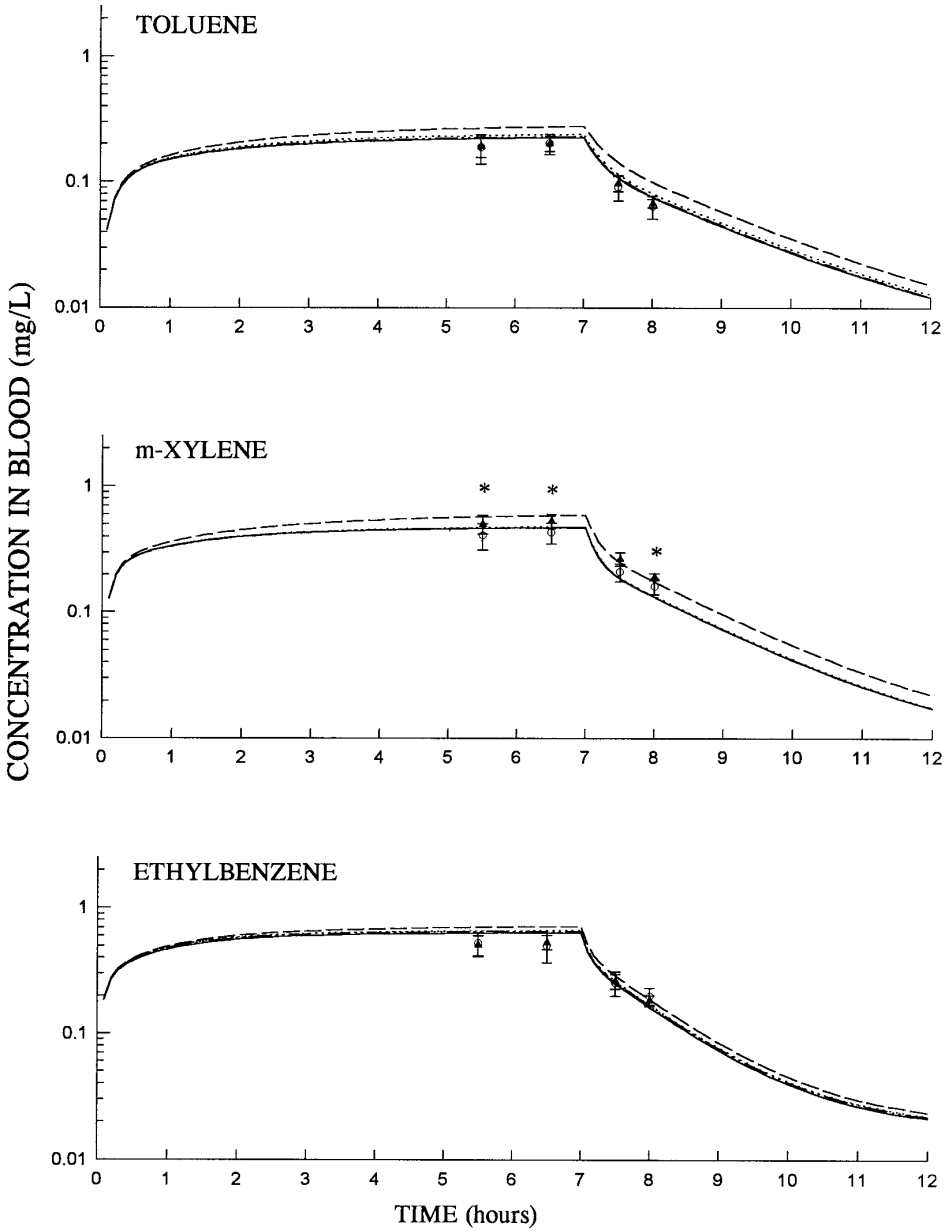

FIG. 9. Comparison of the simulations of venous blood concentrations of toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ) predicted by the individual chemical (solid lines) or a ternary chemical PBPK model (dotted or dashed lines) with corresponding experimental data (symbols) obtained in humans exposed for 7 hr to 17, 33, and 33 ppm, respectively, of these solvents alone or in combination. The ternary model simulations were obtained for three hypothetical mechanisms of interaction [competitive inhibition (---), noncompetitive inhibition (· · ·), uncompetitive inhibition (·-·)]. Each experimental data point represents the mean (±SD) for four adult male volunteers. *Data points for single and mixed alkyl benzene exposures were significantly different ($p < 0.05$).

PBPK modeling framework, all one needs to do is to include the values of K_i for the interaction between C and A, and C and B, as has been done in the present study. There is still the question of how the addition of C alters the way B interacts (i.e., competes in this example) with A. This is actually where the unique usefulness of PBPK modeling becomes evident. Once we describe the effect of C on B, this would result in a change in the rate of B metabolized and therefore its concentration in the venous blood leaving liver (Cvl_B). The C_{vlB} is the numerator of the term representing the inhibitory effect of B on A ($1 + C_{vlB}/K_{iB,A}$). Since exposure to chemical C modifies C_{vlB}, this then translates

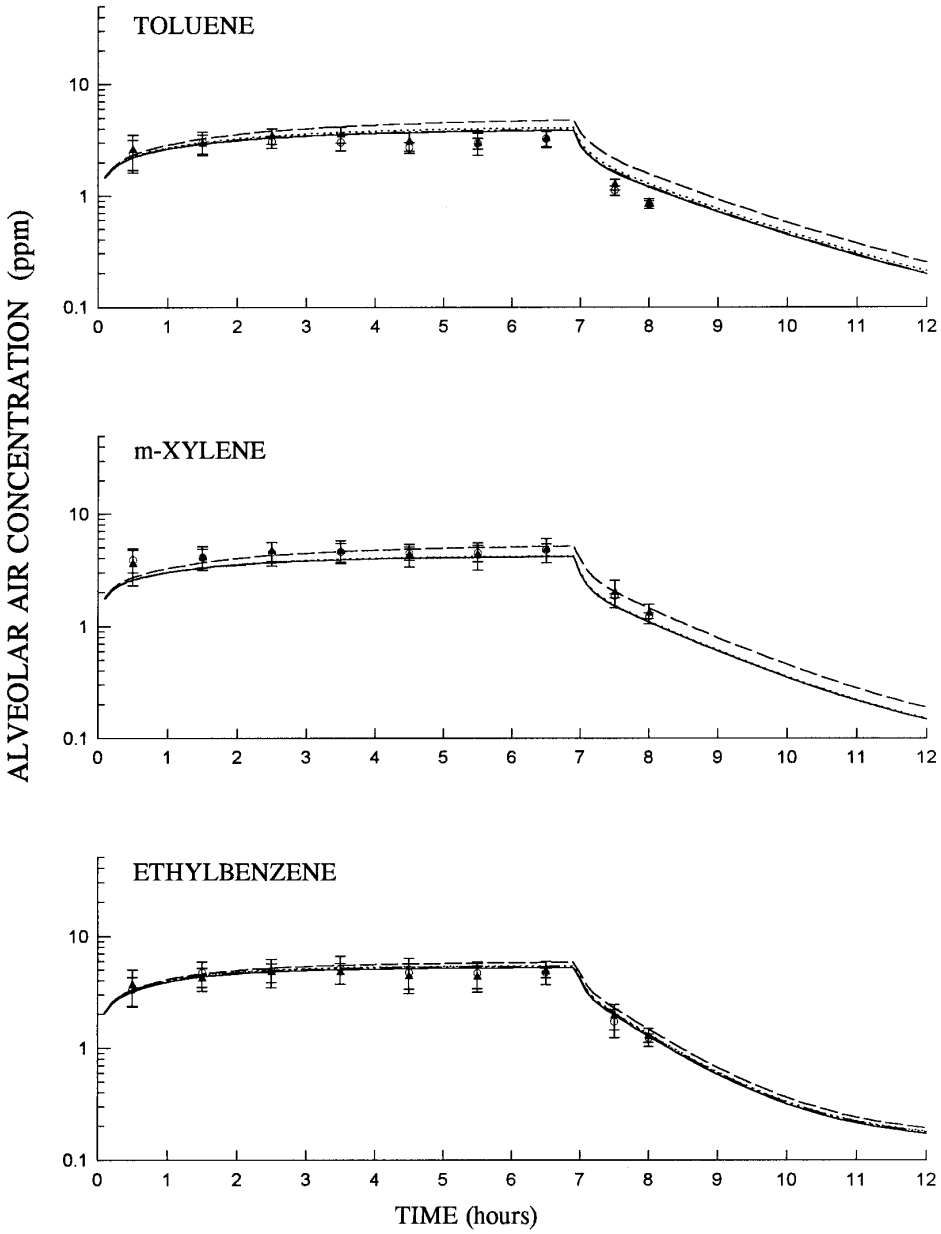

FIG. 10. Comparison of the simulations of alveolar air concentrations of toluene (TOL), *m*-xylene (XYL), and ethylbenzene (EBZ) predicted by the individual chemical (solid lines) or a ternary chemical PBPK model (dotted or dashed lines) with corresponding experimental data (symbols) obtained in humans exposed for 7 hr to 17, 33, and 33 ppm, respectively, of these solvents alone or in combination. The ternary model simulations were obtained for three hypothetical mechanisms of interaction [competitive inhibition (---), noncompetitive inhibition (· · ·), uncompetitive inhibition (·-·)]. Each experimental data point represents the mean (±SD) for four adult male volunteers.

in a modification of the potency of the interactive effect of B on A. Similarly, C may also affect the concentration of A, which then would result in a change in the magnitude of the interactive effect of A on B. This is the basis with which the PBPK model developed in the present study successfully simulated the kinetics of alkyl benzenes in a ternary mixture, taking into account the impact of the occurrence of various binary interactions. Based on the same analogy, it should be possible to predict the influence of the addition of another substrate for P-450 2E1 to this ternary mixture, and so forth.

Logically, the present modeling approach should be applicable to mixtures of any complexity, invoking various kinds of mechanisms of interactions among its components. Of course, the present study was limited to a ternary mixture, in which all compo-

nents compete for the same isoenzyme for metabolism. This simple prototype study demonstrates that the kinetics of the components present in complex chemical mixtures can be predicted by taking into account the inhibition constants for each interacting chemical pair, determined separately. For simulating the kinetics and dynamics of the components of complex mixtures then, data on the quantitative nature of the mechanism of binary interactions among the mixture components are alone needed. Based on mechanistic considerations, there should not be a need to conduct kinetic/dynamic studies with intermediary-size mixtures such as with submixtures of four or five chemicals, solely for enabling the prediction of the tissue dosimetry/response elicited by complex mixtures, because the ''binary to multichemical mixture'' extrapolation of the kinetics/dynamics of chemicals is facilitated by the incorporation of mechanistic interaction data into PBPK/PD models.

Hypothesizing/determining the exact nature of mechanism of interaction may not be easy/feasible in all cases. Even when certain plausible mechanism(s) can be hypothesized, as done in the present study, there is still the question of whether a particular mechanism is the one responsible for the observed interaction. In the present study, the K_i value for each of the various mechanisms was determined by optimization. Therefore, it is only normal that the mixture PBPK model with any of the three mechanistic descriptions along with the corresponding K_i values describes the observed data adequately. In such cases, discriminating one mechanism from another could be impossible without detailed experimentation, consuming significant resources. If more than one mechanism of interaction provide equally good fit to the available human data (Figs. 9 and 10), the ''real'' mechanism cannot be confidently established, even though one of the several mechanisms can be speculated to be more feasible than others, as we have done with the competitive inhibition for the alkyl benzene mixtures. However, we should realize that the fact that the ''real'' mechanism is not known should not affect our ability to make inferences regarding the relevance of interactions to humans, because when the predictions of kinetics for alternative interaction mechanisms (competitive, noncompetitive, and uncompetitive inhibitions) are quantitatively similar at relevant exposure concentrations (i.e., TLVs), the interpretation remains unaffected (e.g., no significant change in biological exposure indices for the alkyl benzenes). This kind of practically useful interpretations for chemical mixtures can be obtained with the PBPK modeling approach, as shown in the present study.

Generally, there appears to be a declining interest in the conduct of binary chemical interaction studies because of the mounting criticism of the value of such studies, particularly of the following type: what if another chemical were present additionally? The results of the present study clearly demonstrate that a quantitative answer to this question can be obtained with a PBPK modeling framework, and that binary chemical interaction data are fundamental for the construction of mechanistic PBPK models for complex mixtures. In light of these observations, we feel that research efforts toward the conduct of descriptive and mechanistic studies of binary chemical interactions should be renewed for making real advances in the areas of predictive toxicology and risk assessment of complex chemical mixtures.

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