

# Physiological Modeling and Extrapolation of Pharmacokinetic Interactions from Binary to More Complex Chemical Mixtures

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The available data on binary interactions are yet to be considered within the context of mixture risk assessment because of our inability to predict the effect of a third or a fourth chemical in the mixture on the interacting binary pairs. Physiologically based pharmacokinetic (PBPK) models represent a potentially useful framework for predicting the consequences of interactions in mixtures of increasing complexity. This article highlights the conceptual basis and validity of PBPK models for extrapolating the occurrence and magnitude of interactions from binary to more complex chemical mixtures. The methodology involves the development of PBPK models for all mixture components and interconnecting them at the level of the tissue where the interaction is occurring. Once all component models are interconnected at the binary level, the PBPK framework simulates the kinetics of all mixture components, accounting for the interactions occurring at various levels in more complex mixtures. This aspect was validated by comparing the simulations of a binary interaction-based PBPK model with experimental data on the inhalation kinetics of *m*-xylene, toluene, ethyl benzene, dichloromethane, and benzene in mixtures of varying composition and complexity. The ability to predict the kinetics of chemicals in complex mixtures by accounting for binary interactions alone within a PBPK model is a significant step toward the development of interaction-based risk assessment for chemical mixtures. **Key words:** mixtures, interactions, PBPK modeling. *Environ Health Perspect* 110(suppl 6):989–994 (2002). <http://ehpnet1.niehs.nih.gov/docs/2002/suppl-6/989-994krishnan/abstract.html>

The current mixture risk assessment methodologies do not facilitate the use of data on interaction mechanisms in a quantitative manner (1,2). The pharmacokinetic and pharmacodynamic interactions can lead to lower toxicity (antagonism) or greater toxicity (synergism, potentiation) of mixtures than that expected based on the knowledge of the potency and dose of the mixture constituents (3). The pharmacokinetic interactions lead to a change in tissue dose of chemicals during mixture exposures compared with single exposures and represent the most common type of interaction observed and reported in the literature (3,4). The extent of the change in tissue dose of chemicals resulting from pharmacokinetic interactions during mixture exposures depends on the concentrations of all components and the mechanism(s) of interactions.

The pharmacokinetic interactions, particularly metabolic interactions, between chemicals have usually been studied under *in vitro* or *in vivo* situations (3). Binary-level interactions at the pharmacokinetic level have been described using physiologically based models (5–9). Basically, these physiologically based pharmacokinetic (PBPK) models describe the organism as a set of tissue compartments interconnected by systemic circulation, and describe the chemical uptake, distribution, metabolism, and excretion with algebraic and differential equations (10). In binary mixtures, the PBPK models for the two chemicals are interconnected at the level of the tissue compartment where the interaction is

hypothesized or shown to occur (Figure 1). In the binary chemical PBPK model, two sets of identical equations are used, one for each chemical, along with an equation that specifically accounts for the interactions (e.g., competitive inhibition for metabolism in liver, induction of hepatic metabolism) (4–5). The latter equation provides the interconnection between the two chemicals circulating at the same time within the unified PBPK model. This modeling framework has been used successfully for elucidating the mechanism of interactions (5,7,8), and also for conducting extrapolations (e.g., high dose to low dose, rat to human) of the magnitude of interactions based on mechanistic considerations (6,11,12).

Despite the availability of PBPK models for conducting extrapolations of the occurrence and magnitude of interactions from laboratory studies to exposure scenarios of interest (11–13), the data on binary chemical interactions have not been used in risk assessment. The primary reason is that the data on binary interactions provide only an incomplete picture of interactions potentially occurring within a complex mixture. Further, there is uncertainty regarding the magnitude and direction of the influence of other mixture components on the interacting chemicals and the binary interaction itself. PBPK models are potentially useful tools for extrapolating the magnitude of interactions from binary to more complex mixtures, based on mechanistic considerations. This article provides an overview of the conceptual basis and validity of PBPK models for extrapolating the occurrence

and magnitude of interactions from binary to more complex chemical mixtures. The descriptions of conceptual basis and validation studies presented in this article relate to mixtures of volatile organic chemicals (VOCs) in which the components interact by competitive inhibition for hepatic metabolism. The reasoning and methodology outlined below should be applicable to other mixtures and interaction mechanisms, provided the required data are available or can be generated.

## Extrapolation of Interactions from Binary to More Complex Mixtures

**Conceptual basis.** In a binary chemical PBPK model, the rate of metabolism of each chemical is calculated using a Michaelis-Menten equation along with a modulation factor reflecting the effect of interaction (e.g., competitive inhibition) (Figure 2). Both chemicals, by competing with each other for the finite number of binding sites, mutually inhibit their metabolism (9). The resulting change in the metabolism rate of one chemical ( $RAM_1$ ) is a function of *a*) its Michaelis-Menten constants ( $V_{max_1}$ , [maximum velocity] and  $K_{m_1}$ ), *b*) its concentration at the site of metabolism ( $C_1$ ), *c*) the concentration of the competing chemical at the site of metabolism ( $C_2$ ), and *d*) the inhibition constant  $K_{i_{21}}$ , which reflects the  $C_2$  at which 50% inhibition occurs:

$$RAM_1 = \frac{V_{max_1} C_1}{C_1 + K_{m_1} \left( 1 + \frac{C_2}{K_{i_{21}}} \right)} \quad [1]$$

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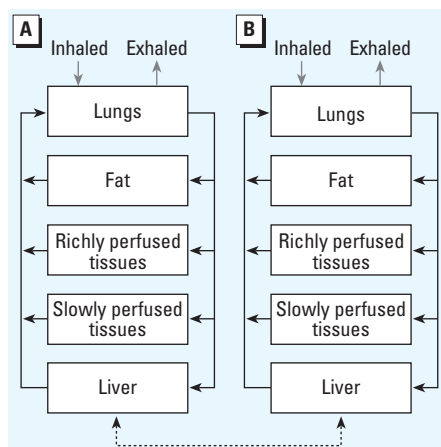
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If there is metabolic inhibition resulting in a significant decrease in RAM of a chemical during mixed exposures compared with that during individual exposures, then this will frequently lead to an increase in the blood concentration of unchanged parent chemicals. The toxicological consequence of such a change in the parent chemical concentration (i.e., increase) or metabolite production (i.e., decrease) depends on which one of these is related to the mode of action of the chemical in mixtures. Incorporating Equation 1 within PBPK models, the change in the rate of metabolism and the ensuing modulation of the tissue concentrations of chemicals in binary mixtures can be simulated. But what happens when the organism is simultaneously exposed to a third or a fourth chemical? How will the other, newer mixture constituents affect the binary interaction between the first two chemicals? The PBPK models are uniquely useful in addressing these questions.

PBPK models facilitate the extrapolation of the occurrence and magnitude of interactions from binary to more complex chemical mixtures on the basis of interaction mechanisms elucidated at binary levels. The calculation of  $RAM_1$  in the presence of several competitive inhibitors can be accomplished using Equation 1 with one modification, i.e., adding to  $C_2/K_{i21}$  in the denominator the sum of  $C_n/K_{in1}$ , representing the effect of each of the inhibitor that interacts with the substrate:

$$RAM_1 = \frac{Vmax_1 C_1}{C_1 + Km_1 \left( 1 + \frac{C_2}{K_{i21}} + \frac{C_3}{K_{i31}} + \frac{C_4}{K_{i41}} + \dots \right)} \quad [2]$$

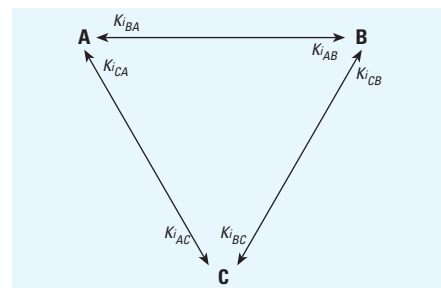


**Figure 1.** Conceptual representation of a PBPK model for a binary mixture of VOCs. Here, the models for the individual chemicals (A, B) are interconnected at the metabolic level in the liver by modifying the RAM according to the interaction mechanism involved.

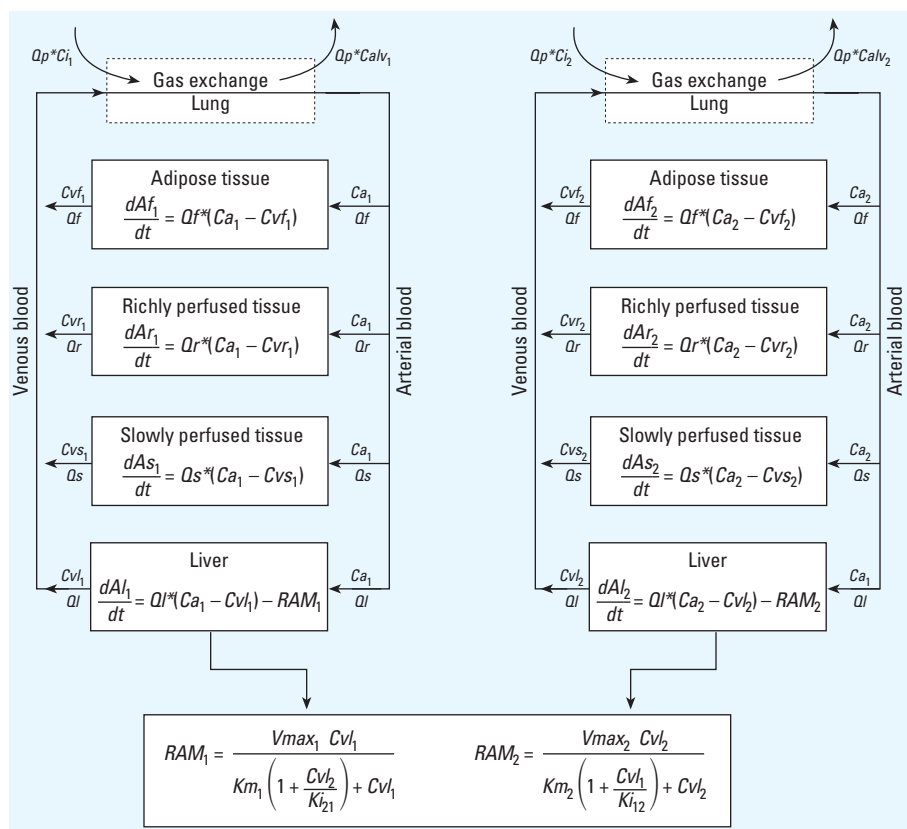
The above equation for calculating RAM in PBPK models then requires only data on binary interactions involving the substrate and each of the inhibitors to predict the consequences in more complex mixture situations. This suggests that binary interaction data can actually be used as the building blocks for mixture modeling. According to this conceptual basis, PBPK models for mixtures of any complexity can be created, as long as the quantitative information on the mechanism of interaction for each interacting pair (e.g.,  $K_i$ ) is available or can be hypothesized. In a mixture of three chemicals (A, B, C), for example, there are three sets of binary interactions (AB, BC, AC) forming an interconnected network (Figure 3). Here, the concentrations of chemical A and chemical B, along with their inhibition constants ( $K_{iAB}$ ,  $K_{iBA}$ ), determine the outcome of the binary interaction AB. With the addition of chemical C to this binary mixture, all one has to do is to estimate the binary-level interaction constants representing metabolic interactions between A and C, and B and C. Chemical C not only interacts directly with chemicals A and B but also indirectly influences the interaction between

chemicals A and B by inhibiting their metabolism (Figure 3).

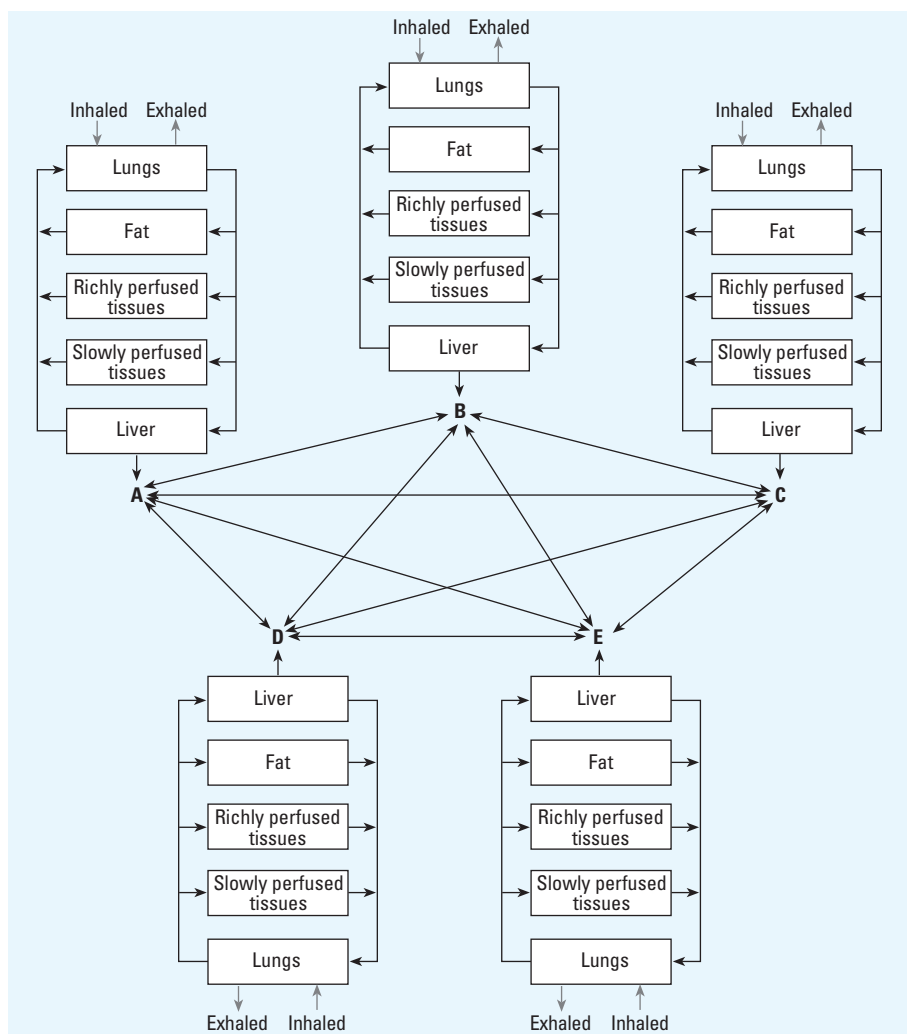
For moving from binary to more complex mixtures, the first step is to create PBPK models for each mixture component, then these single-chemical models should be interconnected at the binary level (Figure 4). If we consider competitive metabolic inhibition as the mechanism of interaction, then the equation for calculating the RAM for each mixture component should be modified appropriately (e.g., Equation 2). Assuming that a PBPK model exists for each mixture



**Figure 3.** Interactions among the components of a ternary mixture.  $K_{iXY}$  refers to the interactive effect of chemical X on chemical Y.



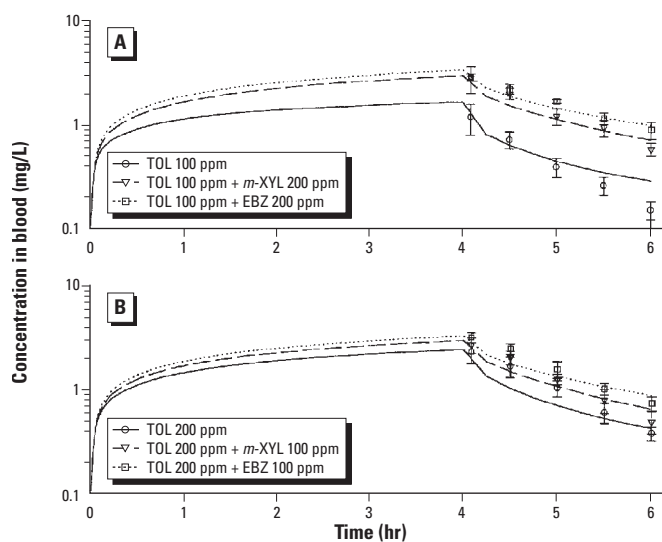
**Figure 2.** Functional representation of a PBPK model for a binary mixture of VOCs. Interactions between components 1 and 2 of this mixture occur at the level of hepatic metabolism.  $C_i$  and  $Calv_i$  refer to inhaled and alveolar air concentrations.  $Cv$  and  $Ca$  refer to venous and arterial blood concentrations.  $Cvi$  and  $Qi$  refer to venous blood concentrations leaving tissue compartments and blood flow to tissues (i.e.,  $f$ , adipose tissue;  $s$ , slowly perfused tissues;  $r$ , richly perfused tissues; and  $l$ , liver).  $K_{ij}$  is the constant describing competitive inhibition of the metabolism of chemical  $j$  by chemical  $i$ .  $Vmax$  is the maximal velocity of metabolism.  $Km$  is the Michaelis affinity constant and RAM is the rate of the amount metabolized.



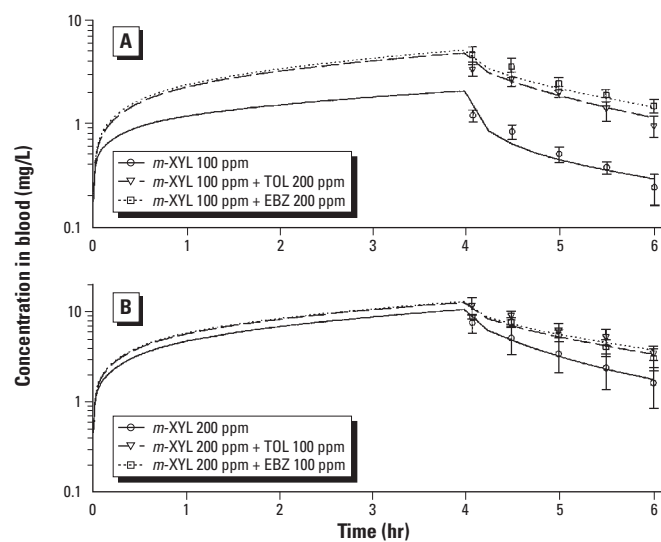
**Figure 4.** Conceptual representation of a PBPK model for a mixture of five VOCs. Here the models for the individual chemicals (A, B, C, D, E) are interconnected at the metabolic level in the liver by modifying the RAM according to the interaction mechanism involved. Note that all interconnections are at the binary level only.

component, logically the proposed modeling approach should be applicable for extrapolation to mixtures of any complexity. It is important to note that all linkages involving mixture components need be only of binary nature (Figure 4).

In the PBPK models, if we include information on interactions at the binary level alone, how is it possible to simulate the consequences of interactions in more complex mixtures? Let us assume that the binary chemical interaction between *A* and *B* has been modeled. After the addition of chemical *C*, the PBPK model simulates not only the consequence of binary interactions involving *C* (i.e., *C*-*A*, *C*-*B*) but also the modulatory effect of *C* on the interaction between *A* and *B*. Once we describe the inhibitory effect of *C* on *B*, this would result in a reduction in the rate of *B* metabolized and consequently an increase in its concentration in venous blood leaving the liver ( $Cvl_B$ ).  $Cvl_B$ , in turn, is the numerator of the term representing the inhibitory effect of *B* on *A* (i.e.,  $1 + Cvl_B/Ki_{BA}$ ). Because the exposure to chemical *C* increases  $Cvl_B$ , this then translates to a modification of the magnitude of the interactive effect of *B* on *A*. Similarly, *C* may also affect the concentration of *A*, which would influence the magnitude of the interactive effect of *A* on *B*. The PBPK model framework can also simulate similar phenomena affecting the concentration of *C*, as all components of the mixture are linked (Figure 3). Based on this analogy, it is possible to predict the influence of the addition of chemical *D* to the ternary mixture, and so forth. When a fourth chemical, *D*, is added to existing ternary mixture PBPK model of chemicals *A*, *B*, and *C*, we need to consider only three



**Figure 5.** Comparison of the PBPK model simulations of venous blood concentrations of TOL after a 4-hr inhalation exposure to 100 (A) and 200 (B) ppm TOL alone or in combination with EBZ or *m*-XYL. Reproduced from Tardif et al. (14) with permission from Academic Press.



**Figure 6.** Comparison of the PBPK model simulations of venous blood concentrations of *m*-XYL after a 4-hr inhalation exposure to 100 (A) and 200 (B) ppm *m*-XYL alone or in combination with EBZ or TOL. Reproduced from Tardif et al. (14) with permission from Academic Press.

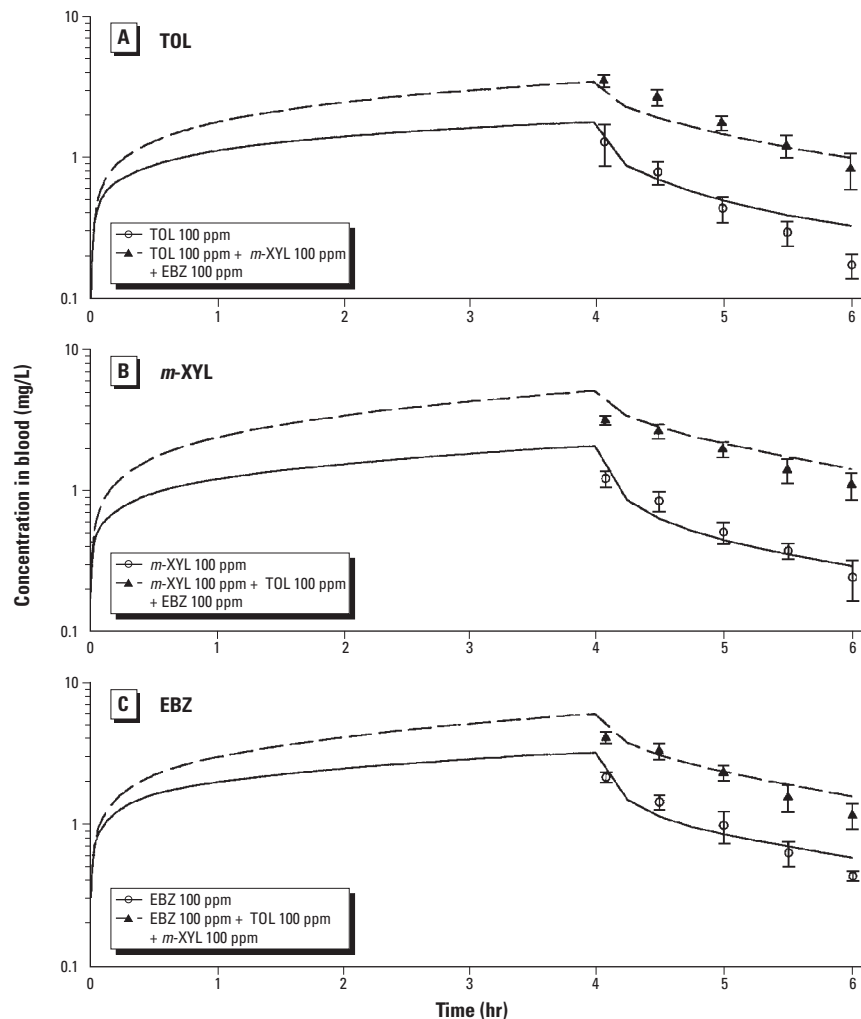
binary-level interactions additionally (i.e.,  $D-A$ ,  $D-B$ ,  $D-C$ ). By doing this, the modulatory effect of  $D$  on  $C-A$ ,  $B-A$ , and  $B-C$  interactions will be automatically simulated, as all components are linked with each other within the PBPK framework. The effect of  $D$  on the kinetics of  $A$  will in turn affect the  $A-C$  and  $A-B$  interactions. Basically, each new binary interaction will affect the kinetics of other chemicals that are part of the network of binary interactions already present in the mixture. The same considerations are applicable while another chemical,  $E$ , is added to the quaternary mixture. With the characterization of four new binary interactions, chemical  $E$  becomes an integral part of the network of the components of the mixture, and any modulation of a binary interaction involving  $E$  will have repercussions on all the others (Figure 4). The novel aspect of this approach is that it requires only data on binary interaction mechanisms for predicting the magnitude and consequences of multiple interactions within

complex mixtures. The validation of the PBPK modeling approach for extrapolation of interactions from binary to more complex mixtures has been accomplished using mixtures containing benzene (BEN), toluene (TOL), *m*-xylene (*m*-XYL), ethyl benzene (EBZ), and dichloromethane (DCM).

**Validation.** The validation of the PBPK modeling approach outlined above has been accomplished in a limited number of studies using mixtures of VOCs (12–16). The overall approach in these studies involved the characterization of the kinetics of chemicals present individually or in binary mixtures for identifying the occurrence and magnitude of binary interactions (e.g., competitive inhibition) as well as for determining the quantitative nature of the interaction mechanism (e.g.,  $K_i$ ). The models for the various individual chemicals were then interconnected at the binary level on the basis of the interaction mechanism (Figure 4). The model was then able to simulate the kinetics of each chemical

in the presence of other chemicals, based solely on binary-level interactions that are interconnected within the PBPK model.

Figure 5 compares the kinetics of TOL in rats exposed for 4 hr to 100 or 200 ppm TOL alone or in the presence of 100 or 200 ppm *m*-XYL or EBZ. Similar data obtained for *m*-XYL are presented in Figure 6. The PBPK models incorporating inhibition constants for competition of hepatic metabolism (13,14) adequately simulated the modulated kinetics of TOL and *m*-XYL in various binary mixtures (Figures 5, 6). The difference in kinetics between single and combined exposures is essentially a function of the modulated venous blood concentrations of both chemicals and the magnitude of the  $K_i$ . When rats are exposed to all three chemicals together (i.e., TOL, *m*-XYL, and EBZ at 100 ppm each), interaction effects on TOL are even greater than in the binary mixtures (Figure 5A vs Figure 7A); EBZ further increases the unchanged concentrations of TOL not only because EBZ interferes directly with the metabolism of TOL but also because it inhibits *m*-XYL metabolism, increases its unchanged concentrations, and thus enhances its inhibitory effect on TOL. Such observations can be made for *m*-XYL as well (Figure 6A vs Figure 7B). To obtain the simulations presented in Figure 7, *a*) the inhibition terms and constants for the two binary interactions EBZ-TOL and EBZ-XYL and *b*) the PBPK model for EBZ were added to the existing TOL-XYL model (13,14). For simulating the kinetics of EBZ, TOL, and XYL in the presence of other chemicals such as DCM and BEN, one had simply to estimate the binary-level interaction constants (Table 1) for the additional pairs (DCM-BEN, DCM-TOL, DCM-XYL, DCM-EBZ, BEN-TOL, BEN-XYL, and BEN-EBZ) and obtain the PBPK models for DCM and BEN for incorporation within the existing TOL-EBZ-XYL PBPK model (14–16). The resulting model simulates the kinetics of TOL, EBZ, XYL, DCM, or BEN in mixtures of varying complexities and compositions, solely on the basis of interconnected information on binary-level interactions (Figures 8, 9). In obtaining these simulations,

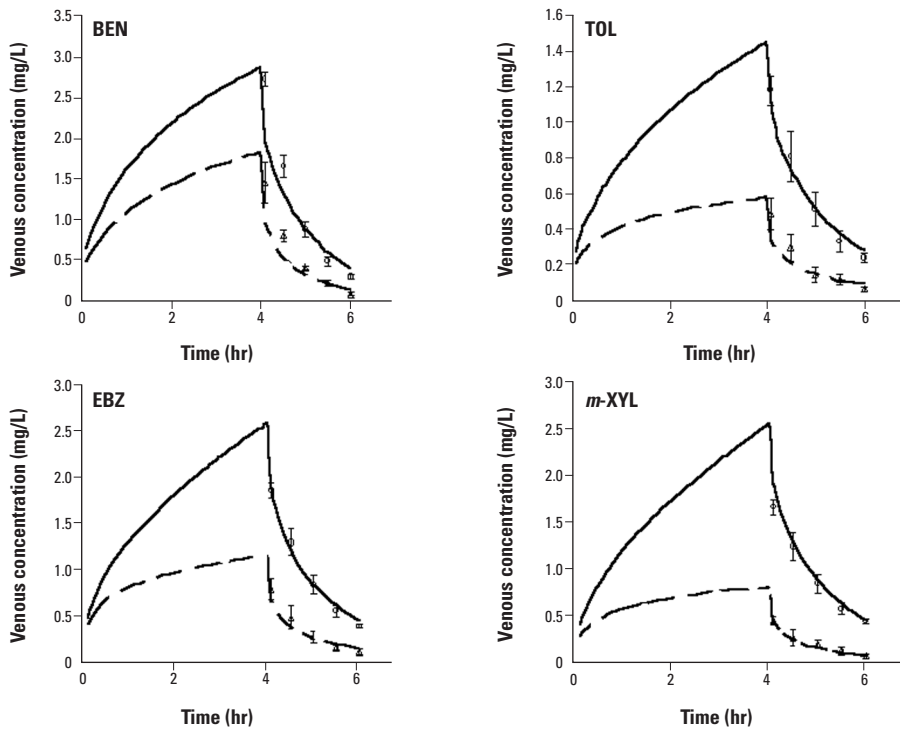


**Figure 7.** Comparison of the simulations of venous blood concentrations of (A) TOL, (B) *m*-XYL, and (C) EBZ predicted by the individual chemical model (solid lines) or a ternary chemical PBPK model (dashed lines) with corresponding experimental data (symbols) obtained in rats exposed for 4 hr to 100 ppm of each of these solvents alone or in combination. Reproduced from Tardif et al. (14) with permission from Academic Press.

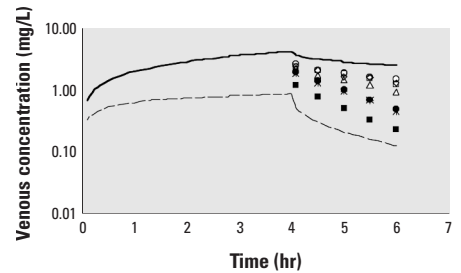
**Table 1.** Biochemical parameters for PBPK modeling of dichloromethane (D), benzene (B), toluene (T), ethyl benzene (E), and *m*-xylene (X).<sup>a</sup>

Parameter	D	B	T	E	X
$V_{max}$ (mg/hr/kg)	6.25	2.11	3.44	6.39	6.49
$K_m$ (mg/L)	0.75	0.10	0.13	1.04	0.45
$K_i$ (mg/L)					
$\alpha_D$	—	0.08	0.16	0.11	0.32
$\alpha_B$	0.30	—	0.14	0.26	0.22
$\alpha_T$	0.35	0.22	—	0.17	0.33
$\alpha_E$	0.99	0.63	0.95	—	1.67
$\alpha_X$	0.45	0.23	0.36	0.51	—

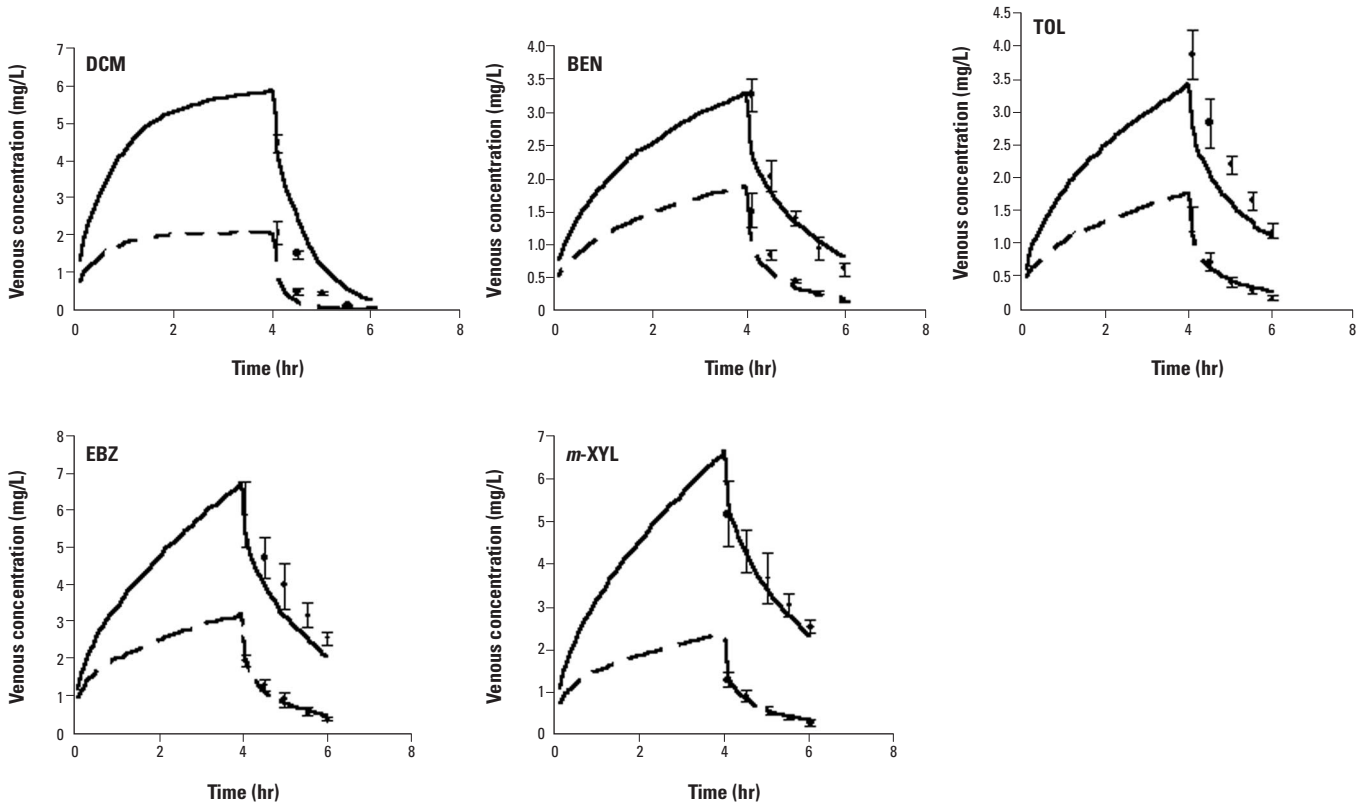
$\alpha$ , inhibitor acting on the substrate's metabolism. <sup>a</sup>Data from Haddad et al. (18).



**Figure 8.** Comparison of experimental data (symbols) with PBPK model simulations (dotted lines, single chemical model; solid lines, quaternary mixture model) of venous blood concentrations of BEN, TOL, EBZ, and *m*-XYL in rats after a 4-hr inhalation exposure. The atmospheric concentrations of the chemicals were 100 ppm each during the single and combined exposures. Reproduced from Haddad et al. (15) with permission from Academic Press.



**Figure 10.** Comparison of PBPK model simulations (lines) with experimental data on venous blood concentrations of *m*-XYL in rats after a 4-hr exposure to this chemical in the presence of DCM, BEN, trichloroethylene (TRI), TOL, tetrachloroethylene (PER), EBZ, *p*-xylene (*p*-XYL), *o*-xylene (*o*-XYL), and styrene (STY) in various combinations (*m*-XYL (50 ppm) + DCM (100 ppm) [■]; BEN + TOL + EBZ + *m*-XYL (50 ppm each) [\*]; DCM (100 ppm) + TOL (50 ppm) + EBZ (50 ppm) + *m*-XYL (50 ppm) [+]; DCM (100 ppm) + BEN (50 ppm) + TOL (50 ppm) + EBZ (50 ppm) + *m*-XYL (50 ppm) [●]; DCM + TOL + PER + EBZ + *o*-XYL + *m*-XYL + *p*-XYL + STY (50 ppm each) [◇]; DCM + TRI + TOL + EBZ + *o*-XYL + *m*-XYL + *p*-XYL + STY (50 ppm each) [□]; DCM + BEN + TRI + TOL + PER + EBZ + *o*-XYL + *m*-XYL + *p*-XYL + STY (50 ppm each + preexposure) [△]; DCM + BEN + TRI + TOL + PER + EBZ + *o*-XYL + *m*-XYL + *p*-XYL + STY (50 ppm each) [○]). The simulations were obtained either using saturable metabolism description in the single-chemical model for *m*-XYL (dotted lines) or by setting hepatic extraction value to zero in the *m*-XYL PBPK model (17).



**Figure 9.** Comparison of experimental data (symbols) with PBPK model simulations (dotted lines, single-chemical model; solid lines, five-chemical mixture model) of venous blood concentrations of DCM, BEN, TOL, EBZ, and *m*-XYL in rats after a 4-hr inhalation exposure. The atmospheric concentrations of the chemicals were 100 ppm each during the single and combined exposures. Reproduced from Haddad et al. (16) with permission from Academic Press.

no change was made to any of the equations or model parameters used to describe the kinetics of single chemicals or the binary interactions within the mixture model. By specifying only the duration and concentration of exposure to each of the mixture constituents, their kinetics was adequately simulated (Figure 8: 100 ppm TOL, XYL, BEN, and EBZ for 4 hr; Figure 9: 100 ppm TOL, XYL, BEN, EBZ, and DCM for 4 hr) (15,16).

According to this methodology, the kinetics of chemicals in increasingly complex mixtures can be modeled by connecting the PBPK models of single chemicals on the basis of the mechanisms of binary interactions. The ability of this mixture-modeling methodology to add/substitute chemicals to the mixture without changing the preestablished PBPK model structure (e.g., adding EBZ to TOL-XYL model) is noteworthy. Because the components of chemical mixtures of interest may differ qualitatively or quantitatively, it is important to have a framework such as this, which can be used as the foundation on which to build. The limitation of this modeling approach is that all existing binary interactions need to be characterized at the mechanistic level for simulating the kinetics of the components of complex mixtures. However, there is no way of predicting a priori the quantitative characteristics of these binary interactions, and therefore they must be determined following experimentation. The number of binary interactions in a mixture ( $N$ ) can be calculated as follows:

$$N = \frac{n(n-1)}{2}, \quad [3]$$

where  $n$  is the number of mixture components.

Considering the complexity of some of the mixtures to which humans are exposed, it will be tedious, if not impossible, to characterize all existing binary interactions in every mixture. In some cases, the mixture composition is ill defined such that all relevant binary interactions cannot even be identified. One way of addressing this problem, until all binary interactions are characterized, would be to estimate the theoretic limits of the modulation of blood concentrations that would arise from metabolic inhibitions. Accordingly, the hepatic extraction ratio in PBPK models of mixture components can be set to zero for predicting the maximal limit of modulation of blood kinetics of chemicals during mixed exposures (17).

Figure 10 compares the PBPK model simulations with experimental data on the blood kinetics of *m*-XYL, determined after inhalation exposure of rats for 4 hr to 50 ppm

of this chemical in increasingly complex mixtures containing up to nine other VOCs (17). Of the two simulations presented in Figure 10, the top line represents the kinetic profile when hepatic extraction ratio ( $E$ ) is equal to zero, which essentially is reflective of the upper limit of the effect of metabolic inhibition. It is obvious that regardless of the number and nature of the inhibitors present in complex mixtures, metabolism cannot be inhibited by more than 100%. As shown by the experimental data on *m*-XYL in Figure 10, with increasing number of inhibitors in a mixture, the impact on the blood kinetics is increasingly more important (i.e., blood concentrations of *m*-XYL increase with mixture complexity). But the increase in blood concentration of the parent chemical form of a substance (e.g., *m*-XYL), as shown in Figure 10, cannot exceed a theoretic maximum resulting from 100% inhibition of hepatic metabolism.

## Conclusions

The methodology reviewed in this article facilitates the addition or substitution of chemicals to existing mixture PBPK models and requires that the binary interactions between the new chemical and preexisting mixture components be characterized. This modeling approach will allow assessors to predict the magnitude of toxicokinetic interactions in mixtures of varying complexity and composition, and in each case permit the prediction of the extent of change in the tissue dose or kinetics of the toxic moiety. The use of this information, along with the data on the tissue dose versus tissue response relationship for each component, should facilitate the conduct of a toxicokinetic interaction-based risk assessment for mixtures (18,19). In cases where the data on binary interactions are lacking or impossible to acquire, a simpler approach of considering maximal inhibition may be used to simulate the theoretic upper bound of the blood concentrations of chemicals that are likely to result during mixed exposures. For risk analysis of mixtures, the maximal impact of metabolic interactions could therefore be estimated by setting  $E = 0$  (in cases of metabolic inhibition) or, alternatively, by setting  $E = 1$  (in cases of metabolic induction). This conservative approach would consider only the consequences of metabolic interaction, which is the most common interaction mechanism elucidated so far (3). Such a pragmatic approach should facilitate the screening and prioritization of chemicals and chemical interactions for further scrutiny to enhance the scientific basis of the health risk assessment of complex chemical mixtures.

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