Physiological Modeling of Toxicokinetic Interactions: Implications for Mixture Risk Assessment

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Most of the available data on chemical interactions have been obtained in animal studies conducted by administering high doses of chemicals by routes and scenarios different from anticipated human exposures. A mechanistic approach potentially useful for conducting dose, scenario, species, and route extrapolations of toxic interactions is physiological modeling. This approach involves the development of mathematical descriptions of the interrelationships among the critical determinants of toxicokinetics and toxicodynamics. The mechanistic basis of the physiological modeling approach not only enables the species, dose, route, and scenario extrapolations of the occurrence of toxicokinetic interactions but also allows the extrapolation of the occurrence of interactions from binary to multichemical mixtures. Examples are presented to show the feasibility of predicting changes in toxicokinetics of the components of complex chemical mixtures based on the incorporation of binary interaction data within physiologically based models. Interactions-based mixture risk assessment can be performed by simulating the change in the tissue dose of the toxic moiety of each mixture component during combined exposures and calculating the risk associated with each tissue dose estimate using a tissue dose versus response curve for all components. The use of such a mechanistic approach should facilitate the evaluation of the magnitude and relevance of chemical interactions in assessing the risks of low-level human exposures to complex chemical mixtures. — Environ Health Perspect 106(Suppl 6):1377–1384 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-6/1377-1384haddad/abstract.html

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Mechanistic risk assessment approaches for single chemicals are fairly well developed but for chemical mixtures they are only in the developmental stage. The default approach in mixture risk assessment assumes additivity in terms of the dose or the response. Whereas the response additivity is applied for carcinogens, the dose additivity is applied in the case of systemic toxicants acting by similar mechanisms (1,2). The additivity assumption is valid only when there is no interaction among the components of the mixture at the exposure, toxicokinetic, and toxicodynamic levels at relevant exposure concentrations in both the test animal species and humans.

Several environmental chemicals interact with each other by various mechanisms that are dependent on the dose, dosing regimen (i.e., single or repeated exposure), exposure pattern (i.e., pretreatment, coadministration, or postadministration), and/or exposure route of one or both chemicals (3). Most of the interaction studies reported to date have been conducted in laboratory animals by administering high doses of one or both chemicals by routes and scenarios often different from anticipated human exposures (4). Further, information on the toxicological consequences of low-level or chronic exposures to binary chemical mixtures, which show significant interactions when administered acutely, is often unavailable (3).

Typically, in the health risk assessment process for chemical mixtures, the available data on toxic interactions among components are not taken into account. The present situation of neglecting data on binary chemical interactions (e.g., synergism, antagonism) will not change unless and until a tool or methodology can be developed for extrapolating the results of animal studies on binary chemical interactions to humans (i.e., by accounting for the route, scenario, exposure concentration, and species differences), and predicting the potential modulation of binary chemical interactions by other chemicals present within complex mixtures.

These concerns can be addressed with the use of a physiologically based modeling approach. Physiologically based modeling refers to the process of reconstructing mathematically the anatomic-physiological characteristics of the organism of interest and describing the complex interplay among the critical determinants of toxicokinetics and toxicodynamics. The biological and mechanistic basis of the physiological modeling approach allows the conduct of various extrapolations (i.e., high dose to low dose, route to route, species to species, scenario to scenario, and binary to more complex mixtures) of the occurrence of toxicokinetic interactions among components of chemical mixtures. This article provides an overview of the conceptual basis of physiological models used for simulating toxicokinetic interactions in chemical mixtures and discusses its implications for developing mechanistic mixture risk assessment strategies.

Physiological Modeling of Toxicokinetic Interactions: Conceptual Basis

The feasibility of using physiologically based toxicokinetic (PBTK) models to describe, predict, and extrapolate the extent and magnitude of the occurrence of interactions for various dose levels, scenarios, species, routes, and mixture complexities arises from the very nature and basis of these models. PBTK models of mixtures correspond to a set of individual chemical models interconnected via the mechanism of interaction. In PBTK models for individual chemicals, the organism is frequently represented as a network of tissue compartments (e.g., liver, fat, slowly perfused tissues, and richly perfused tissues) interconnected by systemic circulation. Absorption of chemicals in the environmental medium may be via pulmonary, dermal, or oral routes. The amount absorbed per unit time can be calculated within the model; alternatively, the change
in chemical concentration in blood or the tissue representing the portal of entry may be simulated using appropriate equations (Table 1). The chemical in the arterial supply binds to blood components and/or enters the various tissue compartments where it may be dissolved in lipid and water components and be bound to tissue macromolecules. The rate of change in the amount of chemical in each tissue compartment is described with mass balance differential equations (MBDEs). In the case of metabolizing tissue these MBDEs accommodate appropriate mathematical descriptions (e.g., saturable or first-order processes). Finally, the concentration of chemical excreted in biological fluids (exhaled air, urine) may be calculated using algebraic or differential equations (5). The equations listed in Table 1 contain several parameters, i.e., critical determinants of chemical uptake and disposition. These determinants can be classified into four categories, namely, physiological, biological, physicochemical, and biochemical (Table 2). If the numerical values of any of these determinants of chemical uptake and disposition are altered during coexposure to other chemicals, then a toxicokinetic interaction is likely to result. Physiological models of toxicokinetic interactions should then account for such alterations at the mechanistic level to provide simulations of blood or tissue concentration profiles of chemicals in mixtures.

To construct a PBTK model for a binary mixture, one must initially construct two single-chemical models. The two models are then linked mechanistically at the level of a tissue or blood compartment. This is accomplished by modifying the numerical value(s) for mechanistic determinant(s) in mathematical expressions of absorption, distribution, metabolism, or excretion. Toxicokinetic interactions can then be viewed as a consequence of one chemical modifying the mechanistic determinant of the toxicokinetics of another chemical in the mixture.

The absorption profile of one chemical may be altered in the presence of another chemical as a result of interference with an active uptake process or, perhaps, of modulation of the critical biological determinants of uptake (e.g., $Q_p, Q_s$). For example, hydrogen cyanide at low exposure concentrations causes an increase in $Q_p$, thus potentially increasing the pulmonary uptake of other chemicals (6). To describe such absorption-level interactions in a PBTK model, chemical-specific quantitative information on the dose dependency of these effects (e.g., $Q_p$, $Q_s$ vs exposure concentration) is required.

The distribution profile of a chemical can be altered by another chemical if it affects certain critical physicochemical, physiological, or biochemical parameters. Some chemicals may alter the solubility characteristics of another chemical. For example, cyanide forms complexes with essential metals, resulting in a change in their tissue concentrations and distribution pattern due to changes in solubility and stability (7–12). Several diethiocarbamates increase the uptake of lead through the blood–brain barrier by forming lipophilic complexes (13). The distribution phase interactions resulting from changes in physiologic parameters such as liver volume ($V_l$) or hepatic blood flow rate ($Q_b$) are invoked by phenobarbital and ethanol. Chemicals in mixture or their metabolites may compete for binding to various macromolecules such as hemoglobin, albumin, metallothionein, or tissue receptors. Competition for plasma protein binding has been modeled by accounting for the number of binding sites, total protein level, dissociation constants, and the concentration of the unbound form of both the substrate and the inhibitor (14). Some chemicals can also increase the number of binding sites through an induction process (e.g., induction of microsomal binding proteins and cytosolic proteins by 2,3,7,8-tetrachlorodibenzo-p-dioxin (15)).

**Metabolic interactions may occur because of one chemical inducing or inhibiting the metabolism of another chemical in the mixture.** Metabolic inhibition occurs when a chemical competes directly with another chemical for an enzymatic binding site (competitive inhibition).

| Pharmacokinetic processes | Equations |
|---------------------------|-----------|
| **Uptake**                |           |
| Pulmonary                 | $C_a = \frac{Q_p C_i + Q_s C_s}{Q_p + (Q_p / P_s)}$ |
| Dermal                    | $\frac{dA_d}{dt} = K_s S(C_{air} - (C_{air} / P_{air})) + Q_s (C_a - (C_{air} / P_{air}))$ |
| Oral                      | $\frac{dA_o}{dt} = K_s D_s e^{-K_w t}$ |
| **Distribution**          |           |
| Protein binding           | $C_{bd} = \frac{n\beta K \cdot C_{bd}}{(1 + \frac{K_s C_p}{C_{bd}})}$ |
| Tissue distribution       | $\frac{dA_t}{dt} = Q_t (C_a - C_{tissue})$ |
| **Clearance**             |           |
| First-order               | $\frac{dA_{clear}}{dt} = Q_c \cdot E \cdot C_a$ |
| Saturable process         | $\frac{dA_{saturable}}{dt} = \frac{V_m \cdot C_a}{K_m + C_a}$ |

*Adapted from Krishnan and Andersen (5). Abbreviations: $A$, amount; $C$, concentration; $D$, dose; $E$, hepatic extraction ratio; $K_w$, rate of oral absorption; $P$, partition coefficient; $PA$, permeation across tissue membrane; $Q$, blood flow rate; $S$, skin surface; $V$, volume. Subscript abbreviations: $a$, arterial blood; $bd$, bound; $fr$, free; $i$, inhaled; $l$, liver; $met$, metabolized; $o$, oral; $s$, skin; $t$, tissue; $v$, venous blood; $vt$, venous blood leaving tissue. All other symbols are defined in Table 2.

**Table 2. Partial list of mechanistic determinants that may be modified during toxicokinetic interactions.**

| Parameters | Mechanistic determinants |
|-----------|--------------------------|
| Physiological | Cardiac output ($Q_c$) |
| Breathing rate ($Q_i$) |
| Glomerular filtration rate (GFR) |
| Biological | Tissue and blood volumes ($V_i, V_j$) |
| Concentration of metabolizing enzymes ($E_i$) |
| Concentrations of binding proteins ($P_j$) |
| Number of binding sites ($n$) |
| Physico-chemical | Blood:air partition coefficient ($P_{ba}$) |
| Tissue blood partition coefficient ($P_{vb}$) |
| Oral absorption rate constant ($K_0$) |
| Dermal permeability coefficient ($K_p$) |
| Biochemical | Maximal velocity for metabolism ($V_{max}$) |
| Binding association constant ($K_0$) |
| Michaelis affinity constant ($K_m$) |
when a chemical binds directly to the enzyme–substrate complex but not to the free enzyme (uncompetitive), or when it does both of these (noncompetitive). The inhibitory effect of one chemical on another is modeled by including a term that describes the quantitative manner in which \( K_m \) and \( V_{max} \) are modified (Table 3).

Once the mechanism of interaction is hypothesized or determined and the two individual chemical PBTK models are interconnected, the integrated binary chemical mixture model is ready to be used for predicting the consequences of toxicokinetic interactions at various dose levels, exposure routes, species, and scenarios. This kind of modeling exercise can help determine the relative importance of an interaction for risk assessment purposes. Of the several interaction PBTK models published so far [reviewed by Simmons (16)], most of them have only been used to evaluate the mechanistic basis of toxicokinetic interactions. Only a few studies have demonstrated the use of PBTK models for conducting extrapolations of the occurrence and magnitude of toxicokinetic interactions (17–23).

### High-Dose to Low-Dose Extrapolations

High-dose to low-dose extrapolations of toxicokinetic interactions can be accomplished by PBTK modeling because the mathematical descriptions used in PBTK models account for the nonlinear kinetic behavior of individual chemicals and the mixture effects. The toxicokinetic nonlinearity is often related to a change in the numerical values of biochemical, physiological or physicochemical determinants that is not strictly proportional to the change in dose or exposure concentration. The ability to conduct high-dose to low-dose extrapolation of toxicokinetic interactions using PBTK models may be examined with metabolic induction/inhibition as the mechanism. In such cases, the binary toxicokinetic interaction model accounts for the nonlinearity arising from two phenomena: the saturable nature of the metabolism of individual chemicals and the relative importance of the metabolic interaction mechanism as a function of substrate concentration. The saturation of metabolism at a high exposure concentration of a chemical leads to a situation that is characterized by the absence of significant inhibitory interaction effects on the hepatic extraction ratio at such concentrations; however, the interactive effect becomes more evident at subsaturation concentrations. In the case of enzyme induction, the effect is more pronounced at high exposure concentrations (i.e., at which metabolism is capacity limited) than at low exposure concentrations (i.e., at which metabolism is perfusion limited). The relative importance and thus the influence of metabolic interactions will depend on the substrate concentration, particularly the range in which saturation occurs. The saturable nature of the metabolism of the inhibitor chemical will also lead to nonlinearity in terms of its effects at a constant exposure concentration of a substrate. Because the saturable nature of the metabolism of mixture components and the magnitude and mechanism of the interactive effects are incorporated within the PBTK models, these models are useful for conducting high-dose to low-dose extrapolations of the consequence of toxicokinetic interactions. This particular application of PBTK models has been explored in the context of determining the relative importance of interaction data for health risk assessments and for establishing interaction thresholds (19–23).

### Route-to-Route Extrapolations

The tissue or blood concentration versus time-course profiles of chemicals may be different depending on the route of exposure (e.g., intravenous, oral, inhalation, dermal). Therefore, the time course of the effective concentration of a chemical at the interaction site may differ according to route of exposure, thus leading to a route-dependent magnitude and profile of the interaction effect. The route-to-route difference in bioavailability and toxicokinetics can be accounted for by using appropriate mathematical descriptions of absorption (Table 1). Route-to-route extrapolations of toxicokinetics using PBTK models have been successfully performed for several individual chemicals. The approach involves shutting off one pathway and introducing the chemical via another route (5). Because equations for absorption via each of the multiple routes are included in the PBTK model for each of the mixture components, all one does is specify chemical concentration in the respective exposure media. In the case of a mixture PBTK model, the same or different routes for the components can be chosen. For example, both chemicals may be administered orally or one by oral ingestion and another by inhalation. Such an approach has been used by Pelekis and Krishnan (23) to conduct route-to-route extrapolation of the occurrence of metabolic interactions between toluene (TOL) and dichloromethane (DCM). The PBTK modeling methodology, in effect, should permit the simulation of the extent and consequence of toxicokinetic interactions following combined multiroute exposures to chemicals.

### Interspecies Extrapolations

The usefulness of PBTK models in the conduct of interspecies extrapolations of single chemicals is well documented (5, 24). However, there have been only limited efforts so far to demonstrate the usefulness of rodent PBTK models in predicting the toxicokinetics of chemical mixture in humans. The procedure involves substituting the numerical values of physiological, physicochemical, and biochemical parameters, including the interaction parameters, with those for the species of interest. This must be done for each component model of the mixture PBTK model. The rat-to-human extrapolation of toxicokinetic interactions using the PBTK modeling approach has been validated for a binary mixture (TOL/m-xylene [XYL]) and a ternary mixture (TOL/XYL/ethylbenzene [EBZ]) (20, 25). In these studies, the values of the interaction parameter (i.e., \( K_i \) for competitive inhibition mechanism) determined in animal studies were kept unchanged in the human PBTK models. Treating the metabolic inhibition constants as species invariant implies that the nature and magnitude of the competition between the alkyl benzenes for binding to cytochrome P4502E1 for metabolism does not change between species. This assumption can be accepted as the default because the substrates (TOL, XYL, EBZ) and the isoenzyme form (2E1) involved are the same despite the fact that the species being considered are different (rat vs human). Although the inhibition potency of a

### Table 3. Mathematical representation of the modifications of maximal velocity for metabolism (\( V_{max}^\text{int} \)) and Michaels affinity constant (\( K_m^\text{int} \)) during metabolic inhibition.

| Mechanism of inhibition | Modification of \( K_m \)  | Modification of \( V_{max} \)  |
|-------------------------|-----------------------------|-----------------------------|
| Competitive             | \( \alpha \times K_m \)     | None                        |
| Uncompetitive           | \( K_m/\alpha \)             | \( V_{max}/\alpha \)        |
| Noncompetitive          | None                        | \( V_{max}/\alpha \)        |

\( \alpha = \left( 1 + \frac{C_{in}}{K_i} \right) \) where \( C_{in} \) represents the liver venous concentration of inhibitor and \( K_i \) represents the inhibition constant.

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*Environmental Health Perspectives • Vol 106, Supplement 6 • December 1998*
chemical is assumed to be the same regardless of the species, it is obvious that the hepatic venous concentration of the chemicals might vary from one species to another, thus accounting for species differences in the overall inhibition effects, if any. Such an approach has recently been used by Pelekis and Krishnan (23) to evaluate the relevance for humans of a toxicokinetic interaction between TOL and DCM characterized in rats. Application of PBTK models along these lines would facilitate a priori characterization of the threshold of interactions in humans and identification of those interactions that may be of concern to humans exposed to low concentrations.

**Scenario-to-Scenario Extrapolations**

Scenario extrapolations to assess the potentially varying nature and magnitude of toxicokinetic interactions are essential because changing the exposure scenarios may involve different mechanisms or alter the kinetic profiles of chemicals. If the change in exposure duration is the sole factor of concern all one must do is adjust the length of exposure (TCHNG) in the PBTK model. Accordingly, the length of time during which a particular tissue concentration is maintained might vary according to the scenario of exposure. This may have direct influence on the extent of interactive effects seen, as the effective concentration of the inhibitor is an important determinant of the outcome of interactions. The application of PBTK models for simulating the kinetics of various chemicals as a function of exposure scenarios is well documented (26–28). The same approach can be used to describe the change in exposure scenarios in the individual chemical models of a mixture PBTK model. In some cases mathematical descriptions to account for time-dependent processes such as induction of a metabolizing or binding protein may have to be additionally incorporated (15,29). The applicability of PBTK models for simulating the kinetics of chemical mixtures in humans following changes in exposure duration and concentration has been demonstrated by Tardif et al. (20).

The inclusion of the information on toxic interactions between two chemicals within the regular risk assessment process (even if they are for a relevant exposure scenario, species, route, and dose level) has been hampered by the fact that such data do not represent the complete picture (30). Chemicals do not just coexist in two but in multiple numbers, and as such the toxicity of two interacting chemicals might further be altered by the other components of the mixture. The toxicity of complex mixtures is determined by the outcome of interactions not only at the binary level but also at other, higher levels (e.g., ternary, quaternary). There has been a lack of useful tools essential for predicting higher order interactions within complex mixtures. The potentially useful tools in this context are statistical, empirical, and mechanistic models. Statistical and empirical models are constructed on the basis of available data and therefore cannot be used for extrapolations. On the other hand, the mechanistic PBTK models are constructed on the basis of the quantitative interrelationships among the critical determinants of the process of interest and therefore are potentially useful in this context.

**Extrapolation from Binary to Higher Order Levels**

One of the approaches for modeling toxicokinetic interactions in mixtures involves identifying and linking all individual chemical models via interaction terms. PBTK models for mixtures of any level of complexity can then be created as long as the quantitative information on the mechanism for each interacting chemical pair is available or can be hypothesized. According to this methodology, for modeling the kinetics of the components of complex mixtures, plausible binary interactions need only be characterized. In a mixture of three chemicals, for example, there are three two-way interactions (Figure 1). The first step is to write the models for each component of the mixture. Then the single-chemical models should be interconnected at the binary level by modifying the appropriate equations. If we consider competitive metabolic inhibition as the mechanism of interaction, the equation for calculating the rate of the amount metabolized (RAM) of each component should be modified appropriately (Table 4). Logically, this PBTK modeling approach should be applicable to predict higher order interactions in mixtures of any complexity, invoking various kinds of mechanisms of interactions among components. It is important to note that all linkages involving mixture components are of binary nature only (Table 4).

If the model considers interactions at the binary level alone, how is it possible to simulate the consequence of a higher order interaction (e.g., involving three chemicals)? This is where the unique usefulness of PBTK modeling becomes evident. Let’s assume that the binary chemical interaction between A and B has been modeled. Following the addition of another chemical, C, the PBTK model not only simulates the binary interactions involving C (i.e., A–C, 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 (C_{out}). C_{out} is the numerator of the term representing the inhibitory effect of B on A (i.e., 1 + C_{out}/K_{BA}) (Table 4). Because the exposure to chemical C increases C_{out}, this translates into a modification of the magnitude of the interactive effect of B on A. Similarly, C may also affect the concentration of A, which would then result in a change in the magnitude of the interactive effect of A on B. The PBTK model framework can also simulate similar phenomena affecting the concentration of C because all components of the mixture are linked. Based on this analogy it will be possible to predict the influence of the addition of another chemical, D, to the ternary mixture, and so forth (Table 4). When D is added to an existing ternary mixture PBTK model for chemicals A, B, and C, we need to consider only three additional binary interactions (Figure 2, small dashed lines). By doing this, the modulating effect of D on the C–A and B–A interactions will be automatically simulated because all components are linked with each other within the PBTK framework. The effect of D on the kinetics of A will in turn affect the kinetics

![Figure 1. Interactions among the components of a ternary mixture. K_{XY} refers to the interactive effect of chemical X on chemical Y.](image-url)
Table 4. Mathematical description of the rate of amount metabolized of a chemical present alone or with one or more chemicals that compete for the same but saturable enzyme catalytic sites.

| Chemicals in mixture, no. | Chemical | RAM equation |
|---------------------------|---------|--------------|
| 1                         | A       | \[
\frac{V_{\text{max}} A \cdot C_{\text{idA}}}{K_{mA} + C_{\text{idA}}} \]
|                           | B       | \[
\frac{V_{\text{max}} B \cdot C_{\text{idB}}}{K_{mB} + C_{\text{idB}}} \]
| 2                         | A       | \[
\frac{V_{\text{max}} A \cdot C_{\text{idA}}}{K_{mA} \left(1 + \frac{C_{\text{idB}}}{K_{iBA}}\right) + C_{\text{idA}}} \]
|                           | B       | \[
\frac{V_{\text{max}} B \cdot C_{\text{idB}}}{K_{mB} \left(1 + \frac{C_{\text{idC}}}{K_{iCB}}\right) + C_{\text{idB}}} \]
|                           | C       | \[
\frac{V_{\text{max}} C \cdot C_{\text{idC}}}{K_{mC} \left(1 + \frac{C_{\text{idD}}}{K_{iCD}}\right) + C_{\text{idC}}} \]
| 3                         | A       | \[
\frac{V_{\text{max}} A \cdot C_{\text{idA}}}{K_{mA} \left(1 + \frac{C_{\text{idB}} + C_{\text{idC}}}{K_{iBA}}\right) + C_{\text{idA}}} \]
|                           | B       | \[
\frac{V_{\text{max}} B \cdot C_{\text{idB}}}{K_{mB} \left(1 + \frac{C_{\text{idC}}}{K_{iCB}}\right) + C_{\text{idB}}} \]
|                           | C       | \[
\frac{V_{\text{max}} C \cdot C_{\text{idC}}}{K_{mC} \left(1 + \frac{C_{\text{idD}}}{K_{iCD}}\right) + C_{\text{idC}}} \]
|                           | D       | \[
\frac{V_{\text{max}} D \cdot C_{\text{idD}}}{K_{mD} \left(1 + \frac{C_{\text{idA}}}{K_{iDA}}\right) + C_{\text{idD}}} \]

A+-----I,----

Figure 2. Interactions among the components of a five-chemical mixture.

Table 4. Mathematical description of the rate of amount metabolized of a chemical present alone or with one or more chemicals that compete for the same but saturable enzyme catalytic sites.

| Chemicals in mixture, no. | Chemical | RAM equation |
|---------------------------|---------|--------------|
| 1                         | A       | \[
\frac{V_{\text{max}} A \cdot C_{\text{idA}}}{K_{mA} + C_{\text{idA}}} \]
|                           | B       | \[
\frac{V_{\text{max}} B \cdot C_{\text{idB}}}{K_{mB} + C_{\text{idB}}} \]
| 2                         | A       | \[
\frac{V_{\text{max}} A \cdot C_{\text{idA}}}{K_{mA} \left(1 + \frac{C_{\text{idB}}}{K_{iBA}}\right) + C_{\text{idA}}} \]
|                           | B       | \[
\frac{V_{\text{max}} B \cdot C_{\text{idB}}}{K_{mB} \left(1 + \frac{C_{\text{idC}}}{K_{iCB}}\right) + C_{\text{idB}}} \]
|                           | C       | \[
\frac{V_{\text{max}} C \cdot C_{\text{idC}}}{K_{mC} \left(1 + \frac{C_{\text{idD}}}{K_{iCD}}\right) + C_{\text{idC}}} \]
| 3                         | A       | \[
\frac{V_{\text{max}} A \cdot C_{\text{idA}}}{K_{mA} \left(1 + \frac{C_{\text{idB}} + C_{\text{idC}}}{K_{iBA}}\right) + C_{\text{idA}}} \]
|                           | B       | \[
\frac{V_{\text{max}} B \cdot C_{\text{idB}}}{K_{mB} \left(1 + \frac{C_{\text{idC}}}{K_{iCB}}\right) + C_{\text{idB}}} \]
|                           | C       | \[
\frac{V_{\text{max}} C \cdot C_{\text{idC}}}{K_{mC} \left(1 + \frac{C_{\text{idD}}}{K_{iCD}}\right) + C_{\text{idC}}} \]
|                           | D       | \[
\frac{V_{\text{max}} D \cdot C_{\text{idD}}}{K_{mD} \left(1 + \frac{C_{\text{idA}}}{K_{iDA}}\right) + C_{\text{idD}}} \]

A+-----I,----

Figure 2. Interactions among the components of a five-chemical mixture.

of B, C, and D. Any modulation of a binary interaction will affect the kinetics of other chemicals that are part of the network of binary interactions present in the mixture. The same considerations are applicable when another chemical, E, is added to the quaternary mixture. After adding the four new binary interactions (E–A, E–B, E–C, and E–D), chemical E will become 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 2).

The novel thing about this approach is that it only requires data on binary interaction mechanisms for predicting the magnitude and consequence of higher order interactions within complex mixtures.

Tardif et al. (25) validated this approach for predicting the kinetics of components of a ternary mixture of alkyl benzenes. These authors hypothesized, based on the possibility that alkyl benzenes are substrates of cytochrome P4502E1, that they are likely to compete for the enzyme catalytic site. Initially, individual chemical PBTK models were constructed in the rat. All individual chemical models were then interconnected by inserting a binary interaction term for metabolic inhibition (i.e., alternate descriptions of competitive, uncompetitive, and noncompetitive inhibitions) in the RAM equations. Once all individual chemical PBTK models were linked by binary interaction terms, the metabolic inhibition constants for each pair of mixture components were obtained by fitting model simulations to experimentally measured venous blood concentrations in rats exposed to mixtures. The Kᵢ values were determined for all three types of inhibition, and competitive inhibition appeared to be the most plausible mechanism of metabolic interaction (25).

With the inclusion of the Kᵢ values for all binary interactions, the mixture PBTK model was used to simulate the kinetics of each component in rats following a 4-hr inhalation exposure to a mixture of 100 ppm each of TOL, XYL, and EBZ. For all three chemicals, the venous blood concentration kinetics simulated by the binary interactions-based mixture PBTK model compared well with experimental data (25). This approach has also been used to predict the carboxyhemoglobinemia resulting from DCM exposure in the presence of aromatic hydrocarbon solvents, i.e., TOL, XYL, and EBZ (19). Based on the results of this simulation study, Krishnan and Pelekis (19) reported that the threshold for DCM–TOL interaction diminished with increasing number of inhibitor chemicals. This observation can be explained with the change in Cᵢ that occurs during multi-chemical interactions at the metabolism site. In the study by Krishnan and Pelekis (19), for example, connecting the PBTK model for TOL to the existing DCM model based on competitive inhibition mechanism increases the liver venous blood concentration of DCM. The addition of a

Environmental Health Perspectives • Vol 106, Supplement 6 • December 1998
third chemical, XYL, to the binary mixture affects the magnitude of the existing binary interaction of DCM–TOL by increasing the $C_{id}$ of TOL and that of DCM (Figure 3). Similarly, the addition of EBZ to the ternary mixture affects the kinetics of all three solvents. The magnitude of the modulation of interactions invoked on the addition of another chemical to an existing network of binary interactions depends on its inhibition potency and also on its $C_{id}$. With increasing complexity of mixtures, the $K_i$ for binary interactions is not modified; rather, the $C_{id}$ is increased according to the potency and number of the inhibitors. The increasing effective concentration of chemicals in a mixture is due to a cascade of inhibitory events at the binary level. This is why we tend to see a more marked inhibitory effect on the metabolism of a substrate at a specific exposure concentration with increasing number of inhibitors.

Though the previous discussion and examples published to date address competitive inhibition as the sole mechanism of interactions, this conceptual modeling approach is applicable for situations involving various other kinds of interaction mechanisms. For example, these can be at the level of absorption (e.g., alteration of ventilation rate, skin permeability coefficient), distribution (e.g., competition for protein binding sites, alteration of the concentrations of binding proteins, alteration of blood flow), and metabolism (e.g., enzyme induction). The methodological approach reviewed in the preceding paragraphs provides a systematic framework for modeling and dealing with interactions occurring in increasingly complex mixtures. Every time a new chemical is added to an existing mixture, one must simply describe the additional binary interactions within the PBTK model framework. This alone would appear to be sufficient for predicting changes in tissue dosimetry due to the higher order interactions occurring in complex chemical mixtures.

### Physiological Modeling of Toxicokinetic Interactions: Implications for Mixture Risk Assessment

The extrapolations issues (i.e., high dose to low dose, route to route, interspecies, scenario to scenario, binary to multicomponent mixtures) relating to the consideration of interaction data in the risk assessment process can be addressed with the use of PBTK models. However, the exact nature in which the PBTK model-based extrapolations can be used for quantitative mixture risk assessment must be clarified. The mixture PBTK models simulate the altered tissue dose of chemicals during mixed exposures. Therefore, the model simulations of change in tissue dose of mixture components can be used along with tissue dose–response curves available for each of the mixture components to assess the risk attributed to each of the mixture components. It may be useful to briefly review the tissue dose-based quantitative risk assessment approach for individual chemicals (31). The following steps represent the methodological aspects involved in the conduct of a tissue dosimetry-based assessment of cancer risk assessment of single chemicals:

1. Determining the quantitative relationship between target tissue dose of the toxic moiety and exposure concentration of the parent chemical in the test animal (using rodent PBTK model);
2. Determining the relationship (e.g., with linearized multistage model) between the target tissue dose obtained in step 1 and the tissue responses seen in animal toxicology studies (e.g., cancer bioassays);
3. Using the relationship in step 2 to determine the tissue dose that corresponds to a given level of risk, one case of excess cancer per million individuals exposed over lifetime; and
4. Using a human PBTK model to estimate the exposure concentration of the chemical that provides tissue dose equivalent to that estimated in step 3. This will then be the environmental concentration of the chemical that corresponds to a predefined level of acceptable risk (i.e., one in a million in the present example).

This approach improves the conventional dose–response relationship by enabling the examination of the relationship between the exposure concentration and tissue dose and tissue dose and tissue response. In the context of complex chemical mixtures, the change in response, i.e., infra-additive or supra-additive toxicities, can be viewed as a consequence of either a change in the target tissue dose of the toxic moiety per unit exposure concentration or a change in the tissue response to unit tissue dose during combined exposures. The former is a consequence of toxicokinetic interactions whereas the latter is the consequence of toxicodynamic interactions. In this paper the examples and methodology discussed are limited to the consideration of toxicokinetic interactions.

Accordingly, toxicological interactions (i.e., increase or decrease in toxicity) result because of an increase or decrease in the target tissue dose per unit exposure concentration of a chemical in complex mixtures. For example, a chemical at exposure concentration of 10 ppm (arbitrary) produces A units of internal dose in humans. The corresponding risk (carcinogenic or non-carcinogenic) can be assessed by using the tissue dose versus response curve (Figure 4). On the other hand, during combined exposure with, for example, 10 other chemicals at 1 ppm (arbitrary), the tissue dose of the toxic moiety of the first chemical may no longer be A; it might be greater than A, i.e., S. Consequently, the risk associated with 10
Appendix: Notation

\( \alpha \) interaction factor  
\( \beta \) concentration of binding proteins  
\( A_{\text{met}} \) amount metabolized  
\( A_o \) amount orally absorbed  
\( A_s \) amount in skin  
\( C_a \) arterial blood concentration  
\( C_{\text{air}} \) air concentration  
\( C_{\text{free}} \) unbound chemical concentration  
\( C_i \) inhaled concentration  
\( C_k \) skin concentration  
\( C_T \) tissue concentration  
\( C_r \) venous concentration  
\( C_{\text{vle}} \) venous concentration leaving liver  
\( C_{\text{vli}} \) concentration of an inhibitor in venous blood leaving liver  
\( C_{\text{vlt}} \) concentration in venous blood leaving tissue  
\( D_r \) oral dose  
\( D_T \) hepatic extraction ratio  
\( E_B \) ethylbenzene  
\( E_{\text{GFR}} \) metabolizing enzyme concentration  
\( G_F \) glomerular filtration rate  
\( K_a \) association constant for protein binding  
\( K_{\text{XY}} \) constant reflecting the inhibitory effect of chemical \( X \) on \( Y \)  
\( K_a \) Michaelis–Menten affinity constant  
\( K_r \) oral absorption rate constant  
\( K_s \) dermal permeability coefficient  
\( \text{MBDE} \) mass balance differential equations  
\( n \) number of binding sites per proteins  
\( P_a \) blood:air partition coefficient  
\( P_{\text{PBTK}} \) physiologically based toxicokinetic model  
\( \text{ppm} \) parts per million  
\( P_{\text{air}} \) skin:air partition coefficient  
\( P_{k,\text{PBTK}} \) skin:blood partition coefficient  
\( P_t \) tissue:blood partition coefficient  
\( Q_c \) cardiac output  
\( Q_l \) liver blood flow  
\( Q_n \) alveolar ventilation  
\( Q_t \) tissue blood flow rate  
\( R_{\text{AM}} \) rate of amount metabolized  
\( S \) exposed skin surface area  
\( T_{\text{CHNG}} \) length of exposure  
\( \text{TOL} \) toluene  
\( V_b \) blood volume  
\( V_l \) liver volume  
\( V_m \) Maximal velocity for metabolism  
\( V_t \) tissue volume  
\( \text{XYL} \) \( m \)-xylene
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