CHAPTER 13

MIXTURES

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13.1 INTRODUCTION

Human exposure to chemicals is rarely, if ever, confined to a single compound. Therefore, the study of chemical mixture toxicology has gained a great deal of momentum in the last two decades. Studying chemical mixtures is an extremely complex task because of the astronomical number of possible combinations. Such numbers certainly preclude any systematic experimental assessment of toxicology of all potentially troublesome chemical mixtures. In the past 15 years or so, physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling has been applied to the toxicological interactions of chemical mixtures. As is generally the case in the evolution of a new area, the progress in the application of PBPK modeling to chemical mixtures has followed different phases from simple binary pharmacokinetic and pharmacodynamic interactions to more and more complex mixtures. First, PBPK modeling of binary chemical mixtures became necessary because of pharmacological or toxicological interactions. Second, as investigators became interested in mechanisms of toxicological interactions, the advances of physiologically based pharmacodynamic (PBPD) modeling formed a natural course of development of this area. Third, when more and more sophistication was incorporated into PBPK modeling, it is inevitable that PBPK modeling of complex chemical mixtures were attempted and novel approaches developed. Since 1996 when the Food Quality and Protection Act was enacted, the United States Environmental Protection Agency (US EPA), under the Congressional mandate, began active

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consideration of cumulative risk assessment. Out of necessity, toxicological interactions must be taken into consideration. Thus, the application of PBPK modeling in cumulative risk assessment has become an active area of research endeavor (US EPA 2003).

13.2 PBPK MODELING OF CHEMICAL MIXTURES

13.2.1 Earlier Days: PBPK Modeling of Binary Mixtures

Although numerous drug interaction studies have been reported in the scientific literature in past decades (Mozayani and Raymon 2004), the earliest applications of PBPK modeling to chemical mixtures did not occur until the mid-1980s. Because of the necessity for extrapolation from animal experimentation to humans for many toxicants, the advances of PBPK modeling in the area of toxicology has far outpaced its application in pharmacology. An earlier review of PBPK modeling of chemical mixtures (Mumtaz *et al.* 1993) indicated that the "first example" of PBPK modeling of a "chemical mixture" actually involved one chemical, *n*-hexane, and its metabolites, methyl *n*-butyl ketone (MnBK) and 2,5-hexanedione (2,5-HD); thus, it is a kind of "one-chemical mixture." This particular PBPK model for *n*-hexane and its metabolites incorporated three inhibitory interactions: (1) hexane and MnBK are competitive substrates for ω-1 oxidation; (2) MnBK and 2,5-HD are competitive substrates for α oxidation; and (3) 2,5-HD acts as a product feedback inhibitor (Andersen and Clewell 1984). The findings of this modeling study were intriguing and they explained some of the most interesting and complex toxicological and pharmacokinetic behaviors of *n*-hexane in animals (Mumtaz *et al.* 1993). However, this PBPK modeling work was never published in a peer-reviewed journal; the principal reason was that the investigators involved were never happy enough with the PBPK modeling results. Interestingly, after about 20 years, the *n*-hexane PBPK modeling work was revisited (Dennison 2004).

Logically, some of the earliest investigations on PBPK modeling were on binary mixtures. Given below and in Table 13.1 are specific examples on PBPK modeling of binary mixtures following a chronological order. Some aspects of PBPK modeling work of higher order of chemical mixtures are given in later sections.

Specific Example 1: Dibromomethane and Isoflurane (Clewell and Andersen 1985) One of the reasons for the delay in the completion of *n*-hexane work was the difficulties encountered during the experimental and PBPK modeling processes. In those earlier days, as an alternative to mimic the interaction between *n*-hexane and its metabolite, MnBK, Clewell and Andersen (1985) studied, using PBPK modeling, a binary mixture of dibromomethane (DBM) and isoflurane. The rationale, as given later in a review (Mumtaz *et al.* 1993) by Clewell, was that (1) DBM was selected as a "surrogate" of MnBK because of its high tissue solubility and the ease of monitoring its metabolism, and (2) isoflurane was selected as a "surrogate" of *n*-hexane because of its poor tissue solubility and its rapid clearance by exhalation. The Clewell and Andersen (1985) publication was, in general, a review

TABLE 13.1 Compilation of Studies on PBPK Modeling of Chemical Mixtures

Chemicals	Interaction mechanisms	References
Binary Mixtures		
Dibromomethane and Isoflurane	Competitive enzyme inhibition	Clewell and Andersen (1985)
1,1-Dichloroethylene and trichloroethylene	Competitive enzyme inhibition	Andersen *et al.* (1987)
Benzene and toluene	Nonompetitive enzyme inhibition	Purcell *et al.* (1990)
Mirex, phenobartital, or chlordecone and bromotrichloromethane	Enzyme induction or interference with tissue repair	Thakore *et al.* (1991)
Ethanol and trichloroethylene	Competitive enzyme inhibition or enzyme induction	Sato *et al.* (1991)
Toluene and *m*-xylene	Competitive enzyme inhibition	Tardif *et al.* (1993, 1995)
1,3-Butadiene and styrene	Enzyme inhibition	Filser *et al.* (1993)
1,3-Butadiene and styrene; 1,3-butadiene and benzene; 1,3-butadiene and ethanol	Competitive enzyme inhibition; enzyme induction	Bond *et al.* (1994)
Vinyl chloride and trichloroethylene	Competitive enzyme inhibition	Barton *et al.* (1995)
Toluene and dichloromethane	Competitive enzyme inhibition	Krishnan and Pelekis (1995)
1,3-Butadiene and styrene	Competitive enzyme inhibition	Leavens and Bond (1996)
Carbon tetrachloride and Kepone	Inhibition of the repair mechanism	El-Masri *et al.* (1996a)
Trichloroethylene and 1,1-dichloroethylene	Competitive enzyme inhibition	El-Masri *et al.* (1996b,c)
Dichloromethane and toluene	Noncompetitive and uncompetitive enzyme inhibition	Pelekis and Krishnan (1997)
n-Hexane and toluene	Noncompetitive enzyme inhibition	Yu *et al.* (1998)
Toluene and *n*-hexane	Competitive enzyme inhibition	Ali and Tardif (1999)
Methyl chloroform and *m*-xylene	Competitive enzyme inhibition	Tardif and Charest-Tardif (1999)
5-Fluorouracil and sorivudine; Triazolam and erythromycin	"Mechanism-based inhibition" or enzyme inactivation	Ito *et al.* (1998), Kanamitsu *et al.* (2000)[a]
Toluene and trichloroethylene	Competitive enzyme inhibition	Thrall and Poet (2000)
Ethylbenzene and xylenes	Competitive enzyme inhibition	Jang *et al.* (2001)

TABLE 13.1 ***continued***

Chemicals	Interaction mechanisms	References
Ternary Mixtures		
Toluene, *m*-xylene, and ethylbenzene	Competitive enzyme inhibition	Tardif *et al.* (1997), Haddad *et al.* (1999a)
Trichloroethylene, tetrachloroethylene, methyl chloroform	Competitive enzyme inhibition	Dobrev *et al.* (2001, 2002)
Toluene, ethylbenzene, and xylenes	Competitive enzyme inhibition	Dennison *et al.* (2004c)
Four-Chemical Mixture		
Benzene, toluene, ethylbenzene, and *m*-xylene	Competitive enzyme inhibition	Haddad *et al.* (1999b)
Five-Chemical Mixture		
Benzene, toluene, ethylbenzene, *m*-xylene, and dichloromethane	Competitive enzyme inhibition	Haddad *et al.* (2000)
Complex Mixtures		
Gasoline and subfractions	Competitive enzyme inhibition	Dennison *et al.* (2003, 2004a,b), Dennison (2004)

[a] The PBPK model in this article is a limited one involving portal vein, systemic blood, and liver; see text for further discussion.

article, although some original experimental and modeling data were apparently included. Many studies described therein utilized the same basic PBPK model of styrene, shown in Fig. 13.1, as a template. A plot of PBPK model simulation versus experimental data showed similar kinetics for the formation of carboxyhemoglobin (HbCO), resulting from the metabolism of DBM to CO, following DBM exposure alone, or in combination with isoflurane in rats. Not much specifics on experimentation and modeling (i.e., animals, exposure regimen, interaction model structure, etc.) were given in this particular publication. Somewhat more details were available in the 1993 review (Mumtaz *et al.* 1993).

Specific Example 2: 1,1-Dichloroethylene and Trichloroethylene (Andersen et al. 1987) The interactive PBPK model of 1,1-dichloroethylene (DCE) and trichloroethylene (TCE), the related discussions on different types of enzyme inhibitions, and the incorporation of competitive inhibition into the liver compartment represent truly the first comprehensive publication (Andersen *et al.* 1987) in the peer-reviewed journal on PBPK modeling of a chemical mixture. Using liver injury [aspartate transaminase (AST)] from DCE as an index, the pharmacokinetics of DCE alone in Fischer 344 rats as well as that in the presence of TCE were compared between PBPK model simulation and experimental results obtained from gas uptake pharmacokinetic studies. For PBPK modeling, Andersen *et al.*

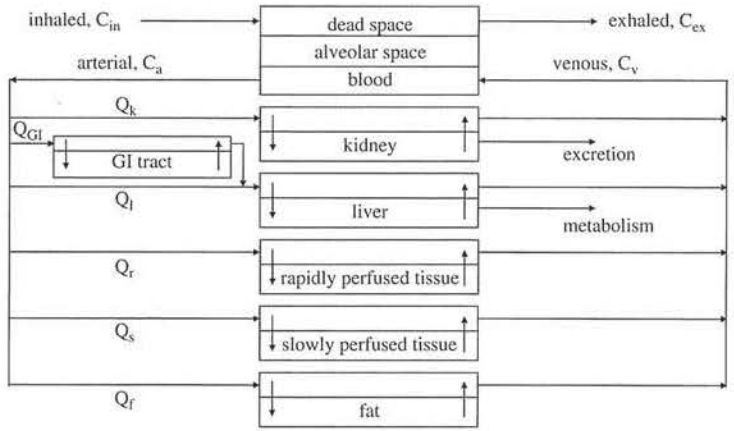

Figure 13.1 A graphical representation of a physiologically based pharmacokinetic (PBPK) model for volatile organic chemicals such as styrene. Modified based on Ramsey, J. C., and Andersen, M. E. (1984). *Toxicol. Appl. Pharmacol.* **73**, 159–175. The variables C_{in} and C_{ex} are the concentrations of chemical in inhaled and exhaled air, respectively, C_a and C_v are the concentrations of chemical in the arterial and venous blood, respectively, and Q_k, Q_{GI}, Q_l, Q_r, Q_s, and Q_f are the flow rate of blood through the kidneys, GI tract, liver, rapidly perfused tissue, slowly perfused tissue, and fat, respectively.

(1987) constructed a PBPK model for each chemical (i.e., DCE or TCE) individually and then linked the two models via the mass balance equation for the liver through enzyme inhibition (see next section for details). The kinetics of each chemical was described by a set of five mass balance differential equations for tissue compartments (fat, muscle/skin, viscera, and liver) and the chamber atmosphere. Thus, the basic template for each PBPK model was again based on that of the styrene model shown in Fig. 13.1 (Ramsey and Andersen 1984). Physiological constants and partition coefficients were either available in the literature (Gargas *et al.* 1986) or, in the case of partition coefficients for TCE, experimentally determined using vial equilibration methods (Sato and Nakajima 1979).

One of the most distinguished features of a PBPK model describing chemical interactions in chemical mixtures in the Andersen *et al.* (1987) article on DCE and TCE, as well as in a large number of subsequent papers on chemical mixtures, is the incorporation of enzyme inhibition as mechanistic basis for interactions. Thus, in many ways, the Andersen *et al.* (1987) article and its related descriptions of different types of enzyme inhibition is the pioneering effort both experimentally and conceptually in the area of PBPK modeling of chemical mixtures. The section below will provide the detailed description of the fundamentals on the mechanistic basis of enzyme inhibition.

Interaction Mechanisms: Enzyme Inhibition Figure 13.2 is a general schematic for multiple mechanisms of enzyme inhibition during coexposure of two substrates (Andersen *et al.* 1987). E is the enzyme in free form; in the case of DCE

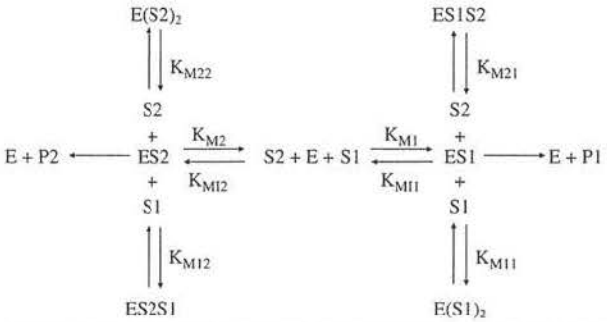

Figure 13.2 A general schematic for multiple mechanisms of enzyme inhibition during exposure to two substrates. E is the free enzyme; S1 and S2 are competing substrates for products P1 and P2; K_{M1} and K_{M2} are the substrate binding constants (they are the same as the inhibitory binding constants K_{MI1} and K_{MI2}). All constants are dissociation equilibrium constants. Redrawn based on Andersen, M. E., Gargas, M. L., Clewell, H. J., III, and Severgn, K. M. (1987). *Toxicol. Appl. Pharmacol.* **89**, 149–157.

and TCE, it is cytochrome P450 2E1 (CYP2E1). S1 and S2 are two substrates; in Example 2 above, they are DCE and TCE. The products formed from S1 and S2, respectively, are P1 and P2. All constants are dissociation equilibrium constants. The enzyme binding constants for substrates 1 and 2, K_{M1} and K_{M2}, are also the inhibitory binding constants when one substrate serves to inhibit the metabolism of a second substrate. In that sense, accordingly, K_{M1} equals K_{MI1} and K_{M2} equals K_{MI2}.

Central to the successful operation of the PBPK model for pharmacokinetic interactions in the binary chemical mixture of DCE and TCE is the incorporation of the set of equations related to different types (competitive, noncompetitive, and uncompetitive) of enzyme inhibition into the liver compartment mass balance differential equation (Andersen *et al.* 1987; note T_1 in the original paper had one term missing) as shown below:

$$V_L \frac{dC_{L1}}{dt} = \frac{d\mathrm{AMT}_{L1}}{dt} = (Q_L C_{a1}) - (Q_L C_{vL1}) - \frac{V_{max1} \times C_{vL1}}{K_m(T_1) + C_{vL1}(T_2)} \tag{13.1}$$

$$T_1 = 1 + \frac{C_{vL2}}{K_{MI2}} + \frac{(C_{vL2})^2}{(K_{MI2} \times K_{M22})} + \frac{(C_{vL1})(C_{vL2})}{(K_{MI2})(K_{MI2})} \tag{13.2}$$

$$T_2 = 1 + \frac{C_{vL2}}{K_{M21}} + \frac{(C_{vL1})}{K_{MI1}} \tag{13.3}$$

Since this set of equations is very important for the understanding of a large portion of publications involving PBPK modeling of chemical mixtures, it is essential to fully appreciate how these equations came about and what do they mean. Therefore, we will go through the conceptual and algebraic derivation of the above equations.

Based on the general schematic for multiple enzyme inhibition during co-exposure to two substrates in Fig. 13.2, we first write the rate equation for the product of interest (in this case, we concentrate on P1 and S1):

$$v = k_1[\mathrm{ES1}] \tag{13.4}$$

Next, we write the conservation equation for enzyme,

$$\mathrm{E_{total}} = \mathrm{E_{free}} + \mathrm{ES1} + \mathrm{ES2} + \mathrm{ES1S2} + \mathrm{ES1S1} + \mathrm{ES2S1} + \mathrm{ES2S2} \tag{13.5}$$

We then solve the conservation equation shown above in terms of ES1, the factor of interest from the rate equation Eq. (13.4), according to the following steps:

We write all the equations for the equilibrium dissociation constants (see Fig. 13.2),

$$K_{M1} = \frac{(\mathrm{E_{free}})(\mathrm{S1})}{(\mathrm{ES1})} \tag{13.6}$$

$$K_{M21} = \frac{(\mathrm{ES1})(\mathrm{S2})}{(\mathrm{ES1S2})} \tag{13.7}$$

$$K_{MI1} = \frac{(\mathrm{ES1})(\mathrm{S1})}{(\mathrm{ES1S1})} \tag{13.8}$$

$$K_{M2} = \frac{(\mathrm{E_{free}})(\mathrm{S2})}{(\mathrm{ES2})} \tag{13.9}$$

$$K_{MI2} = \frac{(\mathrm{ES2})(\mathrm{S1})}{(\mathrm{ES2S1})} \tag{13.10}$$

$$K_{M22} = \frac{(\mathrm{ES2})(\mathrm{S2})}{(\mathrm{ES2S2})} \tag{13.11}$$

Rewrite Eqs. (13.6)–(13.8) in terms of concentrations,

$$(\mathrm{E_{free}}) = \frac{(\mathrm{ES1})K_{M1}}{(\mathrm{S1})} \tag{13.12}$$

$$(\mathrm{ES1S2}) = \frac{(\mathrm{ES1})(\mathrm{S2})}{(K_{M21})} \tag{13.13}$$

$$(\mathrm{ES1S1}) = \frac{(\mathrm{ES1})(\mathrm{S1})}{(K_{MI1})} \tag{13.14}$$

Recasting Eqs. (13.9)–(13.11) is a bit more involved, we start out with rearranging Eq. (13.9), $(\mathrm{ES2}) = (\mathrm{E_{free}})(\mathrm{S2})/K_{M2}$, and substitute Eq. (13.12) (i.e., $\mathrm{E_{free}}$) into this equation, we have

$$(\mathrm{ES2}) = \frac{(\mathrm{ES1})(K_{M1})(\mathrm{S2})}{(\mathrm{S1})(K_{M2})} \tag{13.15}$$

Similarly, Eqs. (13.10) and (13.11) become

$$(\mathrm{ES2S1}) = \frac{(\mathrm{ES2})(\mathrm{S1})}{(K_{\mathrm{M12}})} \tag{13.16}$$

$$(\mathrm{ES2S2}) = \frac{(\mathrm{ES2})(\mathrm{S2})}{(K_{\mathrm{M22}})} \tag{13.17}$$

Substituting Eq. (13.15) into Eqs. (13.16) and (13.17) completes the process of transforming all forms of enzyme and enzyme/substrate complexes in terms of ES1, we obtain

$$(\mathrm{ES2S1}) = \frac{(\mathrm{S1})(\mathrm{ES1})(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M12}})(\mathrm{S1})(K_{\mathrm{M2}})} \tag{13.18}$$

$$(\mathrm{ES2S2}) = \frac{(\mathrm{S2})(\mathrm{ES1})(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M22}})(\mathrm{S1})(K_{\mathrm{M2}})} \tag{13.19}$$

Substituting all these various forms of enzyme and enzyme/substrate complexes, [i.e., Eqs. (13.12)–(13.15), (13.18), and (13.19)] into the conservation equation [i.e., Eq. (13.5)], we get

$$\begin{aligned}\mathrm{E_{total}} &= \mathrm{E_{free}} + \mathrm{ES1} + \mathrm{ES2} + \mathrm{ES1S2} + \mathrm{ES1S1} + \mathrm{ES2S1} + \mathrm{ES2S2} \\ &= \frac{(\mathrm{ES1})(K_{\mathrm{M1}})}{(\mathrm{S1})} + (\mathrm{ES1}) + \frac{(\mathrm{ES1})(K_{\mathrm{M1}})(\mathrm{S2})}{(\mathrm{S1})(K_{\mathrm{M2}})} + \frac{(\mathrm{ES1})(\mathrm{S2})}{(K_{\mathrm{M21}})} + \frac{(\mathrm{ES1})(\mathrm{S1})}{(K_{\mathrm{M11}})} \\ &\quad + \frac{(\mathrm{S1})(\mathrm{ES1})(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M12}})(\mathrm{S1})(K_{\mathrm{M2}})} + \frac{(\mathrm{S2})(\mathrm{ES1})(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M22}})(\mathrm{S1})(K_{\mathrm{M2}})}\end{aligned}$$

Factor out $(E_{S1})/(S1)$:

$$\mathrm{E_{total}} = \frac{(\mathrm{ES1})}{(\mathrm{S1})}\left[\begin{array}{l}(K_{\mathrm{M1}}) + (\mathrm{S1}) + \dfrac{(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M2}})} + \dfrac{(\mathrm{S1})(\mathrm{S2})}{(K_{\mathrm{M21}})} + \dfrac{(\mathrm{S1})^2}{(K_{\mathrm{M11}})} \\ + \dfrac{(\mathrm{S1})(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M12}})(K_{\mathrm{M2}})} + \dfrac{(\mathrm{S2})^2(K_{\mathrm{M1}})}{(K_{\mathrm{M22}})(K_{\mathrm{M2}})}\end{array}\right]$$

Now group all the terms in the bracket in terms of S1 and K_{M1}:

$$\mathrm{E_{total}} = \frac{(\mathrm{ES1})}{(\mathrm{S1})}\left[\begin{array}{l}(\mathrm{S1}) + \dfrac{(\mathrm{S1})(\mathrm{S2})}{(K_{\mathrm{M21}})} + \dfrac{(\mathrm{S1})^2}{(K_{\mathrm{M11}})} + (K_{\mathrm{M1}}) \\ + \dfrac{(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M2}})} + \dfrac{(\mathrm{S2})^2(K_{\mathrm{M1}})}{(K_{\mathrm{M22}})(K_{\mathrm{M2}})} + \dfrac{(\mathrm{S1})(K_{\mathrm{M1}})(\mathrm{S2})}{(K_{\mathrm{M12}})(K_{\mathrm{M2}})}\end{array}\right]$$

Now rewrite blocking for S1 and K_{M1}:

$$\mathrm{E_{total}} = \frac{(\mathrm{ES1})}{(\mathrm{S1})}\left[\begin{array}{l}(\mathrm{S1})\left(1 + \dfrac{(\mathrm{S2})}{(K_{\mathrm{M21}})} + \dfrac{(\mathrm{S1})}{(K_{\mathrm{M11}})}\right) \\ + (K_{\mathrm{M1}})\left(1 + \dfrac{(\mathrm{S2})}{(K_{\mathrm{M2}})} + \dfrac{(\mathrm{S2})^2}{(K_{\mathrm{M22}})(K_{\mathrm{M2}})} + \dfrac{(\mathrm{S1})(\mathrm{S2})}{(K_{\mathrm{M12}})(K_{\mathrm{M2}})}\right)\end{array}\right]$$

Let T_1 and T_2 be the terms, respectively, in the big parentheses above as shown below:

$$T_1 = \left(1 + \frac{(\mathrm{S2})}{(K_{\mathrm{M2}})} + \frac{(\mathrm{S2})^2}{(K_{\mathrm{M22}})(K_{\mathrm{M2}})} + \frac{(\mathrm{S1})(\mathrm{S2})}{(K_{\mathrm{M12}})(K_{\mathrm{M2}})}\right)$$

$$T_2 = \left(1 + \frac{(\mathrm{S2})}{(K_{\mathrm{M21}})} + \frac{(\mathrm{S1})}{(K_{\mathrm{M11}})}\right)$$

Then,

$$\mathrm{E_{total}} = \frac{(\mathrm{ES1})}{(\mathrm{S1})}[\mathrm{S1}(T_2) + K_{\mathrm{M1}}(T_1)]$$

Rearrange to solve for (ES1):

$$\mathrm{ES1} = \frac{\mathrm{E_{total}}(\mathrm{S1})}{[\mathrm{S1}(T_2) + K_{\mathrm{M1}}(T_1)]}$$

Substituting into the original rate equation Eq.(13.4), we obtain

$$v = k_1[\mathrm{ES1}] = k_1\frac{\mathrm{E_{total}}(\mathrm{S1})}{[\mathrm{S1}(T_2) + K_{\mathrm{M1}}(T_1)]}$$

Since $k_1\mathrm{E_{total}}$ is V_{max}, highest possible rate, so

$$v = \frac{\mathrm{V_{max}} \times (\mathrm{S1})}{[\mathrm{S1}(T_2) + K_{\mathrm{M1}}(T_1)]} \tag{13.20}$$

Please note that we have successfully derived the last term in Eq. (13.1) and T_1 and T_2 [i.e., Eqs. (13.2) and (13.3)], the equations published in the Andersen *et al.* (1987) article. Note also K_{M2} here equals to K_{M12} in the Andersen *et al.* (1987) paper.

The short statement below is the most important point to extract for the reader—the role of process—in establishing the sets of equations for this or any other interaction model based on enzyme inhibition.

It should be noted that it always follows this process: (1) Write the rate equation; (2) write the conservation equation; (3) derive dissociation constants; (4) solve conservation equation for enzyme species represented in the rate equation.

Even though there are many different mechanisms for enzyme inhibition and an excellent reference (Segal 1975) is available, the final equation above [Eq. (13.20)] carries, simplistically, the following three types of enzyme inhibition:

1. *Competitive Inhibition*. In Eq. (13.20), when $T_2 = 1$ and the inhibitor (in our case, the second substrate) is only affecting K_{M1}, competitive inhibition results. This type of inhibition includes the following scenarios as described in Segal (1975):
   - Substrate (S) and inhibitor (I, or a second substrate) compete for the same binding site.
   - S and I are mutually exclusive because of steric hinderance.
   - S and I share a common binding group on the enzyme.
   - The binding sites for S and I, though distinct, are overlapping.
   - The binding of I to a distinct inhibitor site causes a conformational change in the enzyme that distorts or masks the S binding site, or vice versa.
2. *Noncompetitive Inhibition*. In Eq. (13.20), when the inhibitor is affecting both K_{M1} and S1, noncompetitive inhibition results. In this type of inhibition, S and I (or a second substrate) are not mutually exclusive but ESI (in our case ES1S2) is catalytically inactive. In this case, S and I don't interfere with each other's binding, but the conformational change of the enzyme affect catalytic center (Segal 1975). There are other variations of the above scenario.
3. *Uncompetitive Inhibition*. In Eq. (13.20), when the inhibitor is affecting only S1 (i.e., $T_1 = 1$), uncompetitive inhibition results. In this type of inhibition, I (or a second substrate) only binds to the ES (in our case ES1) complex. When S binds, a conformational change of the enzyme occurs to unmask the I binding site. The resulting ESI (in our case ES1S2) is catalytically inactive.

Other examples involving PBPK modeling of binary chemical mixtures included benzene and toluene (Purcell *et al.* 1990), mirex/phenobarbital/chlordecone and bromotrichloromethane (Thakore *et al.* 1991), ethanol and trichloroethylene (Sato *et al.* 1991), and toluene and *m*-xylene (Tardiff *et al.* 1993). These mixtures and their respective PBPK modeling have been reviewed previously by Krishnan *et al.* (1994a,b); other than the summary in Table 13.1, they will not be further discussed here. Since those reviews by Krishnan *et al.* (1994a,b), there were at least 14 more binary mixtures studied by various investigators using the PBPK modeling approach in one form or another (Table 13.1). With the exception of two binary mixtures involving two drug pairs, 5-fluorouracil/sorivudine and triazolam/erythromycin (Ito *et al.* 1998; Kanamitsu *et al.* 2000), all of the other studies were on volatile organic solvents (VOCs). Since these VOC studies are quite similar to the ones already described above, we chose not to discuss further the individual publications.

Interaction Mechanisms: Enzyme Inactivation or "Mechanism-Based Inhibition" We will specifically discuss the Ito *et al.* (1998) and Kanamitsu *et al.* (2000) articles on two cases of drug–drug interactions involving 5-fluorouracil/sorivudine and triazolam/erythromycin, because: (1) These studies are two of the relatively few PBPK modeling studies on drugs, and it is specifically on drug–drug interactions; (2) the interactions involved a unique mechanism which was implicated in at least 15 human fatalities in Japan; and (3) the PBPK modeling approach in these articles is somewhat limited involving mainly the liver with two related compartments: portal vein and systemic blood.

In 1993 in Japan, 15 patients with cancer and herpes zoster were treated with 5-fluorouracil (5-FU), an anticancer agent, and sorivudine, an antiviral drug, and died from 5-FU toxicity due to a drug–drug interaction. This drug interaction involved a key enzyme, dihydropyrimidine dehydrogenase (DPD), which is a rate-limiting enzyme in the metabolism of 5-FU. It turned out that sorivudine was converted by gut flora to 5-bromovinyluracil, which is then metabolically activated by DPD. The reactive species binds to DPD and renders the enzyme inactive irreversibly. This type of inactivation of the enzyme, unlike competitive or noncompetitive inhibition, is unique mechanistically. Several terms have been used to describe this unique mechanism: "mechanism-based inhibition," "mechanism-based inactivation," "enzyme-activated irreversible inhibition," "suicide inactivation," and "k_{cat} inhibition" (Ito *et al.* 1998). According to Ito *et al.* (1998), this particular "mechanism-based inhibition" has the following characteristics:

- Preincubation time-dependent inhibition of the enzyme (time-dependence).
- No inhibition if cofactors necessary for producing the activated inhibitor are not present in the preincubation medium.
- Potentiation of the inhibition depending on the inhibitor concentration (saturation kinetics).
- Slower inactivation rate of the enzyme in the presence of substrate compared with its absence (substrate protection).
- Enzyme activity not recovered following gel filtration or dialysis (irreversibility).
- 1 : 1 Stoichiometry of the inhibitor and the active site of the enzyme (stoichiometry of inactivation).

Ito *et al.* (1998) also constructed a physiological model for the general "mechanism-based inhibition" of a number of CYP isozymes. This model, shown in Fig. 13.3, consists of three compartments: the liver, systemic blood, and portal vein. The substrate (S) and inhibitor (I) share the same model structure. The mass balance equations are as follows:

For the Substrate

$$V_h \times \left(\frac{dC_h}{dt}\right) = Q \times C_{\text{portal}} - Q \times \frac{C_h}{K_p} - f_b \times CL_{\text{int}} \times \frac{C_h}{K_p}$$

$$CL_{\text{int}} = \frac{V_{\max}}{K_m + f_b \times \dfrac{C_h}{K_p}}$$

$$V_{\max} = V_{\max}(0) \times \frac{E_{\text{act}}(t)}{E_0}$$

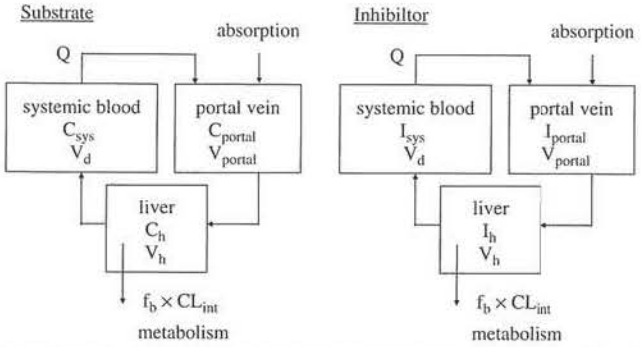

Figure 13.3 A conceptual physiological model for the time profiles of substrate and inhibitor concentrations in the plasma and liver. Q is the blood flow rate; C_{sys} and I_{sys} are substrate and inhibitor concentrations in systemic blood; V_d is the volume of distribution; C_{portal} and I_{portal} are substrate and inhibitor concentrations in portal vein; V_{portal} is the volume of portal vein; C_h and I_h are substrate and inhibitor concentrations in the liver; V_h is the volume of the liver; f_b is the unbound fraction in blood; CL_{int} is the intrinsic metabolic clearance. Redrawn based on Ito, K., Iwatsubo, T., Kanamitsu, S., Ueda, K., Suzuki, H., and Sugiyama, Y. (1998). *Pharmacol. Rev.* **50**, 387–411.

$$V_{portal} \times \left(\frac{dC_{portal}}{dt}\right) = Q \times C_{sys} + V_{abs} - Q \times C_{portal}$$

$$V_{abs} = k_a \times D \times F \times e^{-k_a \times t}$$

$$V_d \times \left(\frac{dC_{sys}}{dt}\right) = Q \times \frac{C_h}{K_p} - Q \times C_{sys}$$

For Inhibitor

$$V_h \times \left(\frac{dI_h}{dt}\right) = Q \times I_{portal} - Q \times \frac{I_h}{K_p} - f_b \times CL_{int} \times \frac{I_h}{K_p}$$

$$CL_{int} = \frac{V_{max}}{K_m + f_b \times \frac{I_h}{K_p}}$$

$$V_{portal} \times \left(\frac{dI_{portal}}{dt}\right) = Q \times I_{sys} + V_{abs} - Q \times I_{portal}$$

$$V_{abs} = k_a \times D \times F \times e^{-k_a \times t}$$

$$V_d \times \left(\frac{dI_{sys}}{dt}\right) = Q \times \frac{I_h}{K_p} - Q \times I_{sys}$$

where Q represents blood flow rate, C_{sys} and I_{sys} represent concentrations of substrate and inhibitors, respectively, in systemic blood, V_d represents the volume of distribution in the systemic blood compartment, C_{portal} and I_{portal} represent concentrations of substrate and inhibitor, respectively, in the portal vein, D represents dose, V_{portal} represents the volume of portal vein, C_h and I_h represent concentrations of substrate and inhibitor, respectively, in the liver, V_h represents the volume of the liver, f_b represents the unbound fraction in blood, CL_{int} represents the intrinsic metabolic clearance, F_a (although the original article did not clarify this, F in the above equations really should have been F_a) represents the fraction absorbed from the gastrointestinal tract, K_m represents the Michaelis constant for the metabolic elimination, V_{max} represents the maximum metabolic rate, and K_p represents the liver-to-blood concentration ratio.

For active and inactive enzymes in the liver, Ito *et al.* (1998) provided the following differential equations:

$$\frac{dE_{act}}{dt} = -\frac{k_{inact} \times E_{act} \times f_b \times \frac{I_h}{K_p}}{K_{i,app} + f_b \times \frac{I_h}{K_p}} + k_{deg}(E_0 - E_{act})$$

$$\frac{dE_{inact}}{dt} = -\frac{k_{inact} \times E_{act} \times f_b \times \frac{I_h}{K_p}}{K_{i,app} + f_b \times \frac{I_h}{K_p}} + k_{deg} \times E_{inact}$$

Ito *et al.* (1998) further defined that k_{deg} is the degradation rate constant (i.e., turnover rate constant) of the enzyme. The initial conditions at $t = 0$ are $E_{act} = E_0$ and $E_{inact} = 0$. In the absence of an inhibitor, the enzyme level in the liver is at a steady state and the degradation rate ($k_{deg}E_0$) is equal to the synthesis rate, which is assumed to be unaffected by an inhibitor.

Subsequently, the same research group reported a similar "mechanism-based inhibition" of CYP3A4 by macrolide antibiotics, erythromycin, in a drug–drug interaction with triazolam (Kanamitsu *et al.* 2000). These investigators used the above three-compartment physiological model and obtained quantitative predictions of the erythromycin/triazolam interaction. The predicted increase in triazolam AUC following erythromycin pretreatment was 2.0-fold (from 61.0 to 119 nM·hr) and 2.6-fold (from 61.0 to 156 nM·hr) from model simulation. These model predictions were very close to the actual observed value, *in vivo* in human, of 2.1-fold increase (from 58.6 to 121 nM·hr) reported in the literature.

It should be noted here that a similar "suicide inhibition" phenomenon (i.e., an enzyme biotransforms a substrate to a reactive species which, in turn, "kills" the enzyme—a "suicide" from the perspective of the enzyme) had also been observed in CYP2E1 catalyzed metabolism of some volatile oraganic solvents such as *cis* and *trans* DCE (Andersen *et al.* 1987; Lilly *et al.* 1998).

PBPD Modeling of Binary Mixtures Since the emphasis of this monograph is on PBPK modeling and PBPD modeling of chemical mixtures is still at its infancy,

we choose to only briefly discuss PBPD modeling by concentrating on two published examples on binary mixtures. For more details on the basic principles of PBPD modeling and specific information on the two case studies discussed below, the readers are encouraged to consult, respectively, El-Masri *et al.* (1996a,c).

One of our earlier examples was the PBPK/PD modeling of a toxicological interaction between Kepone (also known as chlordecone) and carbon tetrachloride (CCl_4) based on mechanisms of interactive toxicity and the application of computer technology in acute toxicity studies. Briefly, CCl_4 is a well-known hepatotoxin. Following free radical formation through the P450 enzyme system, the toxicity of CCl_4 can be an accumulation of lipids (steatosis, fatty liver) and degenerative processes leading to cell death (necrosis). Kepone is found in the environment as a result of photolytic oxidation of mirex, a pesticide used for the control of fire ants, or as a pollutant from careless and irresponsible discharge. At relatively low levels (e.g., 10 ppm in the diet), even repeated dosing of Kepone in the diet up to 15 days caused no apparent toxicity to the liver. The toxicological interaction between Kepone and CCl_4 was elucidated to be the impairment, by Kepone, of the liver's regeneration process. These mechanistic studies were summarized in a number of publications (Mehendale 1984, 1991, 1994).

El-Masri *et al.* (1996a) constructed a PBPD model based on the mechanism of toxicological interaction between Kepone and CCl_4. This PBPD model was verified by literature information, and it was capable of providing time-course computer simulations of mitotic, injured, and pyknotic (dead) cells after treatment with CCl_4 alone or with Kepone pretreatment. This PBPD model was further linked with Monte Carlo simulation to predict the acute lethality of CCl_4 alone and in combination with Kepone.

The second case study involved PBPK/PD modeling of pharmacodynamic interactions between trichloroethylene (TCE) and 1,1-dichloroethylene (DCE) regarding their binding and depletion of hepatic glutathione (GSH) in relation to the intrinsic hepatic GSH systhesis (El-Masri *et al.* 1996c). A PBPK/PD model was used to identify critical time point at which hepatic GSH is at a minimum in response to both chemicals. PBPK models for interactions leading to depletion of hepatic glutathione had been developed by several investigators (D'Souza *et al.* 1988; Frederick *et al.* 1992). Model-directed gas uptake experiments with DCE revealed that DCE was the only chemical capable of significantly depleting hepatic GSH. TCE exposure higher than 100 ppm to the rats obstructed the ability of DCE to deplete hepatic GSH, indicating metabolic competitive inhibition of DCE biotransformation to reactive metabolites. TCE exposure lower than 100 ppm was ineffective in inhibiting DCE from significantly depleting hepatic GSH. El-Masri *et al.* (1996c) further applied these quantitative analyses in establishing an "interaction threshold" (see discussion below in Section 13.2.2) between TCE and DCE.

13.2.2 More Recent Endeavor: PBPK Modeling of Higher-Order Mixtures

In the above section, we provided a glimpse of the development of chemical mixture toxicology in the "early days." As the field of PBPK modeling grows in parallel with

the science of toxicology, the natural progression proceeded in two directions. First, when the toxicology of chemical mixture moves from descriptive work to mechanistic-based research, PBPK modeling transforms into PBPD modeling (discussed above). Second, investigators, driven by innate curiosity and practical need, begin to explore PBPK modeling of more and more complex chemical mixtures. Thus, in the next few sections, we will provide a few examples that reflect the application of PBPK modeling to the toxicology of more complex chemical mixtures.

PBPK Modeling of Ternary and Four-Chemical Mixtures Pioneering efforts in the PBPK modeling of more complex chemical mixtures were from a research group led by Krishnan and various colleagues; two comprehensive reviews of work up to 1994 are available (Krishnan *et al.* 1994a,b). Earlier work from this group concentrated on interactions and PBPK modeling between two chemicals (Tardif *et al.* 1993, 1995; Pelekis and Krishnan 1997). As progress was made, these investigators began to build up the mixtures and devoted their effort to PBPK modeling of more and more complex chemical mixtures (Tardif *et al.* 1997; Haddad *et al.* 1999a,b, 2000).

As shown in Table 13.1, PBPK modeling of a ternary mixture on toluene, *m*-xylene, and ethylbenzene was studied and reported by Tardif *et al.* (1997), and the mechanism involved was competitive inhibition. The details of the conceptual interactive PBPK model and the equations with incorporation of competitive inhibition are provided below under the section for five-chemical mixture. Subsequently, Haddad *et al.* (1999a) applied this interactive PBPK model to the calculation of biological hazard index (BHI). BHI, defined as the biological level tolerable for exposure to mixtures, is traditionally calculated in an analogous way as the hazard index under additivity assumption (Ogata *et al.* 1993; Hadded *et al.* 1999a). However, Haddad *et al.* incorporated toxicological interaction by using PBPK modeling to obtain "simulation concentration" (SC) and modified the BHI calculation according to the following equation:

$$\mathrm{BHI} = \sum_{i=1}^{n} \frac{\mathrm{SC}_i}{\mathrm{BEI}_i}$$

Where BHI and SC were defined before; BEI refers to the concentration or excretion rate of a biomarker in a healthy worker exposed to TLV. In doing so, Haddad *et al.* (1999a) applied interactive PBPK modeling of a chemical mixture into the risk assessment process.

Using the same principle and similar technique, researchers at Colorado State University studied PBPK modeling of two ternary mixtures [trichloroethylene (TCE), tetrachloroethylene (PERC), methyl chloroform (MC) and toluene, ethylbenzene, and xylenes] to respectively enhance the concept of "interaction thresholds" and modify and improve the "Mixture Formula" risk assessment by using an interactive PBPK modeling approach (Dobrev *et al.* 2001, 2002; Dennison *et al.* 2005a).

Haddad *et al.* (1999b) also studied the PBPK modeling of a four-chemical mixture involving benzene, toluene, ethylbenzene, and *m*-xylene. In general, the incorporation of the interaction mechanism, at the level of the liver metabolic enzyme inhibition, is similar to those described above for binary and ternary mixtures, albeit a bit more complicated. We will demonstrate again the principle and techniques involved in a later section on a five-chemical mixture.

The Concept of the "Interaction Threshold" In 1996, El-Masri *et al.* introduced the idea of "interaction thresholds" as the minimal level of change in tissue dosimetry of two or more chemicals associated with a significant health effect (El-Masri *et al.* 1996b). When two or more interactive chemicals are studied together, theoretically there could be infinite interaction thresholds. However, if we specify certain occupational or environmental exposure concentrations for all the other component chemicals in the mixture except one, we may obtain an interaction threshold for that set of exposure conditions. This definition is important because human risk from exposure to multiple chemicals may not always obey the rule of additivity. Dobrev *et al.* (2001) estimated the interaction thresholds of three common volatile organic solvents—TCE, PERC, and MC—under different dosing conditions. First, an interactive PBPK model was built where PERC and MC were competitive inhibitors for TCE, the compound most extensively metabolized among the three. The model was developed and validated by gas uptake pharmacokinetic studies in Fischer 344 rats at relatively high doses of single chemicals, binary mixtures, and the ternary mixture. Using computer simulation to extrapolate from high to low concentrations, Dobrev *et al.* (2001) investigated the toxicological interactions at occupational exposure levels, specifically at around threshold limit value/time-weighted average (TLV/TWA). Since long-term toxicity/carcinogenicity of these three solvents is clearly associated with their metabolism, and TCE is the most extensively metabolized among them, this study focused on changes in internal TCE dose measures related to the mixture co-exposure. Using a 10% elevation in parent compound blood level as a criterion for significant interaction, interaction thresholds were estimated with two of the three chemicals held at constant concentrations. Under the above exposure conditions (i.e., TCE and PERC at their TLVs but varying MC concentrations), the interaction threshold for the ternary mixture was 50, 130, and 25 ppm for TCE, MC, and PERC, respectively. This work was later extended, using computer simulation (i.e., *in silico* toxicology), to human exposure to this three-chemical mixture and the estimation of interaction thresholds for humans (Dobrev *et al.* 2002). Increases in the TCE blood levels led to higher availability of the parent compound for glutathione conjugation, a metabolic pathway associated with kidney toxicity/carcinogenicity. The simulated change in production rates of toxic conjugative metabolites exceeded 17% for a corresponding 10% increase in TCE blood concentration, indicating a nonlinear risk increase due to combined exposures to TCE. This study (Dobrev *et al.* 2002) and the related discussion above reveal that evaluation of metabolic interactions and their thresholds illustrates a unique application of PBPK modeling in risk assessment of occupational exposures to chemical mixtures. It further underscores the importance of incorporating PBPK modeling into the cumulative risk assessment process.

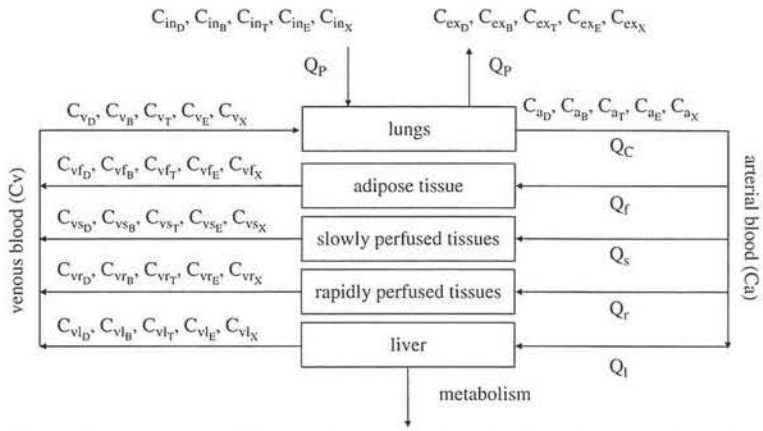

Figure 13.4 Conceptual interactive PBPK model for a five-component chemical mixture. D, dichloromethane; B, benzene; T, toluene; E, ethylbenzene; X, *m*-xylene. Pharmacokinetic interactions occur in the liver as competitive enzyme inhibition. Q_P is alveolar ventilation rate; Q_C is cardiac output; C_v and C_a are venous and arterial blood concentrations of individual component chemicals; C_{vl} is venous blood concentration leaving tissue compartments; Q_i is blood flow to tissues (f, adipose tissue; s, slowly perfused tissues; r, rapidly perfused tissues; l, liver); C_{in} and C_{ex} are inhaled or exhaled concentrations of individual component chemicals. Redrawn based on Haddad, S., Charest-Tardif, G., Tardif, R., and Krishnan, K. (2000). *Toxicol. Appl. Pharmacol.* **167**, 199–209.

Development of a PBPK Model for a Chemical Mixtures Involving Five Components and the Concept of Predicting Pharmacokinetics of Different Chemical Mixtures Based on PBPK Studies of Binary Mixtures An interactive model for 5 chemicals (benzene, toluene, ethylbenzene, *m*-xylene, and dichloromethane) has also been completed (Haddad *et al.* 2000; Krishnan *et al.* 2002), leading to the idea that pharmacokinetic interactions of complex chemical mixtures, regardless of the number of components, may be predicted based on the PBPK modeling of binary mixtures of the component chemicals (Haddad *et al.* 2000; Krishnan *et al.* 2002). Since, to date, their studies represent the most complex interactive PBPK model for a mixture with five reconstituted chemicals, we will provide more detailed discussion below. Also, their concept of predicting the kinetics of complex mixtures based on PBPK studies of binary mixture is interesting and revolutionary, and it warrants further discussion.

As shown in Fig. 13.4, the conceptual PBPK model of the five-chemical mixture (Haddad *et al.* 2000) is similar to the styrene model in Fig. 13.1; the only difference is the incorporation of the individual chemical concentrations in the arterial and venous blood as designated by subscripts D, B, T, E, X, representing the five chemicals. Because the mechanism of interaction was determined to be competitive enzyme inhibition of CYP2E1, the following Michaelis–Menten equations were incorporated into the mass balance differential equations in the liver compartment for the respective chemicals: benzene (B), toluene (T), ethylbenzene (E), *m*-

xylene (X), and dichloromethane (D). RAM here represents rate of metabolism or velocity of the enzyme reaction.

$$\mathrm{RAM}_B = \frac{V_{\max B} \times C_{vlB}}{K_{mB}\left(1+\frac{C_{vlE}}{K_{iEB}}+\frac{C_{vlT}}{K_{iTB}}+\frac{C_{vlX}}{K_{iXB}}+\frac{C_{vlD}}{K_{iDB}}\right)+C_{vlB}}$$

$$\mathrm{RAM}_T = \frac{V_{\max T} \times C_{vlT}}{K_{mT}\left(1+\frac{C_{vlB}}{K_{iBT}}+\frac{C_{vlE}}{K_{iET}}+\frac{C_{vlX}}{K_{iXT}}+\frac{C_{vlD}}{K_{iDT}}\right)+C_{vlT}}$$

$$\mathrm{RAM}_E = \frac{V_{\max E} \times C_{vlE}}{K_{mE}\left(1+\frac{C_{vlB}}{K_{iBE}}+\frac{C_{vlT}}{K_{iTE}}+\frac{C_{vlX}}{K_{iXE}}+\frac{C_{vlD}}{K_{iDE}}\right)+C_{vlE}}$$

$$\mathrm{RAM}_X = \frac{V_{\max X} \times C_{vlX}}{K_{mX}\left(1+\frac{C_{vlB}}{K_{iBX}}+\frac{C_{vlE}}{K_{iEX}}+\frac{C_{vlT}}{K_{iTX}}+\frac{C_{vlD}}{K_{iDX}}\right)+C_{vlX}}$$

$$\mathrm{RAM}_D = \frac{V_{\max D} \times C_{vlD}}{K_{mD}\left(1+\frac{C_{vlB}}{K_{iBD}}+\frac{C_{vlE}}{K_{iED}}+\frac{C_{vlT}}{K_{iTD}}+\frac{C_{vlX}}{K_{iXD}}\right)+C_{vlD}}$$

Experimentally, Haddad *et al.* (2000) did PBPK model simulations of the pharmacokinetics of components under two scenarios: (1) when one of the mixture components was substituted with another such as benzene in the BETX mixture was substituted with dichloromethane and (2) when another chemical was added to an existing four-chemical mixture model such as dichloromethane was added to the existing BTEX model. These investigators also did pharmacokinetic studies and specifically obtained blood kinetic data in rats on all the new binary mixtures with D added; thus, animal experimental data were generated on D-B, D-E, D-T, D-X binary mixtures. When competitive inhibition was incorporated into the interactive PBPK model, the model simulation not only matched the newly obtained binary mixture kinetic data, but also matched earlier data for a variety more complex mixtures beyond binary combinations. Therefore, from their studies emerged the concept that predictability of pharmacokinetic and pharmacodynamic consequences of chemicals in more complex chemical mixtures is possible as long as there is the availability of quantitative data in the literature on binary chemical interactions (Haddad *et al.* 2000; Krishnan *et al.* 2002). So far their approach (Haddad *et al.* 2000; Krishnan *et al.* 2002) has worked for the volatile organic chemicals that they studied. Whether or not this concept has a broader application to mixed classes of chemicals in a mixture remained to be evaluated.

PBPK Modeling of Complex Chemical Mixtures As PBPK modeling of chemical mixtures progresses to involving more and more components, it is a natural course of development that investigators will attempt to tackle the real-world complex chemical mixtures. Verhaar *et al.* (1997) proposed the incorporation of lumping analyses (a chemical engineering technique used in petroleum engineering processes) and QSAR to PBPK modeling. The idea was that each of the three techniques would serve its unique function in the overall goal of predicting some aspects of the chemical mixtures of interest. Thus, QSAR analysis can be used to predict needed physicochemical and toxicological parameters for unknown compounds or for surrogate compounds (from lumping); lumping analysis can drastically reduce the complexity of the description of a mixture; and PBPK/PD modeling can be used to describe the pharmacokinetics, and possibly pharmacodynamics, of an ensemble of compounds or lumped pseudocompounds, including possible interaction effects. A detailed statistical/mathematical treatment on how to minimize errors in lumping was given in an appendix in this article. Verhaar *et al.* (1997) specifically suggested the application of these technologies (i.e., QSAR, lumping analysis, PBPK/PD modeling) to JP-5.

These ideas have now been applied with gasoline as the complex mixture (Dennison *et al.* 2003, 2004a,b, 2005b; Dennison 2004) developing both the PBPK modeling framework and lumping approach. Experimental work involved gas uptake pharmacokinetic studies in male Fischer 344 rats of single, multiple selected target components (benzene, toluene, ethylbenzene, *o*-xylene, and *n*-hexane), and two blends (summer and winter) of gasoline, as well as the volatile fractions of the gasoline. The target components were selected based on the prevalence and toxicological importance; the remainder of the hundreds of component chemicals were lumped into a pseudo chemical (Dennison *et al.* 2003; Dennison 2004). Technological development necessary for this effort included (1) modification of the gas uptake pharmacokinetic chamber system for more efficient incorporation of probes and (2) utilization of more efficient CO_2 absorbent. The PBPK model tracks selected target components and a lumped chemical group representing all nontarget components (Dennison *et al.* 2004a,b, 2005b; Dennison 2004). Competitive inhibition was the principal mechanism of pharmacokinetic interactions among these five selected target single chemicals and a pseudochemical from the lumped components. Computer simulation results from the six-chemical interaction model matched well with gas uptake pharmacokinetic experimental data from single chemicals, five-chemical mixture, and the two blends of gasoline (Dennison *et al.* 2003). Thus, for the first time, we have a PBPK model for a real-world complex chemical mixture.

13.3 FUTURE PERSPECTIVES: SECOND-GENERATION PBPK/PD MODELING

Although the problems and tasks associated with finding a reasonable way to handle, and eventually predict, adverse health effects due to chemical mixtures are complex, human health problems are really the final manifestation of dynamic equilibria of multiple stressors of which chemical mixtures are but one element. Thus, the potential combinations of these multiple stressors may approach infinity. In order to circumvent the study of astronomically large number of combinations, the only logical

way is to concentrate on the finite system—in this case, the human body. Dr. Craig Venter of the human genome fame stated: ". . . If we hope to understand biology, instead of looking at one little protein at a time, which is not how biology works, we will need to understand the integration of thousands of proteins in a dynamically changing environment. A computer will be the biologist's number one tool . . ." (Butler 1999). In line with this thinking, we believe that the only efficient way of studying chemical mixtures or multiple stressors is to understand our body in an integrated manner through biologically based computer simulation such as PBPK/PD modeling and very focused experimentation. In essence, this emphasis leads to a systems biology approach with tools from *in silico* toxicology.

In silico toxicology, by our defintion, means integrating computer modeling with focused, mechanistic, animal experimentation such that experiments which are impractical (e.g., too large, too expensive) or impossible (e.g., human experiments with carcinogens) to perform may be conducted by computer simulation. With this type of approach, once we have a "virtual human" in place, multiple stressors and their integrated adverse human health effects are nothing more than the perturbation of certain processes in the normal systems. In that sense, adverse health effects are therefore the manifestation of parameter changes in the computer simulation of the "virtual human." The classical compartmental pharmacokinetic models may be considered an embryonic form of "Virtual Human" (Yang *et al.* 2004b). Indeed, despite the crude nature of these models as compared with the human body, classical pharmacokinetics has contributed very significantly to the field of medicine. The advancement of PBPK modeling, as a result of "delumping" and the incorporation of physiology into the modeling process, results in a better "Virtual Human."

It is our strong belief that utilization of computer modeling is essential in the studies of toxicology of chemicals, chemical mixtures, and multiple stressors. Biology will be well-served by the application of computer technology as an alternative research method to conserve resources and minimize the use of laboratory animals. In the past few years, tools such as "Reaction Network Modeling" and "Gene Network Modeling" have become available to support computer simulation at the molecular interaction level. For more information on the specifics of these newer modeling approaches in biomedical sciences, the readers are referred to a number of publications (Andersen *et al.* 2002; Klein *et al.* 2002; Liao *et al.* 2002; Reisfeld and Yang 2004; Liao 2004). Looking into the future, linkage of PBPK modeling with "Reaction Network Modeling" and/or "Gene Network Modeling" has the potential of providing a computer simulation platform for modeling complex biological systems from the whole organism down to the molecular interaction level.

13.4 SUMMARY

In studying chemical mixtures in the last two decades or so, and the multiple stressors more recently, we reach the inevitable conclusion that it is impossible to seek knowledge on every possible environmental mixture. A reasonable alternative is therefore to begin with careful study of underlying biology. Although the body is itself a complex chemical system, compared to the infinite combinations of external chemical, physical, biological challenges as well as other multiple stressors, the body represents a finite system and a "constant." Thus, if we understand enough of this underlying biology to be able to carry out computer simulations correctly, we should be able to simulate or even predict, by computer modeling, the potential perturbations caused by these infinite number of external stressors.

With the recent advances in computational technology and biomedical research methodologies and past research on the toxicological interactions of chemical mixtures, the integration of computer modeling and focused laboratory experimentation in an iterative manner should improve modeling and risk assessment with various mixtures. For computer modeling and simulation of complex biological processes to be successful and useful, the integration of biology, chemistry, physics, engineering, and computer science is necessary.

NOTATION

AST	aspartate transaminase
BEI	the concentration or excretion rate of a biomarker in a healthy worker exposed to the TLV
BHI	biological hazard index, a parameter estimating the biological level tolerable for exposure to mixtures
C_a	the concentration of chemical in the arterial blood
C_h	concentration of substrate in the liver
C_{ex}	the concentration of chemical in exhaled air
C_{in}	the concentration of chemical in inhaled air
C_{portal}	concentration of substrate in the portal vein
C_{sys}	concentration of substrate in systemic blood
C_v	the concentration of chemical in the venous blood
CCl_4	carbon tetrachloride
CL_{int}	intrinsic metabolic clearance
CYP2E1	cytochrome P450 2E1
CYP3A4	cytochrome P450 3A4
D	dose
DBM	dibromomethane
DCE	1,1-dichloroethylene
DPD	dihydropyrimidine dehydrogenase
F_a	fraction absorbed from the gastrointestinal tract
f_b	unbound fraction in blood
5-FU	5-fluorouracil
GSH	glutathione
HbCO	carboxyhemoglobin
2,5-HD	2,5-hexanedione
I_h	concentration of inhibitor in the liver
I_{portal}	concentration of inhibitor in the portal vein
I_{sys}	concentration inhibitor in systemic blood
K_m	Michaelis constant for metabolic elimination

K_{M1}	substrate 1: enzyme binding constant
K_{M2}	substrate 2: enzyme binding constant
K_{MI1}	substrate 1: inhibitory binding constant
K_{MI2}	substrate 2: inhibitory binding constant
K_p	liver-to-blood concentration ratio
MC	methyl chloroform
MnBK	methyl *n*-butyl ketone
P1	product 1
P2	product 2
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PD	pharmacodynamic
PERC	tetrachloroethylene
Q	blood flow rate
Q_C	cardiac output
Q_f	the flow rate of blood through the fat
Q_{GI}	the flow rate of blood through the GI tract
Q_k	the flow rate of blood through the kidneys
Q_l	the flow rate of blood through the liver
Q_P	alveolar ventilation rate
Q_r	the flow rate of blood through the rapidly perfused tissue
Q_s	the flow rate of blood through the slowly perfused tissue
S1	substrate 1
S2	substrate 2
SC	simulation concentration
TCE	trichloroethylene
TLV	threshold limit value
TWA	time-weighted average
US EPA	United States Environmental Protection Agency
V_d	volume of distribution in the systemic blood compartment
V_h	volume of the liver
V_{max}	maximum metabolic rate
V_{portal}	volume of the portal vein
VOC	volatile organic solvent

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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING

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Science and Applications

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PREFACE

In recent years, there has been an enormous expansion of uses of physiologically based pharmacokinetic (PBPK) modeling in areas related to environmental chemicals and drugs. For individuals interested in PBPK modeling, it is relatively easy to locate and use the contributions of previous authors on a specific chemical of interest. However, it is more difficult to locate broader sets of contributions containing useful modeling techniques and applications. Our purpose was to provide a broad review of the PBPK modeling literature, before the size of the body of work grew large enough to make such an effort prohibitive, and to provide a resource to contain comprehensive coverage of the PBPK modeling literature from its beginnings in the mid-1900s through the first few years of the twenty-first century. This monograph is meant to be a useful reference and educational tool for those professionals and graduate students in toxicology, pharmacology, computational biology, and risk assessment interested in PBPK modeling as a tool for quantifying tissue doses and for describing the response of organisms to chemical exposures.

Our initial literature search in 2001 and updated in 2002, conducted using the Web of Science, Medline, and Toxline databases and incorporating keywords such as physiologically based pharmacokinetic/PBPK model, physiologically based toxicokinetic/PBTK model, and physiologically based pharmacodynamic/PBPD model, uncovered over 1000 references. As the term PBPK model did not become popular until the 1980s, for earlier contributions we relied on literature searches using the names of authors known by the editors to have made early contributions in the field, followed up by searches on other authors and articles cited in these articles. We chose to organize this diverse body of work based on classes of chemicals (e.g., volatile organics and environmental contaminants) and modeling purposes (e.g., perinatal transfer models and dermal absorption models). Our goal was to be fairly comprehensive, but to stress primary contributions in PBPK model development and in applications of these models to investigate factors that regulate chemical distribution within the body. We have also attempted to include articles that appeared over the past few years during completion of this volume. While we have made attempts to be inclusive in our coverage of the PBPK modeling literature, some important contributions may have been missed in our review process. We apologize to authors whose work may have been inadvertently overlooked in these various chapters and not captured by the editorial review.

This monograph describes the development of PBPK modeling for toxic compounds over the past eight decades and their current uses, providing background on the basics of PBPK modeling for understanding the physical, chemical, and biological properties that determine absorption, distribution, metabolism, and elimination of xenobiotics. Early PBPK modeling applications with volatile anesthetics and

chemotherapeutics paved the way for applying these techniques to a wide range of volatile compounds of occupational and environmental significance. The past 15 years have witnessed extensive application with many other classes of chemicals: metals, inorganic chemicals, pesticides, persistent organics pollutants, drugs, and the metabolites of these classes of chemicals. PBPK models have played important roles in unraveling dose–response behaviors based on estimates of tissue dose and have revolutionized low dose and interspecies extrapolations in risk assessment. Following an introductory chapter on PBPK modeling, a series of chapters reviews PBPK model results for various classes of compounds with coverage of historical development, modeling challenges specific to classes of chemicals, and current practices. Comments are also provided regarding the use of these PBPK models to support pharmacodynamic modeling for various toxic responses and future directions where modeling approaches will be helpful.

This monograph arose through efforts of graduate students, postdoctoral fellows, and professors at Colorado State University to review literature in specific areas and produce a series of chapters. These individuals worked in the Quantitative and Computational Toxicology Program at the Center for Environmental Toxicology and Technology in the Department of Environmental and Radiological Health Sciences. Many of these individuals have graduated from Colorado State and left for other positions. The editors wish to express their sincere appreciation for all the assistance provided by these individuals in developing this monograph. Each of these individuals is cited as the authors on the chapters where they contributed.

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