Chapter 12
Physiologically Based Pharmacokinetic Modeling of Chemical Mixtures

Sami Haddad

Abstract Physiologically based pharmacokinetic (PBPK) modeling is a tool that is increasingly being used for xenobiotics exposure assessment and target tissue dosimetry simulations in risk assessment and in pharmaceutical sciences. Because this tool can use chemical and physiological information/data from different sources (i.e., in vitro, in vivo, in silico), it is also being increasingly used for mixture exposures, especially for mixtures containing chemicals that toxicokinetically interact, at the physiological, physicochemical, and biochemical level. The aim of this chapter is to give an overview of what PBPK modeling is and how it can be used in the context of mixture toxicology. Known mechanisms of toxicokinetic interactions between xenobiotics are described, and mathematical representations are given when available. Existing modeling approaches that are available in the literature are presented for mixtures of various complexities. Current methods and their limitations are reported, and future directions are put forward.

Keywords Pharmacokinetics · Toxicokinetics · Interactions · Mixtures · Metabolism

12.1 Introduction: What Is PBPK Modeling?

Physiologically based pharmacokinetic (PBPK) models (note: toxicokinetics and pharmacokinetics are synonymous in the context of this chapter) are mathematical descriptions of pharmacokinetic processes (absorption, distribution, metabolism, and excretion) of xenobiotics that rely on appropriate physiological, biological, biochemical, anatomical, and physicochemical information. They allow for prediction or simulation of tissue dosimetry as a function of time and exposure scenario (dose and timing) (Krishnan and Andersen 2007). The level of detail of these models

S. Haddad (✉)
Department of Occupational & Environmental Health, School of Public Health, Public Health Research Institute of Université de Montréal, Université de Montréal, Montreal, QC, Canada
e-mail: sami.haddad@umontreal.ca

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C. V. Rider, J. E. Simmons (eds.), *Chemical Mixtures and Combined Chemical and Nonchemical Stressors*, https://doi.org/10.1007/978-3-319-56234-6_12

can vary extensively depending on desired dosimetry (e.g., target tissue or blood AUC, target tissue or blood C_{max}, total amount metabolized, C_{max} of metabolite in target tissue, etc.) and available information. Because of their mechanistic basis, these models are increasingly being used in toxicological risk assessment of chemicals. This is principally due to the fact that PBPK models have the advantage of allowing different types of extrapolations (e.g., animal to human, high to low dose, scenario to scenario, route to route, etc.) with much more confidence than other previously used models (e.g., non-compartmental or compartmental pharmacokinetic models) allow (Krishnan and Andersen 2007; Thompson et al. 2008; Espie et al. 2009).

PBPK models are basically composed of a series of mass-balance differential equations describing the flux of the chemical of interest in the organism (Fig. 12.1).

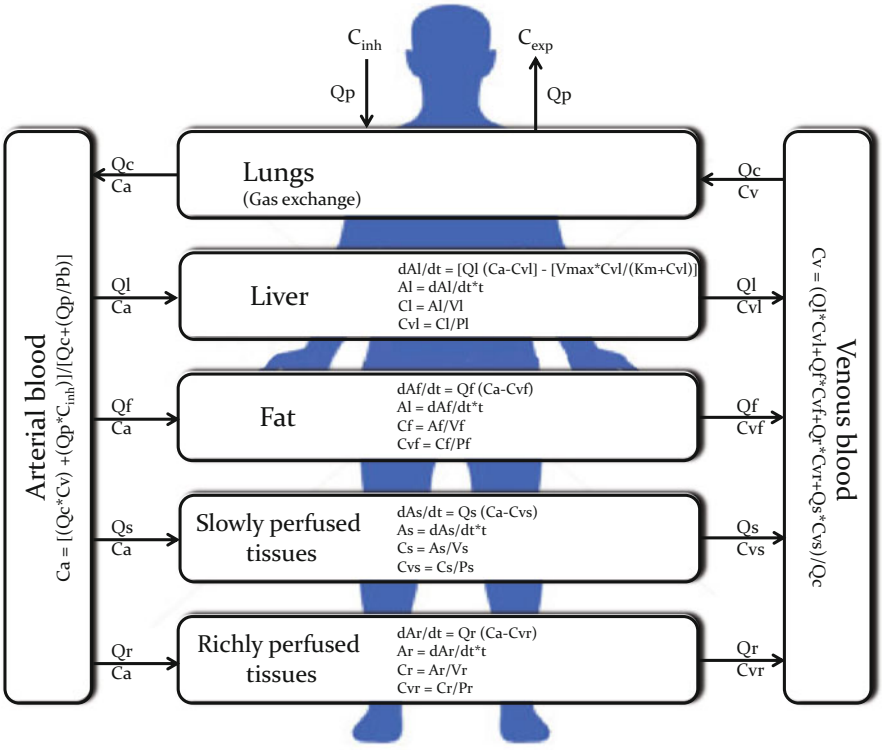

Fig. 12.1 PBPK model conceptual representation. The term d/dt refers to the derivative of the variable over time. Capital letters *A*, *C*, *Q*, *P*, and *V* refer to amount of chemical, chemical concentration, blood flow, partition coefficient, and volume. Lower case letters a, b, c, f, l, p, r, s, t, v, vf, vl, vr, and vs refer to arterial blood, blood/air, cardiac, fat, liver, pulmonary, richly perfused tissues, slowly perfused tissues, time, venous blood, venous blood leaving fat, venous blood leaving liver, venous blood leaving richly perfused tissues, and venous blood leaving slowly perfused tissues, respectively. Subscript inh and exp refer to inhaled and exhaled air. V_{max} and K_m refer to the maximal rate of metabolism and the Michaelis-Menten affinity constant, respectively

When in contact with the skin, lungs, or intestinal walls, a chemical can be absorbed and enter the blood circulation (absorption). With the arterial blood flow, the chemical can then reach the different organs of the body and accumulate, depending on its affinity with the tissue components and its capacity to cross biological membranes (endothelial wall or cellular membrane of parenchymal cells) (distribution). In some tissues, such as liver, metabolism can be an important process contributing to the elimination of the compound. This results in transforming it into another molecule (i.e., a metabolite) which can be more or less toxic than or have the same toxic potency of the parent compound (metabolism). Other processes involved in the elimination of unchanged chemicals which are often described in PBPK models are renal excretion in kidneys, exhalation in lungs, or biliary excretion in the liver (excretion).

In simple PBPK models where the chemical easily crosses tissue or cell membranes, the mass-balance differential equations describing the rate of chemical accumulation (R_acc) in a tissue compartment (subscript t) would be described by the rate of the chemical leaving the tissue with the venous blood (R_out) subtracted from the rate of the chemical entering the tissue with the arterial blood flow (R_in) as follows:

$$R\_\mathrm{acc}_\mathrm{t} = R\_\mathrm{in}_\mathrm{t} - R\_\mathrm{out}_\mathrm{t} \tag{12.1}$$

$$R\_\mathrm{in}_\mathrm{t} = Q_\mathrm{t} \times \mathrm{Ca} \tag{12.2}$$

$$R\_\mathrm{out}_\mathrm{t} = Q_\mathrm{t} \times \mathrm{Cv}_\mathrm{t} \tag{12.3}$$

where Q_t, Ca, and Cv_t are, respectively, tissue blood flow, arterial blood concentration, and venous blood concentration leaving tissue. When elimination occurs in the tissue (e.g., metabolism), an additional rate (e.g., rate of amount metabolized: R_am) must be subtracted from the R_in as follows:

$$R\_\mathrm{acc}_\mathrm{t} = (Q_\mathrm{t} \times \mathrm{Ca}) - (Q_\mathrm{t} \times \mathrm{Cv}_\mathrm{t}) - R\_\mathrm{am}_\mathrm{t} \tag{12.4}$$

The amount of chemical in the tissue can then be estimated by integrating, i.e., numerically solving, the mass-balance differential equations using algorithms available in simulation software (e.g., ACSLX, Stella, MatLab). The concentration is determined by dividing the amount of chemical in tissue by the tissue volume (Eq. 12.5), and the Cv_t is determined by the tissue/blood partitioning (P_t) (Eq. 12.6).

$$C_\mathrm{t} = \frac{A_\mathrm{t}}{V_\mathrm{t}} \tag{12.5}$$

$$\mathrm{Cv}_\mathrm{t} = \frac{C_\mathrm{t}}{P_\mathrm{t}} \tag{12.6}$$

Many toxicokinetic processes in PBPK models can be described with first order mathematical descriptions, such as R_in and R_out in Eqs. 12.2 and 12.3 above. This means that the rate of the process of interest is directly proportional to the appropriate blood or tissue concentration of the chemical (e.g., passive diffusion across membranes). In some instances, kinetic processes must be described using a

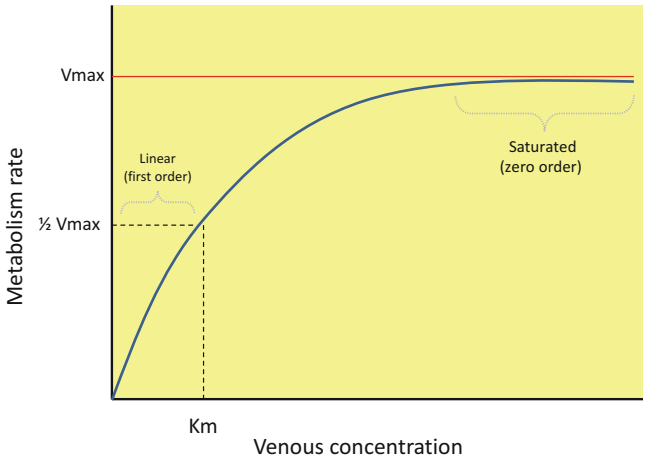

Fig. 12.2 First order process vs saturable process exemplified with metabolic rates

saturation model. This is more likely to happen at higher-dose regions. The same process may be described as a first order process when only low exposure levels are of interest but must be changed to a saturable process when simulating higher exposure levels. In such cases, at low levels of exposure, the simulated rate of the process seems to increase with dose, but at a certain dose range, it levels off to a maximal rate to attain zero order (i.e., rate becomes a constant that is invariable with dose) (Fig. 12.2). This is often the case for describing metabolic rates (R_am). For instance, at low exposure levels, the use of an intrinsic clearance constant (Cl_{int}; first order constant) is often sufficient (Eq. 12.7) for describing the metabolic rate of a chemical, whereas, at higher exposure doses, the metabolic rate becomes saturated and must be described using a maximal rate of metabolism (V_{max}) and a Michaelis-Menten affinity constant (Km) (Eq. 12.8).

$$\text{First order} : R\_\text{am} = \text{Cl}_{\text{int}} * \text{Cvl} \tag{12.7}$$

$$\text{Saturable} \quad : R\_\text{am} = \frac{\text{Vmax} * \text{Cvl}}{\text{Km} + \text{Cvl}} \tag{12.8}$$

where Cvl refers to venous blood leaving the liver.

The equations that compose the PBPK model are populated with parameters that are specific to the organism (physiological parameters), the chemical or chemicals under investigation (physicochemical parameters), and the reactions between the organism and the chemical (biochemical parameters) (Table 12.1).

When the organism is exposed to additional chemicals, the toxicokinetics may be unaffected, and therefore no further considerations in terms of PBPK modeling are necessary. But in many circumstances, co-exposure to one or more chemicals may change the relationship between external dose and internal dosimetry of the chemical of interest.

Table 12.1 PBPK model parameters

Physiological	Physicochemical	Biochemical
Cardiac output[a]	Partition coefficients	Rate constants (V_{max}, K_m) for
Alveolar ventilation rate[a]	Tissue/blood[a]	Enzymatic reactions[a]
Body weight[a]	Blood/air[a]	Active transport in:
Tissue volume[a]	Skin/air	Urinary excretion
Tissue blood flow[a]	Skin/water	Biliary excretion
Tissue blood content	Permeability coefficients	Tissue uptake
Tissue lipid and water content		Tissue efflux
Skin surface area		GI absorption
Glomerular filtration rate		Macromolecular binding constants
		B_{max}
		Kd

[a]Most frequently used PBPK model parameters

12.2 Pharmacokinetic Interactions

When the tissue or blood concentration vs time profile of one chemical (chemical *A*) is modified by co-exposure to another chemical (chemical *B*), it is a clear indication that a pharmacokinetic interaction is occurring. These interactions are the result of a pharmacokinetic mechanism being affected by the other chemical. In terms of PBPK modeling, it can either be the result of an alteration of a physiological parameter value (e.g., increased ventilation rate), a physicochemical parameter (e.g., increased lipophilicity due to complexation), or modified biochemical parameters (e.g., increased V_{max} due to enzyme induction). In the next section of the chapter, common mechanisms of pharmacokinetic interactions will be reviewed.

12.2.1 Chemicals Altering Physiology

Exposure to some xenobiotics can lead to alterations of physiological factors that are critical determinants of toxicokinetic processes. Hence, when such an event occurs, the toxicokinetics of all co-exposed chemicals are modified if the physiological parameter in question plays a role in their absorption, distribution, metabolism, or elimination. Examples of physiological parameters that are altered by exposure to chemicals are provided below.

One parameter that can be altered by the presence of chemicals is the alveolar ventilation rate (Qalv). It has been shown that salicylate poisoning and amphetamines cause increased ventilation by raising carbon dioxide. This increase in ventilation is the body's attempt to compensate for excess carbon dioxide (Crisp and Taylor 2012). Other chemicals can augment the alveolar ventilation rate by diminishing the cellular respiration, for example, carbon monoxide diminishes hemoglobin capacity in oxygen binding, hydrogen cyanide inhibits cytochrome c oxidase which plays a crucial role in the electron transport respiratory chain in the

mitochondria, DDT inhibits ATP-synthase, and dichlorovinyl cystein inhibits pyruvate dehydrogenase in the Krebs cycle (Gregus 2008). In PBPK modeling, a modification of Qalv will lead to changes in the pulmonary absorption rate (and pulmonary elimination) of volatile chemicals as it is used to determine the concentration in arterial blood as follows:

$$\mathrm{Ca} = \frac{\mathrm{Qc} \times \mathrm{Cv} + \mathrm{Qalv} \times \mathrm{Ci}}{\mathrm{Qc} + \mathrm{Qalv}/\mathrm{Pb}} \tag{12.9}$$

where Qc refers to the cardiac output, Ci to the inhaled concentration of chemical, Cv to the venous blood concentration, and Pb is the blood air partition coefficient of the chemical.

Many xenobiotics are known to affect hemodynamics (i.e., blood flow) in humans or animals. This has the result of changing Qc or Qt. Necessarily, the toxicokinetics of all co-exposed chemicals would be affected accordingly, through altered tissue distribution, altered elimination in tissue, or even pulmonary absorption. For instance, ethanol and phenobarbital increase hepatic blood flow (Ql), hence increasing clearance of all co-exposed chemicals that have a high hepatic extraction ratio (Krishnan et al. 1994). Vasodilators and vasoconstrictors will influence the distribution of co-exposed chemicals by altering tissue blood flows. For example, many drugs have been shown to alter renal hemodynamics (e.g., hypertensive agents, nonsteroidal anti-inflammatory drugs (NSAIDs), some immunosuppressants, aminoglycosides, amphotericin *B*) (Hsu and Wu 2012). This may lead to changes in renal clearance of other chemicals due to decreased glomerular filtration rates which can sometimes be irreversible.

Upon exposure to some xenobiotics, gastric emptying may be affected, and, hence, the absorption of ingested chemicals or orally administered drugs can be altered. For example, Nimmo et al. (1975) demonstrated that absorption of orally administered acetaminophen was considerably delayed when subjects were administered pethidine or diamorphine by intramuscular injection. Other drugs have been shown to delay gastric emptying and upper gastrointestinal tract motility (e.g., opioids, anticholinergics, and adrenergic receptor agonists). Several drugs are known to increase the motility of the upper gastrointestinal tract. Among them are the gastrointestinal prokinetic drugs (e.g., metoclopramide, cisapride, domperidone), which may increase rates of absorption but in some instances also decrease bioavailability because of reduced available time for total absorption (Greiff and Rowbotham 1994). Another physiological factor that can be altered to modify the rate of absorption of orally exposed chemicals is the gastric or intestinal pH (De Castro et al. 1996; Budha et al. 2012).

Skin structure or composition can be modified by exposure to a chemical. This can lead to changes in dermal absorption rates of other xenobiotics. Isobutanol has been shown to change skin composition (dehydration) and reduce the absorption of m-xylene (Riihimaki 1979). Dermal permeability of lipophilic compounds has been shown to be increased by dimethyl sulfoxide (DMSO) skin exposure (Hayes and Pearce 1953; Jacob et al. 1964; Choi et al. 1990). DMSO causes swelling of basal

cells of the stratum corneum as well as a disruption of keratin matrices in skin (Kurihara-Bergstrom et al. 1987; Qiao et al. 1996).

Although all these xenobiotic-induced physiological changes have been observed, to our knowledge, none have been described mathematically to ultimately be used in a multichemical PBPK model. Proper dose-response relationships for physiological changes would need to be characterized for them to be incorporated into a PBPK model.

12.2.2 Chemicals Altering Physicochemical Properties

There are very few examples where physicochemical properties of a chemical are modified by the presence of another xenobiotic. A documented example of this is the increased membrane permeability of lead in the presence of dithiocarbamates (Oskarsson and Lind 1985). Indeed, dithiocarbamates can form a complex with lead that is more lipophilic than lead alone, and therefore distribution to brain is increased. Organic chelators such as EDTA can also increase the lipophilicity of ionic metals and therefore alter their capacity to distribute.

Another example of alteration of physicochemical properties is co-exposure to ethanol and mercury. Ethanol is known to depress the conversion of elemental mercury to the ionic form (Kudsk 1965). Elemental mercury, being more volatile than the ionic form, is therefore more easily eliminated by exhalation.

12.2.3 Chemicals Affecting Chemical-Biological Interactions

In terms of published literature on toxicokinetic interactions, chemical-biological interactions are by far the most cited, and many examples of mathematical descriptions exist between multiple xenobiotics. This category of interactions basically results in a modification of biochemical parameters affecting metabolic rates, transport rates, or protein binding. The mechanisms that are affected therefore involve proteins implicated in a critical kinetic process of the xenobiotic of interest. The different types of interactions existing in this category can be divided into two categories: (1) mechanisms affecting the level of active proteins (concentration of enzymes, transporters, or binding proteins) and (2) mechanisms affecting the activity of a protein.

(a) Mechanism Affecting the Level of Active Proteins

The concentration of an active protein (P_a) will basically depend on its synthesis rate (R_P_{synth}), its degradation rate (R_P_{deg}), and its inactivation rate (R_P_{inact}) which is usually as follows:

$$\mathrm{RC\_}[P_a] = R\_P_{synth} - R\_P_{deg} - R\_P_{inact} \tag{12.10}$$

where RC_[P_a] refers to the rate of change in active protein concentration involved in xenobiotic metabolism, transport, or binding. The consequence of a change in [P_a] will be a proportional change in V_{max} for enzymes and active transporters as $V_{max} = K_{cat}$ [P_a] where K_{cat} is the turnover rate or of B_{max} (maximal binding capacity) for binding proteins as $B_{max} = n$ [P_a] where n refers to the number of binding sites.

Increased Protein Synthesis Many xenobiotics are known to increase (i.e., induce) the activity of proteins, which can occur through different mechanisms. One way to achieve this is through an increase in protein concentration. There are many compounds that are known to interact with and activate transcription factors (e.g., PXR, CAR, FXR, AhR, PPARα, etc.) which in turn activate the transcription and synthesis of different enzymes and other proteins (binding proteins or transporters). In terms of mathematical representation of such phenomena, the increased synthesis of CYP1A1 and 1A2 by TCDD was described by a factor representing aryl hydrocarbon receptor binding (Andersen et al. 1997; Leung et al. 1990), and Sarangapani et al. (2002) similarly described induction of CYP2B1/2 by octamethylcyclotetrasiloxane via an unknown receptor. In both cases, the R_P_{synth} is modulated as a function of the fraction of receptor occupancy (F_{RO}) typically modeled using a Hill model as follows:

$$R\_P_{synth} = R\_P_{synth_0} + \left(\left[R\_P_{synth_{max}} - R\_P_{synth_0}\right] \times F_{RO}\right) \tag{12.11}$$

$$F_{RO} = \frac{[FL]^n}{[FL]^n + Kd^n} \tag{12.12}$$

where RP_{synth_0} and $RP_{synth_{max}}$ are the basal and maximal rates of protein synthesis, Kd is the dissociation constant, FL is the free ligand concentration in cells where the receptor is present, and n is the hill coefficient which is dependent on the receptor.

Increased Protein Stability An increase in protein activity can also be achieved by stabilizing the protein, i.e., by reducing the value of R_P_{deg}. Indeed, an example of such a mechanism was described by Chien et al. (1997) where ethanol stabilizes CYP2E1, which consequently increases the overall concentration of the enzyme and therefore its activity. In this example, schematized in Fig. 12.3, the enzyme (i.e., CYP 2E1) would be found in two forms, distinguishable by rate of degradation: the form that is rapidly degraded (P_a^1) and another form which is the slowly degraded enzyme (P_a^2). The authors' hypothesis was that CYP2E1 was synthesized at a given rate into the pool of P_a^1 and it can be converted to P_a^2 according to a transfer rate constant K_{trans}. In the form P_a^1, the degradation rate of the protein is rapid when not bound to ligand and slow when bound. When P_a^1 is highly bound with ligand, the concentration of P_a increases, and therefore the turnover to the P_a^2 form increases. Equation 12.10 can therefore be modified as follows to describe the rate of change in protein concentration for the CYP2E1 in the rapid degradation form:

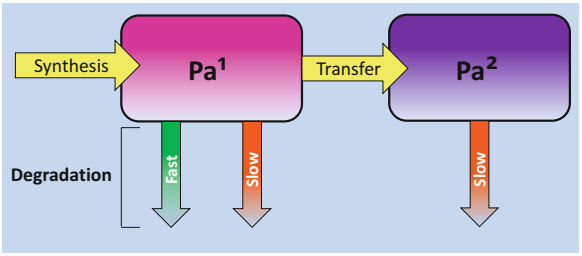

Fig. 12.3 Conceptual representation of CYP2E1 induction by ligand stabilization according to Chien et al. (1997). The CYP2E1 enzyme can occur in the rapidly degraded form ($P_a{}^1$) and the slowly degraded form ($P_a{}^2$) (Modified from Chien et al. 1997)

$$\mathrm{RC\_}\left[P_a{}^1\right] = R\_E_{\mathrm{synth}} - R\_P_{\mathrm{deg}} - R\_P_{\mathrm{trans}} \tag{12.13}$$

$$\text{where } R\_P_{\mathrm{deg}} = \left[P_a{}^1\right] \times \left(f_{\mathrm{unbound}} \times K_{\mathrm{deg}}^{\mathrm{fast}} + f_{\mathrm{bound}} \times K_{\mathrm{deg}}^{\mathrm{slow}}\right) \tag{12.14}$$

$$\text{and } R\_P_{\mathrm{trans}} = \left[P_a{}^1\right] \times K_{\mathrm{trans}} \tag{12.15}$$

The rate of change of concentration of the slowly degraded form of the enzyme is determined as follows:

$$\mathrm{RC\_}\left[P_a{}^2\right] = R\_P_{\mathrm{trans}} - R\_P_{\mathrm{deg}}{}' \tag{12.16}$$

$$\text{and } R\_P_{\mathrm{deg}}{}' = \left[P_a{}^2\right] \times K_{\mathrm{deg}}^{\mathrm{slow}} \tag{12.17}$$

where R_P_{trans} is the rate of transfer, f_{unbound} is the fraction of E_a that is not bound to ligand, and f_{bound} is the fraction of $P_a{}^1$ that is bound to the stabilizing ligand. The slow and fast degradation rate constants are $K_{\mathrm{deg}}^{\mathrm{fast}}$ and $K_{\mathrm{deg}}^{\mathrm{slow}}$, and the transfer rate constant is K_{trans}. The fractions bound and unbound can be calculated using the information of dissociation constant (Kd) for the ligand.

Protein Inactivation Proteins may be inactivated in many ways by xenobiotics. There are several published examples of enzymes being irreversibly inhibited/inactivated by xenobiotics. Generally, the inhibitor or inactivator binds irreversibly to the active site, consequently stopping all catalytic activity. As can be deduced from Eq. 12.10, an increase in R_P_{inact} will lead to a decrease in P_a levels. The same logic would apply for binding proteins or active transporters. Enzyme inactivation has been mathematically described for binary mixtures where a component inhibits the metabolic rate of the other component by this mechanism: triazolam and erythromycin (Kanamitsu et al. 2000a), 5-fluorouracil and sorovidine (Kanamitsu et al. 2000b), and trichloroethylene and its metabolite dichloroacetate (Keys et al. 2004). In the absence of the inactivator, the R_P_{inact} is nil, and levels of P_a remain stable. Upon introduction of the inactivator into the system, the R_P_{inact} becomes positive according to the following equation:

$$R\_P_{\text{inact}} = \frac{K_{\text{inact}} \times [P_a] \times f_{\text{bound}} \times \frac{[I]_t}{P_t}}{\text{Ki}_{\text{app}} + f_{\text{bound}} \times \frac{[I]_t}{P_t}} \tag{12.18}$$

where K_{inact} represents the maximum inactivation rate constant, P_t represents the tissue-to blood partition coefficient, f_{bound} is the unbound fraction in blood, and $[I]_t$ is the inactivator's concentration in tissue where P_a is located.

(b) Mechanisms Affecting the Activity of Proteins

The activity of proteins can be modified without actually changing their concentration. The most frequently published mechanism of toxicokinetic interactions is found in this category, the conventional reversible inhibitions, including competitive, noncompetitive, and uncompetitive inhibition. Other, less frequently reported types of interactions affecting activity of proteins are the allosteric interactions. Also, the depletion of cofactor reserves is another way to alter the rate of protein activity.

Competitive Inhibition When two chemicals compete for the same active site (on an enzyme or active transporter), competitive inhibition occurs. This competition may occur between two substrates for the same active site or between a substrate and another chemical that simply acts as an inhibitor. The consequence of this type of interaction is the apparent decrease in ligand affinity (i.e., increase in apparent Michaelis-Menten affinity constant: $K_{m_{app}}$) as a function of inhibitor concentration ($[I]$) and affinity (K_i) and hence a reduction in rate of activity (R_{activity}) (i.e., metabolism or transport), and V_{max} remains unchanged (Segel 1974), as follows:

$$K_{m_{app}} = K_m \times \left(1 + \frac{[I]}{K_i}\right) \tag{12.19}$$

$$R_{\text{activity}} = \frac{V_{max_{app}} \times \text{Cv}_t}{K_{m_{app}} + \text{Cv}_t} \tag{12.20}$$

There can also be competition between two or more chemicals for a binding site on a binding protein or transporter leading to binding displacement (e.g., tolbutamide and sulfonamides for plasma protein binding) (Sugita et al. 1982). The principle is the same as for metabolism or transport, and the apparent affinity of the xenobiotic for the protein is reduced (i.e., increase in apparent dissociation constant: $K_{d_{app}}$) as a function of inhibitor concentration ($[I]$) and its dissociation constant (K_{d_i}) leading to a decrease in the concentration of the chemical that is bound (C_{bound}), as follows:

$$K_{d_{app}} = K_d \times \left(1 + \frac{[I]}{K_{d_i}}\right) \tag{12.21}$$

$$C_{\text{bound}} = \frac{B_{\max} \times C_{\text{free}}}{K_{d_{\text{app}}} + C_{\text{free}}} \tag{12.22}$$

Noncompetitive Inhibition In some cases, a binding site modulating enzyme or transporter activity, which is different from the active site, may exist on the active protein. A noncompetitive inhibitor may bind this modulating binding site and affect the metabolic or transport rate of another chemical. The change in protein conformation by the inhibitor is mathematically reflected by a reduction in apparent $V_{\max}$, and K_{m} remains unaffected, as follow (Segel 1974):

$$V_{\max_{\text{app}}} = \frac{V_{\max}}{\left(1 + \frac{[I]}{K_{\text{i}}}\right)} \tag{12.23}$$

Uncompetitive Inhibition An inhibitor that binds only the protein-substrate complex (ES) is an uncompetitive inhibitor. Such an inhibitor, observed mostly with enzymes, will affect the catalytic function but not the substrate binding by causing structural distortion of the active site. Because free enzyme is temporarily reduced, $V_{\max_{\text{app}}}$ is reduced as well (as in Eq. 12.23), and the apparent affinity seems to be increased due to a shift of the reaction (Enzyme + Substrate→ES) to the right, as follows (Segel 1974):

$$K_{\text{m}_{\text{app}}} = \frac{K_{\text{m}}}{\left(1 + \frac{[I]}{K_{\text{i}}}\right)} \tag{12.24}$$

An interesting study by Barton et al. (1995) modeled the disappearance of trichloroethylene (TCE) and vinyl chloride from a closed vapor uptake chamber during concomitant rat exposure and showed how the three types of inhibition descriptions (competitive, noncompetitive, and uncompetitive) best fit the co-exposure data. This allowed elimination of noncompetitive inhibition as a mechanism of interaction between both chemicals but could not discriminate between competitive and uncompetitive inhibition with this particular exposure data set. The authors further pointed out that competitive inhibition could simulate all data sets with the same parameter values. In contrast, with uncompetitive inhibition, although multiple data sets were well simulated using the same kinetic parameters, several key mixture data sets were simulated only by varying parameter values.

Allosteric Interactions There are other examples of increased enzyme activity related to co-exposure that do not implicate a change in concentration of protein. A few enzyme kinetic studies on interacting xenobiotics have shown that some enzymes with multiple binding sites, particularly CYP 3A4, demonstrate unusual kinetics in the presence of another substrate or inhibitor. Different models were proposed for such cooperative binding (Kenworthy et al. 2001). The authors describe three different allosteric interaction models: (a) a two-site model with

competition between substrate and effector which can activate the enzyme at low concentrations but inhibit it at high concentrations; (b) a three-site model for heteroactivation where two substrates can bind cooperatively and stimulate metabolism at the activator site; and (c) a three-site model with inhibition including a substrate and an inhibitor (a more detailed description and complex equations can be found in (Kenworthy et al. 2001)). As an example of such cooperative activation and inhibition interactions, the rate of formation of 3-hydrodiazepam from diazepam increases up to nearly almost 400% in the presence of testosterone, and the formation of 6β-hydroxytestosterone from testosterone is inhibited 45% by diazepam.

Cofactor Depletion The cellular reserves of cofactors for phase 2 metabolic reactions (UDPGA, PAPS, GSH, etc.) are usually considered to be amounts well over saturation levels, making the description of reaction rate limited only by substrate concentrations. In some instances, the reserves of cofactors may be depleted well below saturation levels, rendering the reaction rate of substrate also dependent on levels of cellular concentrations of cofactors. The reaction rate, therefore, must be described as a bi-enzyme kinetic reaction where both cofactor and substrate concentration must be considered for the calculation of the reaction rate (Marangoni 2003). Of course, co-exposure to chemicals utilizing cofactors will affect the metabolic rate of other compounds using the same cofactor, independently of isoenzyme used. A description for this inhibition mechanism was used in a PBPK modeling study by (Zurlinden and Reisfeld 2015) for acetaminophen and its major metabolites (i.e., the sulfo-conjugate and the glucurono-conjugate) in humans. In this particular example, substrate and co-substrate inhibition (Forrest et al. 1982; Mutlib et al. 2006; Nagar et al. 2006) must also be considered, and the description of conjugation is as follows:

$$R_{\text{conjugation}} = \frac{V_{\max} \times C_t^S \times F_t^{cf}}{\left(\mathrm{K_m}^S + \times C_t^S + \times \frac{\left(C_t^S\right)^2}{\mathrm{Ksi}}\right)\left(K_m{}^{cf} + F_t^{cf}\right)} \tag{12.25}$$

where $F_t^{cf}$is the fraction of available cofactor in the metabolizing organ and K_{si} is the inhibition constant for the substrate inhibition. Superscripts S and cf are for substrate and cofactor, respectively. Although this particular example with acetaminophen very nicely describes the cofactor depletion phenomena, it is not in the context of co-exposures to other chemicals. But clearly, concomitant, or even subsequent, exposure of acetaminophen to another chemical metabolized by UGTs would be affected by the depletion of the UDPGA cofactor, and the rate of conjugation would have to be described accordingly.

12.3 PBPK Modeling Strategies for Mixtures

In this section, an overview of techniques or strategies is presented for pharmacokinetic modeling of mixtures and for describing or predicting the kinetics of mixture components. Having a multichemical exposure does not necessarily mean that pharmacokinetic interactions occur between all mixture components. None may actually occur, or some or all components may be affected by the presence of others. A simple way of determining which mixture components' kinetics are affected by the other mixture constituents is by comparing the pharmacokinetics (e.g., blood concentration vs time profiles) of the mixture's constituents when administered as a mixture with the kinetics of each constituent administered alone (at the same dose and exposure scenario). The components that show the same pharmacokinetic profiles in mixture and single chemical exposures are not affected by mixture constituents, unless the impact of multiple interactions caused by different chemicals cancel each other, which is rather unlikely. When developing a PBPK model for a mixture, it is important to identify the components that interact with each other and those that do not. The simplest situation is a mixture with no interaction between constituents. In this case, the PBPK model for the mixture can be developed exactly in the same way as if each chemical component were administered alone (single chemical exposures). When interactions occur (i.e., toxicokinetic profiles differ between single and mixture exposure), then chemical culprits (inhibitor, inducer, chelator, etc.) and mechanisms of interaction (see previous section) should be identified, if possible, to develop the mixture model appropriately. This may become very tedious depending on the level of complexity of the mixture, or even the level of knowledge of chemical components within the mixture.

12.3.1 Binary Mixtures

To date, many PBPK models have been published for binary mixtures for all sorts of chemicals, e.g., aromatic and chlorinated solvents (Andersen et al. 1987), petroleum products (Ali and Tardif 1999; Jang et al. 2001), drinking water contaminants (Niu et al. 2015; Tan et al. 2007; Isaacs et al. 2004), and medicinal drugs (Ishigam et al. 2001; Boom et al. 1998; Sugita et al. 1982; Russel et al. 1987, 1989), among others. When developing a model for such mixtures, it is common practice to start with single chemical PBPK models of both mixture components or to develop them if not available.

The next step would be to link both models together by the hypothesized mechanism of interaction using the appropriate mathematical description (see Chap. 9). The hypothesis can be supported by available information from in vitro experiments, known biotransformation pathways, pharmacokinetic experiments, etc. In a pharmacokinetic interaction, a mixture component may be the culprit (chemical modifying the pharmacokinetics of the other chemical) or the victim (the chemical

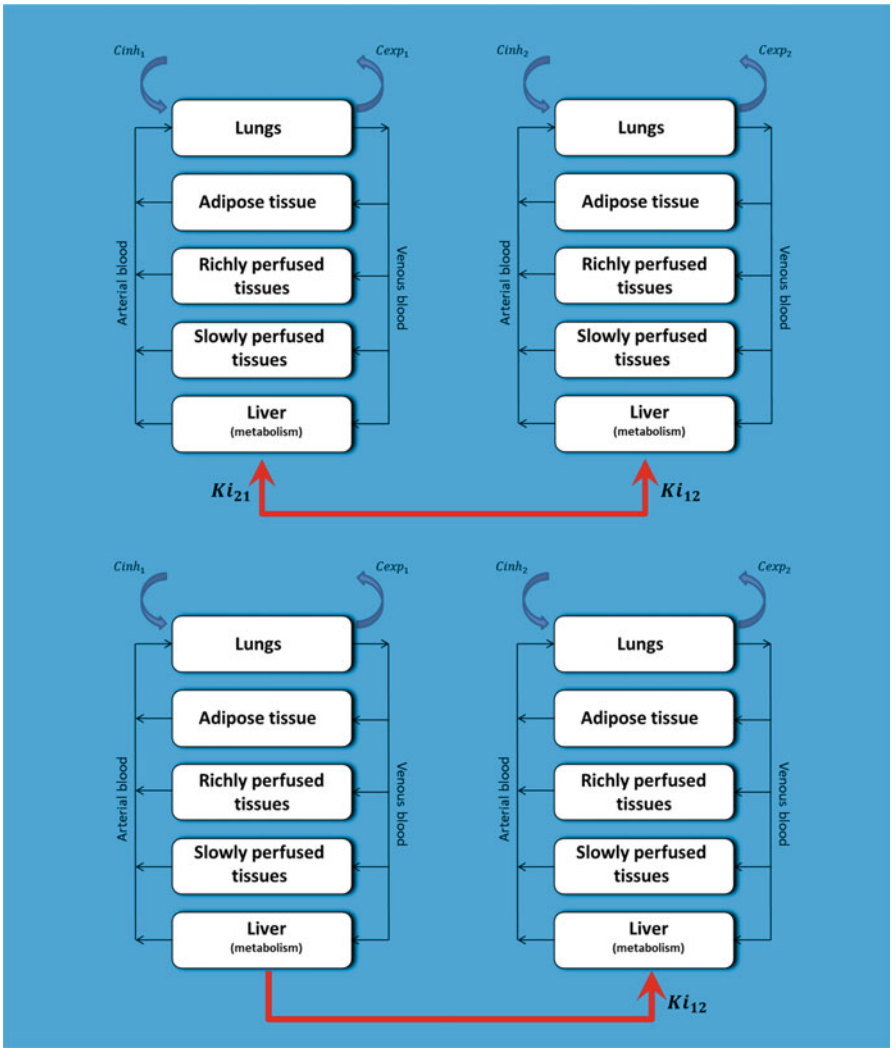

Fig. 12.4 Illustration of two examples of PBPK models for interacting pairs of chemicals. Top portion illustrates a mutual interaction, whereas bottom portion illustrates a one-way interaction where the chemical on the left is the culprit and the chemical on the right is the victim of the interaction

whose pharmacokinetics is being modified by the culprit) or both (Fig. 12.4). If chemical *A* is the culprit and chemical *B* is the victim in the interaction, then only the mathematical description of the pharmacokinetics of *B* will be modified to change absorption, metabolism, distribution and/or excretion as a function of the appropriate tissue dose of chemical *A* (e.g., liver concentration of *A* will affect hepatic metabolic rate of *B* if metabolic inhibition occurs). Alternatively, if both chemicals affect the

kinetics of the other chemical, both their mathematical descriptions will be modified according to the mechanism of interaction as a function of the culprit's appropriate tissue dose.

12.3.2 Mixtures of Greater Complexity

When pharmacokinetic interactions occur between more than two chemicals, it can become more complicated to describe the situation. An impact on the tissue dose of one chemical (chemical *A*) by the presence of another (chemical *B*) will affect all other chemicals with which chemical *A* interacts. The chemicals with modulated tissue dose will in turn affect the tissue levels of all chemicals they interact with and so on.

The situation can become even more complicated when a mixture component is biotransformed into a metabolite which interacts with its parent compound (e.g., product inhibition) or even other mixture components/metabolites. Another hurdle to tackle in mixture toxicology is that mixture components are not always fully identified. The following subsections relate to approaches that have been proposed to deal with these problems.

12.3.2.1 Extrapolating In Vivo Binary Interactions to Complex Mixtures

To resolve the problem of PBPK modeling of mixtures with more than two interacting components, Krishnan's laboratory at Université de Montréal proposed and validated an approach that allows the interlinkage of the pharmacokinetics of all interacting chemicals in a single model (Tardif et al. 1997; Haddad et al. 2001; Haddad and Krishnan 1998; Haddad et al. 1999a, 1998; Krishnan et al. 2002). This is done by linking each of the chemical models by the description of the binary interaction, forming a "web of interactions" (Fig. 12.5). This generates a web of pharmacokinetic interconnections, and all chemicals in that web are affected by a modulation of tissue dose of one of the mixture components. This also applies to metabolites that interact with the mixture components. In the latter case, the metabolite needs to be added to this chemical web (an additional PBPK model should be made for this metabolite) and linked appropriately. When a chemical in the mixture does not interact, it can be in the web but without any linkage to other chemicals.

This binary interaction-based PBPK modeling of mixtures has been validated in vivo in rats with ternary and quaternary mixtures of aromatic hydrocarbons, i.e., toluene, ethylbenzene, and m-xylene by Tardif et al. (1997) and benzene, toluene, ethylbenzene, and m-xylene by Haddad et al. (1999a) and with the addition of a fifth component, dichloromethane (Haddad et al. 2000b), as well S-8 and JP-8 jet fuel mixtures (Martin et al. 2012). Although this approach offers an accurate model to describe the mixture, it has high data requirements. Because all binary interactions

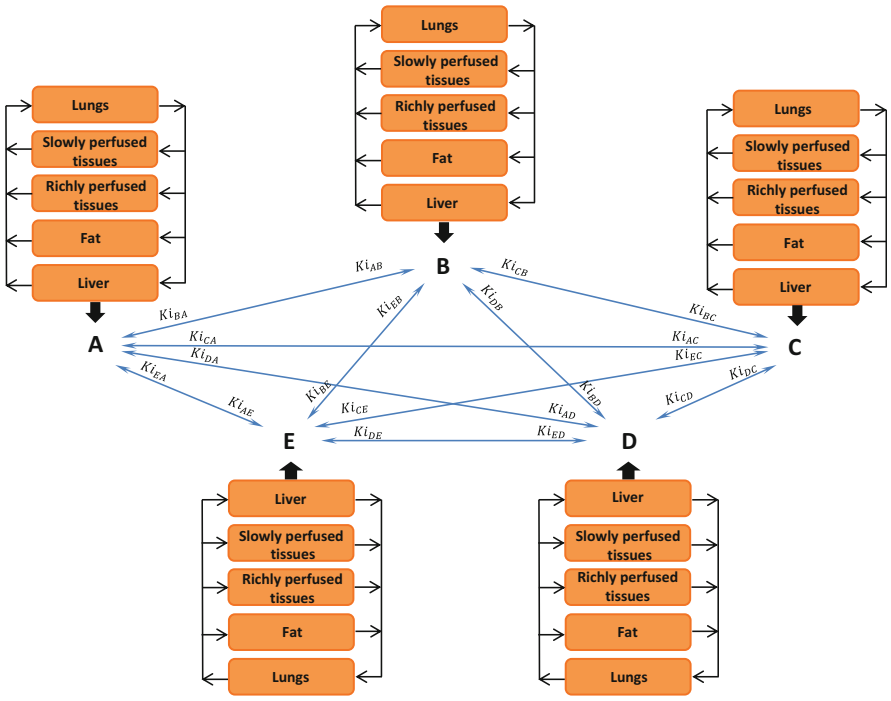

Fig. 12.5 Conceptual representation of a web of binary interactions in a quinary PBPK mixture model

must be described between all interacting components, they must all be characterized. This task may become time-consuming, animal intensive, and costly when the mixture components are numerous. The number of binary interactions (N) to characterize in a whole mixture of "n" components is determined as follows:

$$N = n\,(n-1)/2 \tag{12.26}$$

According to this equation, for a mixture of ten interacting chemicals, 45 binary interactions should be characterized to apply this interaction-based approach. To overcome this hurdle in mixture PBPK modeling, alternative methods have been proposed for mixtures with large number of components. In these mixtures, it is simply currently too costly to characterize all in vivo binary interactions, and oftentimes the identity of all components has simply not yet been determined.

12.3.2.2 QSAR Approach

Building a mixture PBPK model can prove to be difficult when data on binary interactions, chemical biotransformation, and partitioning are not fully characterized. Instead of going through full parameter characterization to develop a PBPK

model, a useful initial step can be to use quantitative structure-activity relationships (QSAR) to estimate the model parameter values. Price and Krishnan (Price and Krishnan 2011) developed QSAR algorithms for volatile organic chemicals to estimate partition coefficients, V_{max}, and K_m based on chemical structures of 53 different chemicals. Using estimated parameters, they predicted the toxicokinetics of different mixtures by assuming competitive inhibition and assuming K_i values were equal to K_m. The use of QSAR approaches can rapidly give health assessors an idea of the amplitude of interactions if competitive inhibition is the expected mechanism between mixture constituents.

12.3.2.3 Unidentified Components: Chemical Lumping

Many or most complex environmental mixtures to which humans are exposed to are not completely characterized, but some constituents may be of interest to estimate exposure. Such is the case with gasoline where a few components, such as benzene, toluene, ethylbenzene, xylene, and n-hexane, are chemicals of toxicological interest, and thousands of other hydrocarbons (isoalkanes, n-alkanes, aromatic derivatives, and smaller amounts of alkenes and alkynes) have lesser or no known toxicological significance. Simulating all constituents of such mixtures would be an enormous task and is not feasible due to resource and time constraints. In addition to difficulties in determining all parameter values for all components, validation would also prove to be nearly impossible because of limitations in chromatographic separation of all mixture components.

To circumvent such a problem, Dennison et al. (2003) devised an original strategy for gasoline mixtures. In their study, they proposed to lump most of the gasoline chemical components together by considering them as a single chemical entity and leaving the known toxicologically relevant components as separate entities, assuming competitive inhibition among components. The approach was similar to the binary mixtures approach assuming competitive inhibition between all mixture components (Haddad and Krishnan 1998). Known parameter values for partition coefficients, V_{max} and K_m, were used (i.e., for benzene, toluene, ethylbenzene, xylene, and n-hexane), and K_i values were set equal to K_m values. All other parameters related to the chemical lump were mathematically optimized to observed values, and again K_i was set equal to K_m. The characteristics of the lumped compartment changed with gasoline blend (winter blend vs summer blend). This study demonstrated the feasibility of reducing the number of model parameters in a mixture model enabling a targeted focus on toxicologically relevant mixture components.

12.3.2.4 Physiological Limits of Interactions

An alternative to the interaction-based PBPK model approach is to simply consider the physiological limits to determine the plausible range of internal exposure

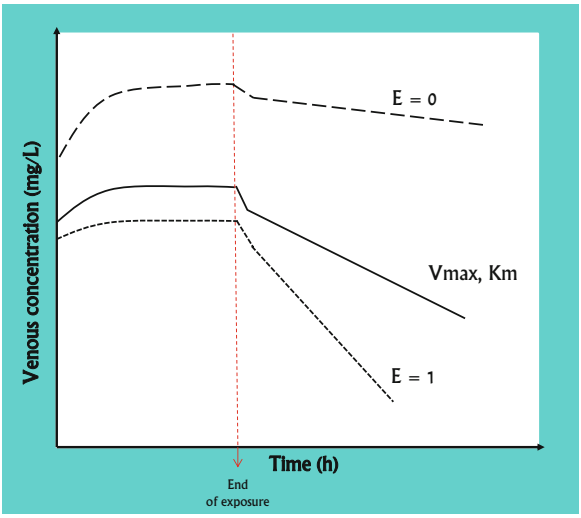

Fig. 12.6 Simulations of blood concentration vs time profiles of toluene in rats following a 4 h inhalation exposure considering average rat clearance (solid line), maximal inhibition of hepatic clearance (dashed line), and maximal induction of hepatic clearance (dotted line). Hepatic extraction ratio is represented by E (Modified from Haddad et al. 2000a)

(Haddad et al. 2000a). The logic is simple: if the sole mechanism of interaction occurs essentially at the level of hepatic metabolism, then we can determine the maximal and minimal tissue dose that a mixture component can attain if its pharmacokinetics are modulated by co-exposure. These limits are determined by fixing chemical hepatic clearance equal to hepatic blood flow (i.e., maximal impact of enzyme induction on clearance leading to a hepatic extraction ratio of 1) and to zero where the biotransformation is totally inhibited (i.e., hepatic extraction ratio = 0) (Fig. 12.6).

Although this method does not allow the risk assessor to determine with precision the concentration time profile of mixture constituents, it does allow clear estimation of the maximal value of tissue dose which would be protective/conservative in terms of health risk assessment toward potential increased internal exposure due to combined exposures. Furthermore, this method is independent of the number of mixture components and identity of mixture components. Such an approach would also be applicable to other types of interactions where physiology can be rate limiting (e.g., renal excretion, biliary excretion, extrahepatic metabolism, etc.). A limitation of this approach is for compounds having only biotransformation as a mode of elimination and only one metabolic pathway; under such circumstances, the estimated limits would yield very large concentration intervals. It works well with VOCs because they are also eliminated by exhalation.

12.3.2.5 IVIVE of Interactions

In vitro to in vivo extrapolations are acknowledged as the way toxicity testing for environmental agents should be conducted in the twenty-first century (NRC 2007). Obtaining in vivo data for interactions is not always feasible or desirable because

(i) the workload and time associated with experiments create practical limitations, (ii) the associated costs are important, and/or (iii) the number of animals required for such studies is incompatible with the call for animal reduction in research. In the pharmaceutical industry, in vitro assays for screening and preclinical research in drug metabolism and pharmacokinetics are conducted on a routine basis. Large amounts of in vitro data are collected on drug-drug metabolic interactions using cell cultures, cell suspensions, or subcellular fractions (e.g., microsomes, S9 fractions, etc.). Metabolic constants V_{max}, K_m, or intrinsic clearances are often measured as well as inhibition constants (K_i) for drug-drug interactions for different enzymes associated with drug clearance rates. Many empirical clearance models have been proposed to extrapolate in vitro clearance and metabolic interactions data to the in vivo situation, but they have had varying success rates (Wilkinson 1987; Robinson et al. 1991; Robinson 1992; Saville et al. 1992). To increase predictability of these models, several studies have proposed adjusting equations to account for in vitro non-specific binding in the incubation medium to better reflect the free concentrations of substrates and/or inhibitors at enzyme active sites, both in vitro and in vivo (Obach 1997, 1999; Mclure et al. 2000).

$$\mathrm{CL}_{\mathrm{invivo}} = \frac{Q_{\mathrm{liver}} \times \mathrm{RBP} \times \mathrm{CL}_{\mathrm{int,met}} \times \mathrm{Fu_p}/\mathrm{Fu_{inc}}}{Q_{\mathrm{liver}} \times \mathrm{RBP} + \mathrm{CL}_{\mathrm{int,met}} \times \mathrm{Fu_p}/\mathrm{Fu_{inc}}} \tag{12.27}$$

where Q_{liver}, RBP $CL_{int,met}$, Fu_p, and Fu_{inc}, respectively, refer to blood flow in the liver, blood to plasma ratio, metabolic intrinsic clearance, fraction unbound in plasma, and fraction unbound in incubation medium.

Recently, a physiologically based model for hepatic metabolic interactions has been proposed (Theil et al. 2003; Haddad et al. 2010), where the liver is described as a multicompartmental model, representing the vascular, the interstitial, and the cellular matrix (Fig. 12.7). Exchanges between these compartments consider active transport and passive diffusion. Metabolism and metabolic interactions are considered to occur inside the hepatocytes and are related to unbound concentrations in the cells. This unbound concentration is a result of different input and output processes that influence intracellular concentrations (i.e., active efflux, biliary excretion, active uptake, simple diffusion, metabolism, intracellular and extracellular protein binding, and solubility in lipids). Additionally, the chemical concentration gradient along the sinusoids of the liver lobule has also been simulated by representing the liver as seven segments linked in series. Compared to other models described above, this model fared best in predictions of drug-drug binary interactions between three cytochrome 2D1 substrates (i.e., bufuralol, bunitrolol, and debrisoquine) in an isolated perfused liver system. Accordingly, for extrapolation to work, data taken from in vitro assays must also be adjusted to eliminate bias from non-specific binding. A very recent study suggested a more complex hepatic model that incorporates hepatic lobule geometry and many of the processes described here in order to predict the magnitude of metabolic interactions (Cherkaoui-Rbati et al. 2017). These models should be compared to assess their predictive power.

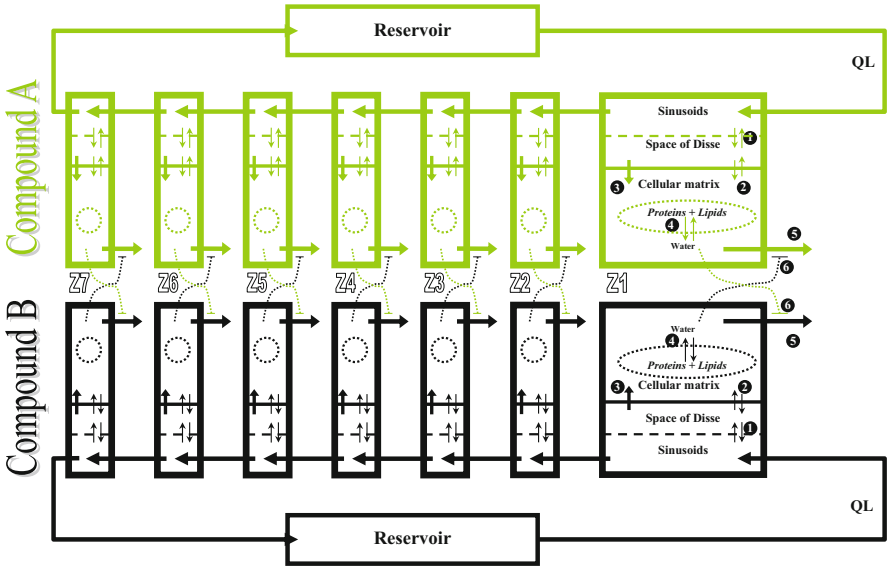

Fig. 12.7 Conceptual representation of the physiologically based pharmacokinetic model of a binary mixture (compounds *A* and *B*) in an isolated perfused liver (IPRL) system. The liver is separated into seven segments (Z1–Z7) connected in series, and each segment is further subdivided into three subcompartments (sinusoids, space of Disse, and cellular matrix). Chemicals enter the sinusoidal space of the first segment by the hepatic portal vein. From the sinusoidal space, the chemical can go to the next liver segment or distribute to other subcompartments. The exchange between the sinusoid and the space of Disse (1) is very rapid due to the presence of large fenestrae. The chemicals in the space of Disse can then enter the cellular matrix by partitioning processes (2) or by active uptake. In the cellular matrix, the chemicals freely distribute between the lipids and proteins and the water (4). The compounds are eliminated from the cellular matrix subcompartment by metabolism (5). The rate of metabolism is dependent on the substrate unbound concentration in the cell and influenced by the intracellular unbound concentration of competing substrate (6). Before leaving the liver, the chemical must pass through all liver segments where all the same processes occur. QL refers to the perfusate flow in the recirculating IPRL system (Modified from Haddad et al. 2010)

Current methods for estimating hepatic clearance have been shown to be quite ineffective in predicting in vivo clearance of compounds that are highly bound to albumin (i.e., $Fu_p < 0.05$) (Poulin et al. 2012). Recent studies have shown that to accurately predict the clearance of this category of compounds, it must be assumed that there is a mechanism facilitating the distribution of the bound drug in the organ by albumin, hence leading to an apparent unbound fraction in the organ that is greater than in blood (Poulin et al. 2012). A clearance algorithm (Eq. 12.27) that adjusted Fu_p in Eq. 12.28 was proposed and validated for IVIVE of in vitro metabolic rate from microsomes (Poulin and Haddad 2013) and hepatocytes (Poulin and Haddad 2013).

$$Fu_{p-adjusted} = \frac{PLR \times Fu_p \times \frac{Funionized_{plasma}}{Funionized_{cells}}}{1 + (PLR - 1) \times Fu_p \times \frac{Funionized_{plasma}}{Funionized_{cells}}} \quad (12.28)$$

where PLR and Funionized refer, respectively, to the plasma to liver albumin concentration ratio and the chemical's fraction that is the unionized form in the matrix.

Although nothing has been published for the impact of albumin binding on the prediction of metabolic interactions, interactions between naproxen and bisphenol A for glucuronidation were studied in vitro (Verner et al. 2010) and in isolated perfused rat liver (IPRL) (Bounakta et al. 2017; Poulin et al. 2017). In this IPRL study, liver co-exposure to both compounds in the presence and absence of albumin showed that competitive inhibition was observed. But in the presence of albumin, the clearance and interaction of these two highly albumin-bound compounds were clearly affected confirming the occurrence of an albumin-facilitated uptake mechanism, suggesting that predictions of metabolic interactions for highly albumin-bound compounds from in vitro data must be treated in a similar fashion (i.e., inhibition constants should be adjusted by $Fu_{p\text{-adjusted}}$).

12.4 PBPK Modeling and Mixture Risk Assessment

PBPK modeling can prove to be practical and useful in mixture risk assessment. Exposure assessment of mixture components and their potential for toxicokinetic interactions are among the many challenges that mixtures pose to risk assessors. As shown above, mixture PBPK modeling can allow for prediction of internal exposure, or target tissue dose, even in contexts where toxicokinetic interactions occur.

Unless toxicodynamic interactions are known to occur, the PBPK modeling approaches discussed above can be used in the context of mixture risk assessment. Haddad et al. (Haddad et al. 1999b, 2001) demonstrated an approach in which risk assessors could use PBPK modeling to estimate biological/target organ hazard indices (BHI or THI) in lieu of calculating hazard indices (HI) for mixtures using external exposures concentrations. This approach consists of summing the internal doses of mixture constituents that have similar modes of actions or the same target tissues (refer to Chap. 14). The internal doses of mixture components are normalized by the internal dose obtained during single exposure to guideline values (e.g., *T*hreshold *L*imit *V*alues, *Ref*erence *C*oncentrations). If the sum is greater than unity, exposure to the mixture is considered to pose a health risk. This allows for consideration of the pharmacokinetic interactions between mixture components in the health risk evaluation.

PBPK modeling is also useful in mixture risk assessment because it confers greater confidence in different types of extrapolations. A lot of data come from animal studies, and the kinetics of chemicals (mixtures or single) can be translated to the human situation by changing the animal parameter values to those of humans.

Tardif et al. (1997) extrapolated a ternary PBPK model for toluene, ethylene, and xylene from rat to human, and simulations successfully predicted experimental data from exposed human volunteers.

PBPK models incorporating Monte Carlo simulations can also be used to estimate exposure in the population of interest and determine the range of internal exposure levels in the population to protect sensitive populations (Niu et al. 2015; Hinderliter et al. 2011). Additionally, these models can be extrapolated to different lifestages (e.g., neonates, infants, teenagers, etc.) and sexes and to different polymorphisms to further characterize exposure in different subpopulations (Haddad et al. 2006; Verner et al. 2008, 2009; Hinderliter et al. 2011).

12.5 Conclusions, Current Needs, and Research Perspectives

Although many advances have been made in recent years to predict exposure resulting from toxicokinetic interactions and to use this information in risk assessment, hurdles still remain. In vivo characterization remains the gold standard for identifying and characterizing binary interactions but is too costly and time-consuming. Although not 100% accurate, we can now put more confidence in the prediction of in vivo metabolic interactions from in vitro data, but toxicokinetic interactions can still occur at other levels, and we cannot, therefore, solely rely on metabolic in vitro data. Effects on absorption, distribution, and excretion processes of chemicals, especially for chemicals that interact with proteins (in binding or transport processes), are frequent, and additional research emphasis should be put on IVIVE of these processes to increase capacity in high-throughput data generation and PBPK model predictions. Development of QSAR for toxicokinetic interactions is also very much needed, but the generation of such knowledge is limited by the available data on toxicokinetic interactions. Predictive environmental toxicology could certainly rely on data available from the pharmaceutical industry to generate QSAR algorithms, but more data for ADME of environmental contaminants are required. Data generated from projects such as TOXCAST (Dix et al. 2007; Judson et al. 2010) offer promise in this context.

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