CHAPTER FIVE

THE APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING TO RISK ASSESSMENT

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Learning Objectives

Students who complete this chapter will be able to

1. Understand why PBPK modeling is needed in risk assessment
2. Know what PBPK modeling is
3. Know how PBPK modeling is done, particularly in its application to risk assessment
4. Learn how PBPK modeling of chemical mixtures is done
5. Follow what are some of the latest development in the application of PBPK modeling in risk assessment

The area of science called *physiologically based pharmacokinetic (PBPK) modeling* can be traced back to the 1920s. In June 2005, the first book on PBPK (Reddy, Yang, Clewell, and Andersen, 2005) was published and its contents encompassed over one thousand publications on PBPK modeling. Despite the fact that it is a mature science with almost one hundred years of history, active development is still going on in this area and a later section of this chapter provides a glimpse of some of these latest advances. It is important to emphasize that this chapter, though a learning tool, only provides some of the fundamentals to stimulate your interests. Alone, it will not make you a PBPK modeler. To be proficient, there is no alternative but

to attend specific training workshops, and most important, to get your hands dirty by doing PBPK modeling yourself. Only through repeated practice, reading, and making all the mistakes everyone else has made before you will you then open the window to a very useful and powerful technology.

The Need for PBPK Modeling in Risk Assessment

Conventionally, risk assessment is done based on exposure dose or administered dose. This is neither accurate nor satisfying because an exposure or administered dose will go through absorption, distribution, metabolism, and elimination (ADME) in our bodies before a sufficient amount of the dose reaches the target organ to exert its toxicity. To be able to follow, on a time course basis, the ADME processes of a given chemical in our bodies and, further, to follow an active component (e.g., from a technical formulation) or a reactive species (e.g., from metabolic transformation) require the understanding of pharmacokinetics of that chemical. PBPK modeling is a very useful tool for the integrated computer simulation of pharmacokinetics of a chemical or chemicals. Therefore, the need for PBPK modeling in risk assessment arises when we want to incorporate the state-of-the-science technology to conduct a more accurate risk assessment. Additional arguments in favor of incorporating PBPK modeling into the risk assessment process include deliberations from the following perspectives.

Toxicological Interactions of Multiple Chemicals

Present EPA risk assessment guidelines on chemical mixtures, including the recent effort on cumulative risk assessment of organophosphrous (OP) pesticides (Environmental Protection Agency, 2002a, 2002b), advocate the additivity approach. For instance, in the "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity" (Environmental Protection Agency, 2002a), it was assumed that at lower levels of exposure typically encountered environmentally no chemical interactions are expected (i.e., simple additivity). For additivity to hold true, a further assumption must be that all the common mechanism chemicals behave the same pharmacokinetically and pharmacodynamically (i.e., having the same PK and PD) (Environmental Protection Agency, 2002a). In reality though, a case study of cumulative risk assessment of thirty-three organophosphorus pesticides provided BMDL (lower bound benchmark dose at ED_{10}) with a range of a 3,977- to 5,528-fold difference between the highest BMDL for malathion and the lowest BMDL for dicrotophos (Environmental Protection Agency, 2002b). These three to four orders-of-magnitude

differences among common mechanism chemicals suggest strongly that the PK and PD are not the same among these chemicals—thus the probability of toxicological interactions at the level of PK and PD. That being the case, PBPK modeling will be a most useful, if not the only, tool available for the integration of PK and PD interactions of multiple chemicals.

Minimizing Animal Experiments

PBPK modeling, as a form of *in silico* toxicology, minimizes animal usage by avoiding unnecessary animal experiments or extremely complex animal experiments. In essence, these complex experiments can be conducted on computer instead. Once a PBPK model is constructed, tested, and validated, an immense number of computer simulations (i.e., *in silico* experiments) can be performed by varying exposure scenarios including different routes, doses, species, and the involvement of different chemicals. This is particularly relevant in considering toxicological interactions of chemical mixtures, which is an essential element in cumulative risk assessment.

Food Quality Protection Act (FQPA) and the Subsequent Development of Cumulative Risk Assessment at EPA

In 1996, the U.S. Congress passed the Food Quality Protection Act (FQPA). Among other mandates, FQPA requires that EPA consider *cumulative risk*. As such, EPA is required to evaluate pesticides in light of similar toxic effects that different pesticides may share or involving chemicals with "a common mechanism of toxicity" (Environmental Protection Agency, 1999). Pioneering efforts were provided by the Office of Pesticide Programs (OPP), at EPA. Scientists at OPP took the lead and developed and conducted cumulative risk assessment on organophosphorus (OP) pesticides (Environmental Protection Agency, 2002a, 2002b). Subsequently, an interoffice endeavor on "Physiologically-Based Pharmacokinetic/Pharmacodynamic Modeling: Preliminary Evaluation and Case Study for the *N*-Methyl Carbamate Pesticides: A Consultation" (Environmental Protection Agency, 2003a) at the EPA had been peer-reviewed by the FIFRA Science Advisory Panel in December 2003 (Environmental Protection Agency, 2003b). A further effort, supported by the Office of Drinking Water and National Center for Environmental Assessment (NCEA), Office of Research and Development (ORD), EPA, is a complementary endeavor to earlier development to further advance a framework approach of incorporating PBPK modeling, particularly incorporating credible human tissue studies, into the cumulative risk assessment process (Environmental Protection Agency, 2005b). These scientific activities illustrate the progressive incorporation of PBPK modeling into the cumulative risk assessment process.

Internal Dose

Internal dose, sometimes referred to as *tissue dose* or *target dose*, can be thought of as the integrated dose level over time (i.e., area under the curve [AUC]) of a biologically effective chemical form (the parent compound or a reactive species) in a given tissue. Some consider the maximal concentration (C_{max}) in the blood or plasma versus time curve as a convenient form of internal dose. In either case, the internal dose takes into consideration the ADME processes, and it is therefore a more accurate dose metric, which should be more closely related to the toxic endpoint(s) than the exposure or administered dose. Once again, PBPK modeling is a very useful tool for computer derivation of the internal dose.

Exposure Dose Reconstruction and Human Biomonitoring

One of the missing links of human biomonitoring results such as those published in the Centers of Disease Control and Prevention (CDC) third biannual report (Centers for Disease Control and Prevention, 2005) is that we do not know what exposure conditions and levels were in the environment for those chemicals. When we have a robust and validated PBPK model for one or more chemicals, we can theoretically carry out a large number of computer simulations for numerous hypothetical exposure scenarios to reach the internal dose levels (i.e., human biomonitoring data reported). This is a form of *back-extrapolation* or *back-calculation* to estimate the possible exposure scenarios. It is not that we are trying to emphasize the importance of exposure dose when we have just stressed the importance of internal doses in the last section. We are interested in exposure scenarios leading to human biomonitoring results for a different reason. Possible environmental remedial actions may be taken when we are quite certain how and where the chemicals inside our body (i.e., human biomonitoring results) are coming from.

Systems Biology

The recent emphasis on the application of systems biology to biomedical research frequently traces its origin to cybernetics, as advanced by Norbert Wiener in the mid-twentieth century (Wiener, 1961). Even then, the integration of "computing machines" and biology was already advocated by a handful of visionaries. *Systems biology* integrates computational and experimental sciences in an effort to describe and understand entire biological systems (Kitano, 2002). PBPK modeling is a form of a systems biology approach toward toxicology where the physiology and biochemistry of a given chemical in an organism are integrated with computational modeling. In two of our recent publications (Yang and others, 2006b, 2006c) we

further present a systems biology representation of the integration of different scales of biologically based computer modeling across a number of biological levels of organization. The ADME model, as exemplified by PBPK modeling for whole-body pharmacokinetics, is linked with biochemical reaction network (BRN) modeling, a form of predictive xenobiotic metabolomics (or metabonomics). We can further link these integrated models with the genomic model, which is a general representation of the gene and/or protein expressions related to the toxicological processes being studied. In doing so, we can link a complicated metabolic pathway model with an ADME model and a genomic model to capture the full systems biology of toxicological interactions and effects.

What Is Physiologically Based Pharmacokinetics?

The concept of PBPK had its embryonic development in the 1920s. PBPK modeling blossomed and flourished in the late 1960s and early 1970s in the chemotherapeutic area due mainly to the efforts of investigators with expertise in chemical engineering. In the mid-1980s, work on PBPK modeling of volatile solvents started yet another revolution in the toxicology and risk assessment arena. Today, there are more than one thousand publications directly related to PBPK modeling of industrial chemicals, drugs, environmental pollutants, and simple and complex chemical mixtures. Our laboratory has recently published a book on PBPK modeling in collaboration with others (Reddy, Yang, Clewell, and Andersen, 2005).

Differences Between Classical Pharmacokinetic Models and PBPK Models

Classical pharmacokinetics refers to those empirical noncompartmental or compartmental pharmacokinetic studies routinely practiced in the pharmaceutical industry (van de Waterbeemd and Gifford, 2003). As illustrated later, the compartments of a PBPK model have anatomical and physiological significance. This is a major difference from *empirical* noncompartmental or compartmental pharmacokinetic modeling approaches. PBPK models can be used to describe concentration-time profiles in an individual tissue or organ and in the plasma or blood. When the concentration of a certain target tissue, rather than the plasma concentration, is highly related to a compound's efficacy or toxicity, PBPK modeling is a more useful tool than classical pharmacokinetic models for describing the PK-PD relationship; thus, it better predicts the time course of drug effects resulting from a certain dose regimen for the compound of interests. Furthermore, PBPK models in combination with absorption simulation and quantitative structure-activity relationship (QSAR) approaches bring us closer to a full prediction of drug disposition for new

pharmaceutical entities, and help streamline the selection of the lead drug candidate in the drug discovery process (van de Waterbeemd and Gifford, 2003). Last, unlike empirical noncompartmental and compartmental pharmacokinetics, PBPK modeling is a powerful tool for extrapolation, whether for interspecies, interroutes, interdoses, or interlife stages.

Conceptual Model: Graphic Representation

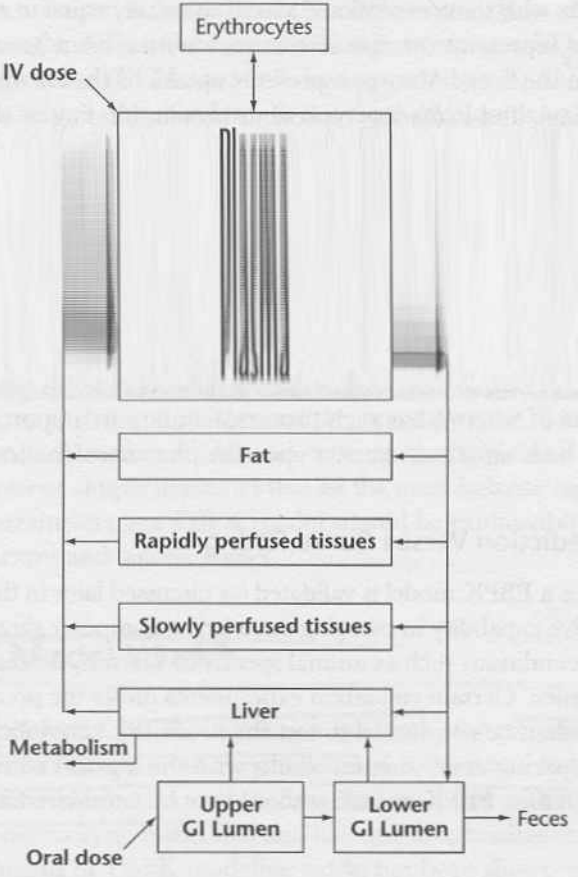
A PBPK model, graphically and conceptually illustrated in Figure 5.1, reflects the incorporation of basic physiology and anatomy. The compartments correspond to anatomic entities, such as the liver and fat, while the blood circulation conforms to basic mammalian physiology. In the specific model in Figure 5.1, a PBPK model for hexachlorobenzene (HCB) in the rat (Lu and others, 2006), the exposure routes of interest are either oral gavage or intravenous (IV) as indicated. Depending on the need, other routes of exposures can be added easily. Some tissues are "lumped" together, such as richly (rapidly) or poorly (slowly) perfused tissues in Figure 5.1, when they are kinetically similar for the specific chemical(s) studied. On the other hand, a given tissue can be split as needed. In this case, HCB is known to bind with erythrocytes and the blood compartment is split into two subcompartments, the erythrocytes and plasma. Similarly, because of the complexity related to the absorption and exsorption (plasma-to-gastrointestinal [GI] lumen passive diffusion) processes of HCB, the GI lumen compartment is split into upper and lower portions. In conceptualizing the PBPK model, the Law of Parsimony should always be applied to keep the model as simple as possible. When the needs arise, complexity can be incorporated as illustrated.

Mathematical Model: Mass-Balance Differential Equations

A mathematical model, regarding PBPK modeling, is computer-code-formulated in such a way that it can be executed by the computer software to simulate the kinetic behavior of a chemical(s) in the body of an organism such as a rat, mouse, fish, or human. A key element of such a mathematical model is a set of mass-balance differential equations representing all of the interlinked compartments such as liver or fat. This set of mass-balance differential equations is formulated to express a mathematical representation, or model, of the biological system. This model can then be used for computer simulation to predict the time course behavior of any given chemical included in the model.

These mass balances are essentially molecular accounting statements that include the rates at which molecules enter and leave the compartment, as well as

FIGURE 5.1. A GRAPHIC OR CONCEPTUAL PBPK MODEL FOR HCB FOLLOWING IV OR ORAL EXPOSURE.



Note: For an IV exposure, the uptake in the upper GI lumen was turned off, and the excretion of metabolites was tracked. For oral exposures, the reverse was true.

Source: Adapted from Lu and others (2006).

the rates of reactions that produce or consume the chemical. For instance, a general equation, for chemical *j* in any tissue or organ, is

$$V_i \frac{dC_{ij}}{dt} = Q_i (CA_j - CV_{ij}) - \text{Metab}_{ij} - \text{Elim}_{ij} + \text{Absorp}_{ij} - \text{Pr}\,Binding_{ij} \quad (5.1)$$

where V_i represents the volume of tissue group *i*, Q_i is the blood flow rate to tissue group *i*, CA_j is the concentration of chemical *j* in arterial blood, and C_{ij} and CV_{ij} are the concentrations of chemical *j* in tissue group *i* and in the effluent venous blood from tissue *i*, respectively. Please note that C_{ij} here refers to the "free," un-

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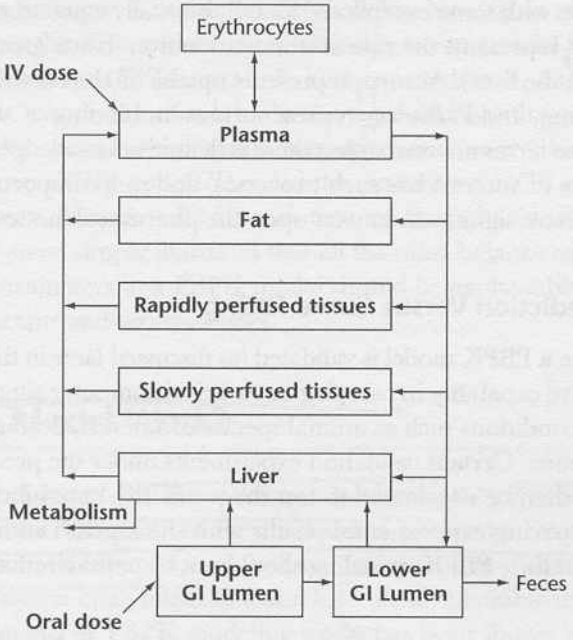

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PBPK model (Leung, Ku, Paustenbach, and Andersen, 1988; Mills and Andersen, 1993). $Metab_{ij}$ is the rate of metabolism for chemical j in tissue group i; liver, being the principal organ for metabolism, would have significant metabolic rates, while, with some exceptions, $Metab_{ij}$ is usually equal to zero in other tissue groups. $Elim_{ij}$ represents the rate of elimination from tissue group i (e.g., biliary excretion from the liver), $Absorp_{ij}$ represents uptake of the chemical from dosing (e.g., oral dosing), and $PrBinding_{ij}$ represents protein binding of the chemical in the tissue. These terms are zero unless there is definitive knowledge that the particular organ-tissue of interest has such processes, and more importantly, that such processes will have significant impact upon the pharmacokinetics of the chemical(s).

A Priori Prediction Versus Curve Fitting

Once a PBPK model is validated (as discussed later in this chapter), it has the predictive capability in carrying out a priori computer simulations given a set of initial conditions such as animal species of interest, dosing route, dosing levels, and regimen. Certain validation experiments under the precise simulation conditions can then be conducted to test the predictive capability of the PBPK model by comparing experimental results with the a priori computer simulation results. Therefore, PBPK modeling should not be considered as curve fitting exercises.

Biological Relevance

As the name *physiologically based* implies, another important consideration in PBPK modeling is that whenever an equation and its related parameter(s) are introduced into the model, they must have biological relevance. In many ways, the mass-balance differential equations in PBPK modeling can be translated into simple English. For instance, the mass-balance equation for the liver compartment in Figure 5.1 is

$$VL \times \frac{dCL}{dt} = QL \times (CA - CVL) - KMET \times CVL + KGILV1 \times AGIUp + KGILV2 \times AGILow \tag{5.2}$$

Equation 5.2 looks like a rather formidably long equation. However, the English translations for both sides of the equation are really quite easy to follow.

Left side: A small change in the amount of chemical (HCB in this case) with respect to a small change in time. We talk about "amount" because when volume of the liver (VL in ml) multiplies the concentration in the liver (CL in mg/ml), it becomes amount (mg). Note the unit on the left side is finally amount/time or more specifically, mg/hr.

Right side: Amount coming into the liver from general circulation (first term) minus the amount metabolized (second term) plus amount absorbed from the upper GI lumen (third term) plus amount absorbed from the lower GI lumen (fourth term). The first term is derived from blood flow rate (*QL* in ml/hr) to and from the liver times the differential concentration between arterial blood (*CA* in mg/ml) and venous blood (*CVL* in mg/ml). In the second term, *KMET* is metabolic rate constant with a unit of ml/hr. In the third and fourth terms, *KGILV1* and *KGILV2* are absorption rate constants from upper and lower GI lumen with a unit of 1/hr whereas *AGIUp* and *AGILow* are the amounts of HCB in the two GI lumen compartments. Note the unit for each term on the right side is also mg/hr.

The above exercise simply illustrates that all the mass-balance equations and their respective parameters in a PBPK model should be explainable by biologically relevant concepts and terminologies.

How Does a PBPK Model Work?

The fundamental object of PBPK modeling is to identify the principal organs or tissues involved in the disposition of the chemical of interest and to correlate the chemical absorption, distribution, metabolism, and excretion within and among these organs and tissues in an integrated and biologically plausible manner. How individual components of PBPK modeling works has been shown in a previous section. However, we will briefly summarize the process in its entirety.

After a conceptual model is developed as shown in Figure 5.1, time-dependent mass-balance equations are written for a chemical(s) in each compartment. A set of such mass-balance differential equations representing all of the interlinked compartments are formulated to express a mathematical representation, or model, of the biological system. This model can then be used for computer simulation to predict the time-course behavior of any given chemical included in the model. Computer simulations may be developed for any number of desired time-course end points such as the blood levels of the parent compound, liver level of a reactive metabolite, and similar information on different species at lower or higher dose levels and/or via a different route of exposure. The experimental pharmacokinetic data may then be compared with a PBPK model simulation. If the model simulation does not agree with the measurements, the model might be deficient because critical scientific information is missing or certain assumptions are incorrect. The investigator, with knowledge of the chemical and a general understanding of the physiology and biochemistry of the animal species, can design and conduct critical experiments for refining the model to reach consistency with

experimentation. This refinement process may be repeated again and again when necessary; such an iterative process is critically important for the development of a PBPK model. In that sense, PBPK modeling is a very good hypothesis-testing tool in toxicology, and it may be utilized to conduct many different kinds of experiments on the computer, such as *in silico* toxicology. Note that there is always the possibility that a good model may not be obtained at the time because of the limitation of our knowledge about the chemical. Validation of the PBPK model with datasets other than the working set (or training set) to develop the model is necessary. Remember—a model is usually an oversimplification of reality: "all models are wrong; some are useful," as stated by George Box. The more the datasets against which a model is validated, the more robust is that model in its predictive capability. Once validated, the PBPK model is ready for extrapolation to other animal species, including humans.

Data Requirements for PBPK Modeling

What are the specific data needed for building PBPK models? Obviously, well-conducted in vivo pharmacokinetic data are essential and usually the more the datasets (e.g., different doses, routes, species), the better. In each study, time-course blood and tissue concentration data are essential. These time-course data should include at least the following tissues and organs: blood (or plasma if blood cell binding is not an issue), liver (organ of metabolism), kidney (representing rapidly perfused organs/tissues), muscle (representing slowly perfused organs/tissues), and target organ(s)/tissue(s).

Three sets of parameters are needed for PBPK model building: physiological parameters (e.g., ventilation rates, cardiac output, organs as percentage of body weight), thermodynamic parameters (e.g., tissue partition coefficients, protein binding), and biochemical parameters (e.g., Michaelis-Menten metabolism parameters K_m and V_{max}). Most, if not all, of the parameters for laboratory animals are available in the literature (Brown and others, 1997). When information gaps exist, needed data can be obtained via experimentation or through allometric extrapolation, usually based on a power function of the body weight (Lindstedt, 1987).

Datasets Used for Model Building and Model Validation

When building a PBPK model, certain experimental datasets are necessary for comparing with simulation results to see if the theoretical data (computer simulations) are superimposable to the observed data (experimental results). During

this phase of the work, we are trying to (1) test our hypotheses of pharmacokinetic fate of the chemical(s) of interest in the given biological system, (2) assess the appropriateness of the assumptions that we made for the PBPK model, and (3) find appropriate values for those parameters that can neither be derived experimentally nor extrapolated allometrically. The datasets used in this model-building phase should be considered as a *training set* or *working set*. Once a PBPK model is constructed, the next phase is model validation. This is where a priori simulations under a specific exposure scenario can be carried out and the simulation results then compared with available experimental data. Superimposition of the two suggests validity of model prediction under that set of conditions. The more datasets there are against which a model is validated, the more robust is that model in its predictive capability. Validation of the PBPK model with datasets other than the training set (or working set) used to develop the model is essential.

Available Software Comparison

A PBPK model generally is a system of coupled ordinary differential equations, which are solved with the aid of computer tools. The available computer tools for PBPK modeling include programming languages, simulation software, and spreadsheets. An excellent list of these tools, along with their developers/vendors, salient features, and application examples, has been compiled in a recent report on PBPK modeling (Environmental Protection Agency, 2005a). Earlier, Rowland, Balant, and Peck (2004) presented a somewhat different list. Certain commonly known examples in these lists are MATLAB (MathWorks, Natick, Massachusetts), Berkeley Madonna (University of California-Berkeley), SAAM II (University of Washington-Seattle), SCoP (Simulation Control Program, Simulation Resources, Inc., Redlands, California), SimuSolv (Dow Chemical Company, Midland, Michigan), and ACSL, ACSL Tox, and acslXtreme (AEgis Technologies Group, Huntsville, Alabama). The available software for PBPK modeling varies in flexibility and user friendliness. Regardless of the variation in flexibility, PBPK software should at least have proper algorithms for integration, optimization, and sensitivity analysis. Given the diversity in the software in use, concerns have been expressed about standardizing the software for PBPK modeling (Rowland Balant, and Peck, 2004). In the toxicology community, ACSL, ACSL Tox, and acslXtreme, closely related, are the most commonly used software.

Two PBPK simulation programs used in our laboratory, Berkeley Madonna (version 8.3.6) and acslXtreme (version 2.0.1.6), are briefly introduced here. Both programs are general-purpose differential-equation solvers with high flexibility. The modeling process in each program follows the procedure of representing a

model graphically or in equations, compiling model equations into machine code, and reporting results. Berkeley Madonna is more affordable, easier to learn, more user-friendly, and requires less programming knowledge.

The critical components of a model in both Berkeley Madonna and acslXtreme are the equations and statements that represent the parameter settings, model structure, integration method, and other related conditions. In Berkeley Madonna, the equations need not follow a particular order or structure. They will be automatically sorted into a proper order for execution. For readability and ease to debug, however, coding in the following order is recommended: integration method and related conditions, parameters, parameter scaling, exposure conditions, and mass balance for each compartment.

In the following sections, we first provide a general explanation of the blocks for a model written with ACSL. We then provide a detailed explanation of a PBPK model written with Berkeley Madonna. This seemingly preferential treatment of Berkeley Madonna is due to the fact that Berkeley Madonna is more affordable to students and easier to use. We believe that the readers of this book are more likely to be interested in starting their PBPK modeling experience with Berkeley Madonna.

In acslXtreme, the model equations, saved in a CSL (continuous simulation language) file, are organized to a specific structure with several blocks (*acslXtreme Language Reference Guide*, 2005):

```
PROGRAM
    INITIAL
    Statements executed before the run begins.
    State variables do not contain the initial conditions yet.
    END

  DYNAMIC
      DERIVATIVE
      Statements to be integrated continuously.
      END

  DISCRETE
  Statements executed at discrete points in time.
  END
  Statements executed at each communication interval.
  END

  TERMINAL
  Statements executed after the run terminates.
  END

END
```

Equations should be placed in the appropriate blocks; misplacement of equations may prevent the code from running or produce wrong results. In the Derivative block, however, the equations can be grouped in whatever way the modeler likes. Although no acslXtreme code is available in the literature, a reader can refer to Thomas and others (1996a) and Easterling, Evans, and Kenyon (2000) for the codes in ACSL and SimuSolv that are structurally very similar to those in acslXtreme.

After a model code is executed, both Berkeley Madonna and acslXtreme are amenable to *in silico* experimentation, including, but not limited to, tabulating and plotting simulation results, examining the effects of a parameter on model outputs, visual optimization, statistical optimization, sensitivity analysis, and Monte Carlo analysis. In this regard, Berkeley Madonna offers a user-friendly interface so that those manipulations can be achieved by selection of the self-explanatory options from the tool menu. AcslXtreme, however, requires some acquaintance with the specific command language, which is a challenge to a new user.

Explanation of an Example of Computer Code for a PBPK Model in Berkeley Madonna

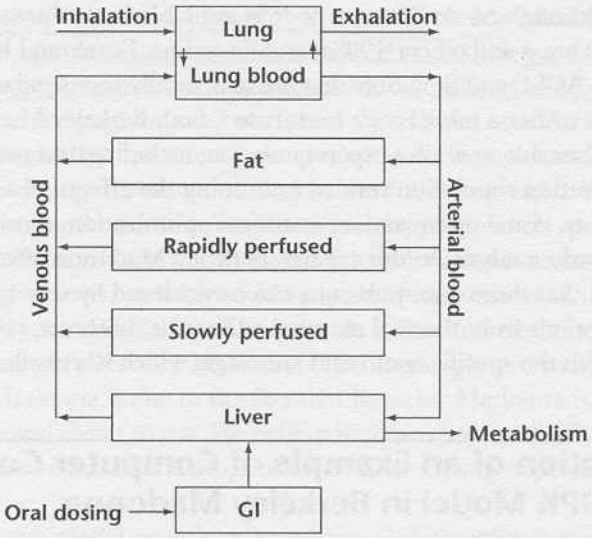
A PBPK model code written in Berkeley Madonna simulates the exposure and pharmacokinetics in the rat of 1,1,1-trichloroethane, a volatile organic chemical, which is lipophilic and slowly metabolized in the liver. Prior to explaining the code, we need to sequentially define the foundations on which the code is based: (1) exposure conditions, (2) PBPK model structure, and (3) necessary assumptions/simplifications and mass-balance differential equations for all compartments.

Exposure conditions: Two exposure pathways, not taking place simultaneously, are involved in this case. At time zero, a rat is orally administered a 1,1,1-trichloroethane water solution at the dose of 14.2 mg/kg body weight; or else it starts inhaling 1,1,1-trichloroethane vapor at 150 ppm continuously for six hours. That determines the time-course concentrations of 1,1,1-trichloroethane in the exhaled air and venous blood.

PBPK model structure: The model structure (Figure 5.2) is determined according to the exposure conditions and the pharmacokinetic characteristics of 1,1,1-trichloroethane. As 1,1,1-trichloroethane is lipophilic and slowly metabolized in the liver, the fat and liver are included in the model structure. The other organs and tissues have no individually distinct impact on the pharmacokinetics, and are thus lumped to a rapidly and slowly perfused compartment. The lung/lung blood and gastrointestine (GI) compartments accommodate the inhalation and oral dosing exposures.

We assume that each of the compartments is homogeneous, that the chemical uptake in each tissue compartment is perfusion-limited, that is the diffusion of

FIGURE 5.2. PBPK MODEL STRUCTURE FOR 1,1,1-TRICHLOROETHANE IN THE RAT.



the chemical into the tissue is rapid and the rate-limiting step is the blood perfusion rate, and that 100 percent of the oral dose in the GI compartment is absorbed. The amount of change of 1,1,1-trichloroethane in a small time interval (dt) in the fat (F) compartment can be expressed as

$$\frac{dAF}{dt} = QF \times (CA - CVF) = QF \times (CA - \frac{CF}{PF}) \qquad (5.3)$$

where AF is the amount in fat, QF is the blood flow rate into fat, CA is the arterial blood concentration, and CVF is the concentration in the effluent blood from fat, which is related to the fat concentration (CF) divided by the fat partition coefficient (PF). Equation 5.3 can be applied to the rapidly (R) and slowly (S) perfused compartments by replacing the F with R and S, respectively.

The differential equation for the liver is a little more complicated than in Equation 5.3 because the absorption from the GI compartment and the metabolism should be considered therein.

$$\frac{dAL}{dt} = QL \times (CA - CVL) + \frac{dAB}{dt} - \frac{dAM}{dt} \qquad (5.4)$$

where dAB/dt represents the rate of absorption from the GI compartment into the liver, and dAM/dt represents the rate of metabolism that results in a negative change in the chemical amount in the liver.

The differential equation for the GI lumen is

$$\frac{dAGI}{dt} = -\frac{dAB}{dt} = -KAB \times AGI \tag{5.5}$$

where AGI stands for the amount in the GI compartment, and KAB is the rate constant of absorption from GI to blood and then to the liver. The minus sign indicates that the amount left in the GI compartment decreases with time.

The venous blood concentration, CV, can be expressed using an algebraic equation:

$$CV = (QF \times CVF + QL \times CVL + QR \times CVR + QS \times CVS)/QC \tag{5.6}$$

where QC is the cardiac output. For the calculation of arterial blood concentration, CA, assumptions are involved that steady state in the lung is quickly reached upon inhalation, that the exhaled concentration is in equilibrium with CA, and that the chemical is only absorbed in the alveolar region. In the blood flowing through the lung, the amount of change over time can be expressed as:

$$\frac{dABlood}{dt} = QC \times (CV - CA) + QP \times (CIN - \frac{CA}{PB}) \tag{5.7}$$

where QP is pulmonary ventilation rate, CIN is the concentration inhaled, PB is blood:air partition coefficient, and CA/PB is the concentration exhaled. At steady state, $dABlood/dt = 0$. Thus, Equation 5.7 is reduced to

$$QC \times (CV - CA) + QP \times (CIN - \frac{CA}{PB}) = 0 \tag{5.8}$$

Solving Equation 5.8 for CA,

$$CA = \frac{QC \times CV + QP \times CIN}{QC + \frac{QP}{PB}} \tag{5.9}$$

Now that the exposure conditions, model structure, and mass-balance equations are clarified, let us turn to the Berkeley Madonna code for this case. Like a

typical PBPK model code, the 1,1,1-trichloroethane includes documentation, integration method, parameters, mass-balance equations, and error-check equations. We will go through it line by line. The code contents are followed by brief explanations; these are not meant to replace a PBPK modeling course or workshop. In a Berkeley Madonna code, documentation is composed of the text strings confined in paired curly brackets or preceded by semicolons.

```
{1,1,1-Trichloroethane code originally supplied by Dr. Reitz. Converted into a Berkeley Madonna form for the 2005 Colorado State University Beginner's PBPK Workshop by Yasong LU and Ray Yang. 7/16/2005. Reference: Reitz RH, McDougal JN, Himmelstein MW, Nolan RJ, Schumann AM. 1988 Physiologically based pharmacokinetic modeling with methylchloroform: Implications for interspecies, high dose/low dose, and dose route extrapolations.

Source: Toxicology and Applied Pharmacology, 95, 185–199}.
```

These sentences are part of the documentation of this code. Different from the other components, documentation is not essential for code execution. However, it records important information pertinent to the code, such as the purpose(s) of the modeling, experimental conditions being simulated, date and author(s) of the code, history of the modifications to the code, rationale of the modeling structure and parameter value selections, and explanation of the terminology in the code. Documentation is critical for model code maintenance. Therefore, it is always good practice to provide documentation as thoroughly as possible.

Method Stiff

The METHOD statement defines the numerical integration method for model calculation. For PBPK modeling, STIFF is a frequently used method that automatically finds the appropriate integration intervals over time. See the next section for more details on numerical integration methods.

```
STARTTIME = 0
STOPTIME = 12
```

The STARTTIME and STOPTIME statements define the starting and ending times of the simulation. The former is usually 0; the latter varies depending on the experimental duration.

```
{Physiological Parameters}
{Constants set for the rat}
BW = 0.233;Mean body weight (kg); Reitz et al. code.
```

```
QCC = 15.;Cardiac output constant [L/(hr*kg^0.74)]; Reitz et al., 1988.
QPC = 15.;Alveolar ventilation constant [L/(hr*kg^0.74)]; Reitz et al., 1988.
{Blood flow fractions}
QLC = 0.24;Fractional blood flow to liver; Reitz et al., 1988.
QFC = 0.05;Fractional blood flow to fat; Reitz et al., 1988.
QSC = 0.18;Fractional blood flow to slowly perfused; Reitz et al., 1988.
QRC = 1.0-(QFC+QSC+QLC);Fractional blood flow to rapidly perfused;
      Reitz et al., 1988.
{Volume fractions}
VLC = 0.04;Fraction liver tissue; Reitz et al., 1988.
VFC = 0.07;Fraction fat tissue; Reitz et al., 1988.
VRC = 0.05;Fraction rapidly perfused tissues; Reitz et al., 1988.
VSC = 0.91-VLC-VFC-VRC;Fraction slowly perfused; Reitz et al., 1988.
```

This block defines the physiological parameters necessary for the modeling. Each parameter statement is followed by a semicolon and text string (documentation) explaining the meaning of the parameter symbol and the source of the parameter value. These statements, either following a semicolon or in between curly brackets, are for our own record or information and they are ignored by Berkeley Madonna.

```
{Chemical specific parameters}
{Partition coefficients}
PB = 5.76;Blood/air; Reitz et al., 1988.
PLA = 8.6;Liver/air; Reitz et al., 1988.
PFA = 263.;Fat/air; Reitz et al., 1988.
PRA = 8.6;Rapidly perfused/air; Reitz et al., 1988.
PSA = 3.15;Slowly perfused/air; Reitz et al., 1988.
PL = PLA/PB
PF = PFA/PB
PR = PRA/PB
PS = PSA/PB
```

The tissue:air partition coefficients were experimentally measured; they are divided by a blood:air partition coefficient to convert to tissue:blood partition coefficients, which govern the distribution of the chemical in each compartment.

```
{Metabolism; saturable; estimated from Schumann et al. data and Reitz et al. drinking
water study}
VMAXC = 0.419;Capacity of saturable metabolism [mg/(hr*kg^0.7)]; Reitz et al., 1988.
KM = 5.75;Affinity of saturable metabolism (mg/L); Reitz et al., 1988.
```

These lines define the Michaelis-Menten kinetic parameters for 1,1,1-trichloroethane metabolism in the liver.

```
{Scaled parameters}
QC = QCC*BW^0.74;Cardiac output (L/hr); Reitz et al., 1988.
QP = QPC*BW^0.74;Alveolar ventilation (L/hr); Reitz et al., 1988.
VF = VFC*BW;Fat volume (L)
VL = VLC*BW;Liver volume (L)
VR = VRC*BW;Rapidly Perfused volume (L)
VS = VSC*BW;Slowly Perfused volume (L)
QL = QLC*QC;Liver blood flow (L/hr)
QF = QFC*QC;Fat blood flow (L/hr)
QR = QRC*QC;Rapidly Perfused blood flow (L/hr)
QS = QSC*QC;Slowly Perfused blood flow (L/hr)
VMAX = VMAXC*BW^0.7;Capacity of saturable metabolism (mg/hr); Reitz et al., 1988.
```

In this block, the physiological parameters and maximum metabolic velocity are scaled by the body weight.

```
{Exposure conditions: oral dosing}
BDOSE = 14.2;Oral bolus dose rate (mg/kg)
KA = 1.25;Rat GI absorption rate constant (/hr); Reitz et al., 1988.
ODOSE = BDOSE*BW;Oral bolus dose (mg)
```

These statements define the oral exposure dose and the GI absorption rate constant.

```
{Exposure conditions: inhalation}
TCHNG = 6.;Length of inhalation exposure (hrs)
;Unit conversion: from ppm to mg/L; often necessary for inhalation exposure scenarios.
MW = 133.5;Molecular weight (g/mol)
CONC = 0.0;Inhaled concentration (ppm)
CIN0 = CONC*MW/24450.;Convert ppm to mg/L
CIN = IF TIME<TCHNG THEN CIN0 ELSE 0;Turn off inhalation after exposure interval
```

The inhalation exposure conditions are defined in this block. Two features here deserve some elaboration:

1. *Unit conversion.* In inhalation experiments, chemical concentrations are frequently expressed in parts per million (ppm), which must be converted to mg/L or something similar for further calculations. The theoretical basis of the unit conversion is the ideal gas law.

2. *The "if-then-else statement."* This statement is used to change a parameter under certain condition(s). In this case, the inhalation exposure is turned off, since the TIME hits six hours. Please note that although this code accommodates both oral and inhalation exposure, they do not coexist. Thus, the inhaled concentration (CONC) is set as zero here to avoid the undesirable double-dosing; when running the code for inhalation, we can turn off the oral dosing and give CONC an appropriate value.

At this point all parameters have been defined in the code. The following sections demonstrate how the chemical amount and/or concentration in each compartment are calculated. For each compartment, there is a mass-balance differential equation coupled with a statement (INIT, which also signifies integration of the parameter that follows) defining the initial value of the amount in the compartment. When necessary, the concentration in a compartment is calculated as the ratio of the amount therein over the compartment volume.

```
{Chemical distribution—mass balances}
;AS = Amount in Slowly Perfused (mg);AS' = dAS/dt
AS' = QS*(CA-CVS);Mass-balance differential equation.
INIT AS = 0.;Initial amount in slowly perfused.
CS = AS/VS;Concentration in slowly perfused, mg/L.
CVS = CS/PS;Effluent blood conc, in equilibrium with tissue conc, mg/L.
```

These lines calculate the amount and concentration in the slowly perfused compartment and the concentration in the venous blood flowing out of that compartment.

```
;AR = Amount in Rapidly Perfused (mg)
AR' = QR*(CA-CVR);Mass balance in rapidly perfused
INIT AR = 0.;Initial amount in rapidly perfused
CR = AR/VR;Conc in rapidly perfused, mg/L
CVR = CR/PR;Effluent blood conc, mg/L
;AF = Amount in fat (mg)
AF' = QF*(CA-CVF);Mass balance in fat
INIT AF = 0.;Initial amount in fat
CF = AF/VF;Conc in fat, mg/L
CVF = CF/PF;Effluent blood conc, mg/L
```

The chemical amount and concentration in the fat and the rapidly perfused compartment are calculated in the same way as for the slowly perfused compartment.

```
;AL = Amount in liver (mg)
AL' = QL*(CA-CVL) – AM' + AO';Mass balance in liver
```

```
INIT AL = 0.;Initial amount in liver
CL = AL/VL;Conc in liver, mg/L
CVL = CL/PL;Effluent blood conc, mg/L
;AM = Amount metabolized (mg)
AM' = VMAX*CVL/(KM+CVL);Rate of metabolism, mg/hr
INIT AM = 0.;Initial amount metabolized, mg
;AO' = Rate of input to liver from stomach after oral bolus (mg/hr)
AO' = KA*MR;Rate of GI absorption, mg/hr
INIT AO = 0.;Initial value of absorbed amount, mg
```

This block shows the calculations for the liver compartment. Different from the fat and the rapidly and slowly perfused compartment, the mass balance in the liver includes metabolism and absorption from the GI compartment.

```
;MR = Amount remaining in stomach after oral bolus (mg)
;First-order absorption
MR' = -KA*MR;Absorption rate, mg/hr
INIT MR = ODOSE;Initial value of the amount in stomach = given dose, mg
```

These lines demonstrate the calculation of the amount in the GI compartment.

```
;Blood concentrations (mg/L)
CV = (QL*CVL+QS*CVS+QF*CVF+QR*CVR)/QC;Venous blood conc, mg/L
CA = (QC*CV+QP*CIN)/(QC+QP/PB);Arterial blood conc, mg/L
CEX = CA/PB;Conc leaving the alveolar region, mg/L
CEXMGL = 0.667*CEX+0.333*CIN;Conc in exhaled air, mg/L
CEXPPM = CEXMGL*24450./MW;mg/L converted to ppm, for comparing with data
```

The venous and arterial blood concentrations are calculated algebraically. By convention, the alveolar respiration has been assumed to account for two-thirds of total respiration (Ramsey and Andersen, 1984); hence the concentration in the exhaled air is a weighted average of the inhaled concentration (CIN) and the concentration leaving the alveolar region (CEX).

```
;Error check
;Total amount of chemical delivered should equal to the amount calculated by the code.
;Amount inhaled
AIN' = QP*CIN
INIT AIN = 0.
;Amount exhaled
AEX' = QP*CEX
INIT AEX = 0.
;TOTAL = Total amount delivered
```

```
TOTAL = ODOSE + AIN – AEX
;Calculated = Total amount calculated
Calculated = AF+AL+AS+AR+AM+MR
ERROR = (TOTAL – Calculated)/(TOTAL+1E-30)*100;ERROR should be close to 0.
```

This final block is set to check the potential error(s) in the code. A small value (1E-30) is added to the denominator in the ERROR equation to avoid a situation where the denominator might end up being zero. If the total amount of chemical delivered experimentally is different from the summed amount in all compartments and eliminated, it would suggest that there is an error(s) in the code. This error-check tool, however, cannot uncover all errors in a code; thorough examination of a code is strongly encouraged.

Numerical Integration

Numerical integration, as opposed to finding an exact solution, is the basis for computer simulation in PBPK modeling. In essence, it is an approach for approximating very closely the true solution of a calculation in much the same way as we approximate an area under the curve (AUC) using the trapezoidal rule. In this latter case, the smaller the trapezoids (i.e., the step size), the more accurate the approximation of the AUC. In Berkeley Madonna, there are five numerical integration methods available for use. They are Euler's Method, Runge-Kutta 2, Runge-Kutta 4, Auto-stepsize, and Rosenbrock (stiff). Detailed explanation of these methods is beyond the scope of this chapter. We will simply point out two things: (1) a very popular method for approximating solutions to first-order initial-value problems is the *fourth-order Runga-Kutta method* (i.e., Runge-Kutta 4 in Berkeley Madonna; Runga-Kutta refers to two German mathematicians); (2) for some differential equations, application of standard numerical integration methods such as the Euler and Runge-Kutta methods exhibit instability in the solutions. This instability or difficult-behavior in the equation is described as *stiffness* and is often caused by the presence of different time scales in the underlying problem. Stiff problems are ubiquitous in many areas of science, including biology. One of the methods in Berkeley Madonna, Rosenbrock (stiff), is specifically to be used for the stiff problems.

Sensitivity and Uncertainty

A PBPK model provides pharmacokinetic profiles of a chemical given physiological, biochemical, and thermodynamic parameters. For various reasons, it is valuable to identify the sensitivity of an output to the model parameters and to

measure the effect of the variability or uncertainty in a parameter on model outputs. These evaluations involve sensitivity analysis and uncertainty analysis.

Sensitivity Analysis

Sensitivity analysis examines the influence of model parameters on outputs. Conceptually, there are two kinds of sensitivity analyses in mathematical modeling: local and global (Blower and Dowlatabadi, 1994; Nestorov, Aarons, and Rowland, 1997; Saltelli, Tarantola, and Chan, 1999). *Local sensitivity* refers to the response of model outputs to the perturbation of a single parameter, whereas *global sensitivity* refers to the response of outputs to the simultaneous alterations in all parameters. Sensitivity analysis in the PBPK community is currently predominantly limited to local sensitivity (Clewell, Lee, and Carpenter, 1994; Easterling, Evans, and Kenyon, 2000; Emond, Birnbaum, and DeVito, 2004; Evans and Andersen, 2000; Evans, Crank, Yang, and Simmons, 1994; Sweeney, Gargas, Strother, and Kedderis, 2003).

The sensitivity of an output to a parameter can be quantitatively reflected by a sensitivity coefficient (SC). Considering an output R is a function of a parameter x, that is, $R = F(x)$, then:

$$SC = \frac{F(x + \Delta x) - F(x)}{\Delta x} \tag{5.10}$$

where Δx is a perturbation in x. When the Δx is sufficiently small, the SC is a partial derivative of R with respect to x. Thus, Equation 5.10 can be reformulated into:

$$SC = \frac{\partial R}{\partial x} \tag{5.11}$$

Since parameters and outputs have distinct units and magnitudes, the SC should be properly normalized for interparameter or interoutput comparisons. Thus:

$$SC = \frac{\frac{\partial R}{R}}{\frac{\partial x}{x}} = \frac{\partial \ln R}{\partial \ln x} \tag{5.12}$$

where SC can be recognized as the sensitivity of the logarithm of an output R ($\ln R$) to the logarithm of a parameter x ($\ln x$), hence it is also known as a *log-normalized sensitivity coefficient* (LSC). An LSC identifies the percentage change in an

output due to a percentage change in a parameter. It has been suggested that LSCs should be in the range of −1 to 1; a value substantially beyond the range indicates that the error in a parameter is greatly amplified in the output, and hence implies undesirable feature(s) in the model (Clewell, Lee, and Carpenter, 1994).

The utilities of sensitivity analysis include

- Identifying the most sensitive parameters for an output, which helps us understand a pharmacokinetic behavior of interest (Emond, Birnbaum, and DeVito, 2004; Evans and Andersen, 2000).
- Evaluating the necessity of carefully measuring unknown parameters. If the output of interest is sensitive to an unknown parameter, precise measurement of this parameter is required.
- Directing targeted experimentation and improving study design. For example, sensitivity analysis may suggest optimal exposure conditions, necessary data to be collected, and the frequency of data collection (Evans, Crank, Yang, and Simmons, 1994; Schlosser, 1994).

Uncertainty Analysis

The term *uncertainty* is often used along with *variability* although they are distinct concepts. *Uncertainty* is defined as the possible error in estimating a true value of a parameter; it is a defect in knowledge and can be reduced by improving experimental methods (Clewell and Andersen, 1996). *Variability*, however, refers to the difference of a parameter among individuals; it is a fact that can be measured but not be changed (Clewell and Andersen, 1996).

For the purpose of risk assessment, average pharmacokinetic information is not very useful because it does not take into account the uncertainty and variability of the parameters (Clewell and Andersen, 1996). Uncertainty analysis measures the effects of uncertainty and variability in model parameters on predicted pharmacokinetics. Monte Carlo simulation is a common technique for uncertainty analysis. Before conducting a PBPK simulation, the statistical distributions of all parameters are determined. A set of the parameters is sampled from those distributions using Monte Carlo simulation. These parameters are then input into a PBPK model, which is executed and generates a set of outputs. Then another set of parameters is sampled, the PBPK model is reexecuted, and the outputs are recorded. This process is repeated many times (e.g., 1,000) until many sets of outputs are generated. The outputs are statistically analyzed to get the means and variances. As such, the effects of the uncertainty and variability of parameters on outputs are measured (Blower and Dowlatabadi, 1994; Clewell and Andersen, 1996; Hetrick, Jarabek, and Travis, 1991; Thomas and others, 1996b). Recently a more advanced statistical approach, *Bayesian analysis*, has been applied in PBPK

modeling to explore the effects of uncertainty and variability in model parameters (Bois 2001; David and others, 2006; Jonsson, 2001; Jonsson and Johanson, 2001a; Marino and others, 2006). This approach can separate uncertainty from variability. More information on the Bayesian approach is introduced in a later section.

PBPK Models for Chemical Interactions (Interactive PBPK Models) in Chemical Mixtures

Since humans are rarely, if ever, exposed to a single chemical, a key feature of PBPK modeling is that it can be used to integrate information on toxicological interactions. The most ideal and scientifically defensible data requirement for establishing an interactive PBPK model is that an established, validated PBPK model is available for each component chemical in the mixture. Furthermore, there are many pharmacokinetic datasets in laboratory animals as well as in humans available for each of these component chemicals. We use the term of *interactive PBPK model* to mean a PBPK model that is capable of simulating interactions between and among chemicals in a mixture. The interactive PBPK model is then built on the basis of known pharmacokinetic interactions. For instance, one chemical may inhibit the biotransformation of other mixture components. The individual PBPK models may then be linked together at the liver compartment by introducing competitive (or other) inhibition terms in the mass-balance differential equation. In our opinion, the application of PBPK modeling to toxicological interactions of chemical mixtures is necessary in cumulative risk assessment. However, this area is very complex, and it is still an emerging field. For a more thorough discussion, see the chapter on PBPK modeling of chemical mixtures in Reddy, Yang, Clewell, and Andersen (2005), as well as the chapter on the application of PBPK modeling in cumulative risk assessment in Yang and others (2006b). It should be emphasized here that PBPK modeling handles only part of the chemical mixture issue in cumulative risk assessment (i.e., the pharmacokinetic interactions at the whole body level). PBPK modeling must be integrated with "biochemical reaction network modeling" in order to go to the molecular interaction reaction network level, and further to the linkage with toxic end points to fully address the chemical mixture issue in cumulative risk assessment (Mayeno, Yang, and Reisfeld, 2005; Yang and others, 2006b, 2006c).

A research group led by Professor Kannan Krishnan, Université de Montréal, Canada, pioneered efforts in the PBPK modeling of more complex chemical mixtures. Earlier work from this group concentrated on interactions and PBPK modeling between two chemicals (Pelekis and Krishnan, 1997; Tardif and others, 1995; Tardif, Lapare, Krishnan, and Brodeur, 1993). As progress was

made, these investigators began to build up the mixtures and devoted their efforts to PBPK modeling of more and more complex chemical mixtures (Haddad, Charest-Tardif, Tardif, and Krishnan, 2000; Haddad, Tardif, Charest-Tardif, and Krishnan, 1999; Tardif, Charest-Tardif, Brodeur, and Krishnan, 1997). So far, these investigators have successfully carried out PBPK modeling on the pharmacokinetic interactions on chemical mixtures involving up to five chemicals (Haddad, Tardif, Charest-Tardif, and Krishnan, 2000; Krishnan, Haddad, Beliveau, and Tardif, 2002); however, they have advanced the hypothesis 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, Charest-Tardif, Tardif, and Krishnan, 2000; Krishnan, Haddad, Beliveau, and Tardif, 2002). Thus, according to their concept, PBPK models for mixtures of any complexity can be created as long as the quantitative information on the mechanism of interaction for each interacting pair (e.g., competitive inhibition rate constant) is available (Krishnan, Haddad, Beliveau, and Tardif, 2002).

Applying the same approach created by Krishnan and coworkers, investigators in our laboratory have studied PBPK modeling of a ternary mixture of trichloroethylene (TCE), tetrachloroethylene (PERC), and 1,1,1-trichloroethane (methyl chloroform, MC) in rats and humans (Dobrev, Andersen, and Yang, 2001, 2002). Furthermore, Dennison, Andersen, and Yang (2003) in our laboratory characterized the pharmacokinetics of gasoline, a very complex mixture, in rats using an integrated PBPK modeling and lumping approach. The PBPK model tracks selected target components (benzene, toluene, ethylbenzene, *o*-xylene, and *n*-hexane) and a lumped chemical group representing all nontarget components. Competitive inhibition was the principal mechanism of pharmacokinetic interactions among these five selected target single chemicals and a pseudo-chemical from the lumped components. Computer-simulation results from the six-chemical interaction model matched well with gas uptake pharmacokinetic experimental data from single chemicals, a five-chemical mixture, and the two blends of gasoline. The PBPK model analyses indicated that metabolism of individual components was inhibited up to 27 percent during the six-hour gas uptake experiments of gasoline exposures.

The Current Status of PBPK Modeling of Chemical Mixtures

For a more comprehensive discussion of PBPK modeling of chemical mixtures, we refer you to Chapter Thirteen in a recently published book on PBPK (Yang and Andersen, 2005). Currently, the largest number of chemical components incorporated into a PBPK model is five individual chemicals and one lumped pseudo-chemical (Dennison, 2004; Dennison, Andersen, and Yang, 2003). Mechanistically,