CHAPTER 6

PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS IN TOXICITY TESTING AND RISK ASSESSMENT

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Abstract: Physiologically-based pharmacokinetic (PBPK) modeling offers a scientifically-sound framework for integrating mechanistic data on absorption, distribution, metabolism and elimination to predict the time-course of parent chemical, metabolite(s) or biomarkers in the exposed organism. A major advantage of PBPK models is their ability to forecast the impact of specific mechanistic processes and determinants on the tissue dose. In this regard, they facilitate integration of data obtained with in vitro and in silico methods, for making predictions of the tissue dosimetry in the whole animal, thus reducing and/or refining the use of animals in pharmacokinetic and toxicity studies. This chapter presents the principles and practice of PBPK modeling, as well as the application of these models in toxicity testing and health risk assessments.

INTRODUCTION

Toxicity tests and risk assessments improve our understanding of "how much chemical is too much", for human safety. Given the ethical considerations associated with human testing, animals have been employed as surrogates. With the highest level of emphasis placed on biologically relevant and cost-effective mammals, rodents are most often used in toxicity testing. While data from humans can be used in establishing safe exposure levels, human data are more frequently available for therapeutic and industrial compounds than for some classes of chemicals, such as pesticides (compounds developed

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and marketed based on their ability to produce toxic, even lethal, responses) and other environmental contaminants. In many instances, estimates of acceptable human exposure limits are developed from the results of tests in animals.[1-4] Studies with laboratory animals can be conducted to identify the toxic responses observed and to estimate the potency of the chemical; their results are considered to be valuable both from a qualitative and a quantitative perspective for extrapolation to humans exposed to low doses.[5-7]

Initial studies conducted for the purpose of "Hazard Identification" facilitate the identification of the organs, tissues and systems that are adversely affected by the chemical.[8] For the dose–response assessment, data describing the responses are interpreted in the context of dose—most often in the context of the applied (external) dose.[8-9] This dose is typically reported as mg/m^3 in air for inhaled toxicants and in mg/kg/day for orally ingested toxicants. Because chemicals are subject to pharmacokinetic processes (such as absorption, distribution, metabolism and elimination (ADME)) differently in animals and humans, a detailed understanding of the interspecies differences in these processes is essential to confidently extrapolate biological response data from animals to humans.[10-12]

The biological response results from the interaction between the toxicant and the target tissue. For this reason, models that can predict the target tissue concentration of the toxicologically-active chemical species (parent compound or metabolite) are especially useful and have been applied in what is referred to as the "exposure–dose–response" paradigm (Fig. 1).[9,13] Here, the "dose" refers to the target tissue concentration of the putative toxic moiety of a chemical. This exposure–dose–response paradigm is critically important for establishing conditions where humans are at risk for adverse outcomes defined in animal models. Due to their strong biological underpinnings, biokinetic models have become the preferred approach for conducting extrapolations of potential internal dose surrogates associated with toxicity.[14-19] In essence, biokinetic modeling, when linked with dynamic biological responses, serves as a systems biology tool at the whole-organ/whole-body level. Once validated, model-predicted target tissue concentrations should be reliable for the extrapolation of dosimetry across dose, route, time and species. The ability of the biokinetic models, especially the physiologically-based pharmacokinetic (PBPK) or toxicokinetic models, to calculate target tissue dose contributes to addressing and/or reducing some sources of uncertainty in risk assessments.[15,18]

This chapter introduces the principles and practice of PBPK modeling as applied in toxicity testing and risk assessment.

MODEL DEVELOPMENT

PBPK modeling refers to the development of quantitative descriptions of the ADME of chemicals, on the basis of interrelationships among the critical determinants of these processes.[14,20-22] The critical determinants of ADME include tissue volumes, physiological flow rates, rates of absorption, diffusion across cell membranes, tissue:blood partition coefficients and rates and affinities for biochemical reactions. These models are more useful than the conventional data-based pharmacokinetic models, particularly for the conduct of various extrapolations central to predictive toxicology applications.[23-25] The biological and mechanistic basis of the PBPK models enables them to be used, with limited animal experimentation, for extrapolation of the kinetic behavior of chemicals from test animal species to humans, from one exposure route to another and from high dose to low dose.[21,26] Initial work on the development of PBPK models dates back to the research work of Haggard on volatile organics

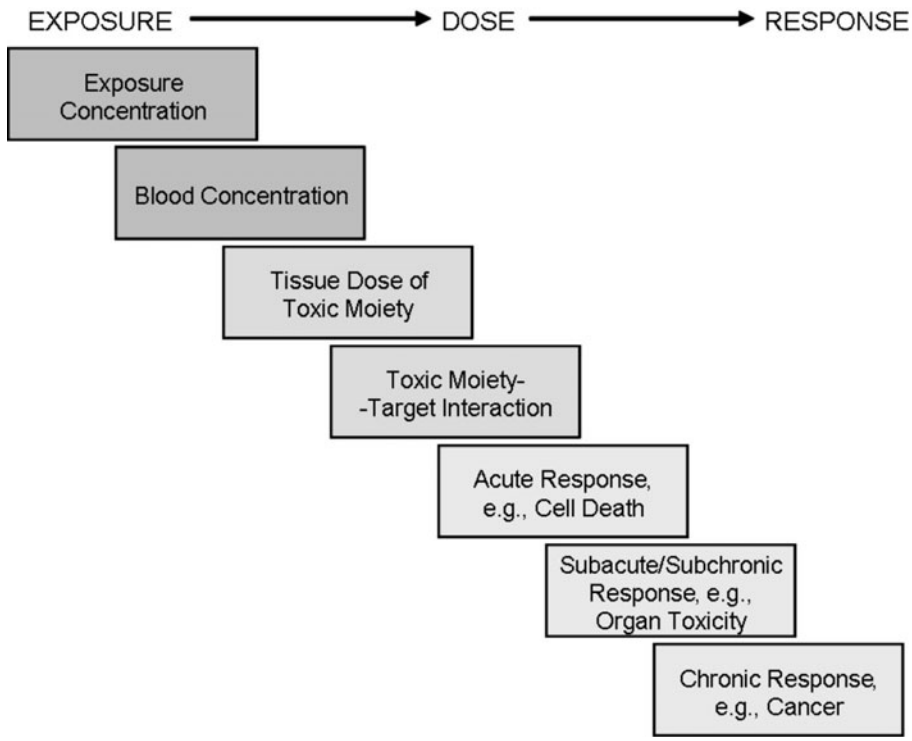

Figure 1. The exposure–dose–paradigm. Based on references 9 and 13.

and anaesthetics.[27] Further developments in the PBPK modeling of volatile chemicals, as well as pharmaceuticals, ensued.[28-35] Subsequently, the interest in the development of PBPK models has increased, due to their capacity to facilitate various extrapolations to enhance the scientific basis and efficiency of toxicity testing, as well as risk assessment.

At the most fundamental level, the PBPK model must be properly designed. Considerations include the biology of the animal species and the toxicity of the chemical. Failure to consider systematically the biology of the organism and the toxicity of the chemical of interest in guiding the model development process will prove detrimental. Flaws in the understanding of the key points of either will lead to incongruence and the failure of the developed model to meet expectations. Parsimony should be followed and the model should be only as complex as is necessary to address the key issues and tissues related to the toxicity of the chemical of interest.[36-37] Once the model structure has been established, values for physiological, physicochemical parameters and biochemical rate constants must be identified. Then, once the model has been structured and parameterized, the practitioner must determine its suitability through a process called evaluation or validation. This exercise demonstrates the fit between model predictions and data describing pharmacokinetic information (e.g., blood concentration–time-course data for the parent chemical, concentrations of metabolite in a given tissue). The success of this is critical to model application and is a function of the model structure, the appropriateness of the

parameter values and the reliability of the in vivo toxicokinetic data.[36-37] These various aspects are discussed in the following sections.

Model Structure

The structure of a PBPK model corresponds to a diagrammatic representation of the organism (i.e., species or individual) on the basis of the critical elements, in terms of tissues and ADME processes. Accordingly, the following aspects are considered to guide the selection of specific tissues for inclusion in the PBPK model:[37]

- Target organ or a surrogate compartment (e.g., blood)
- Portals of entry or uptake of chemicals (e.g., lungs, skin and gastrointestinal tract)
- Sites of significant metabolism (e.g., liver)
- Sites of significant storage capacity (e.g., adipose tissue, bone)

The tissue compartments are then interconnected via a systemic circulation (i.e., arterial and venous blood supplies), such that the mass balance of the cardiac output in the organism is maintained at all times in the model (Fig. 2). Tissues can be regrouped, if the concentration versus time-course of a chemical is comparable. Table 1 lists frequently used compartments in PBPK models, as well as the tissues/organs that are grouped together. The development of a reasonable model structure for a chemical then requires an understanding of the qualitative and quantitative determinants of ADME in the species of interest.

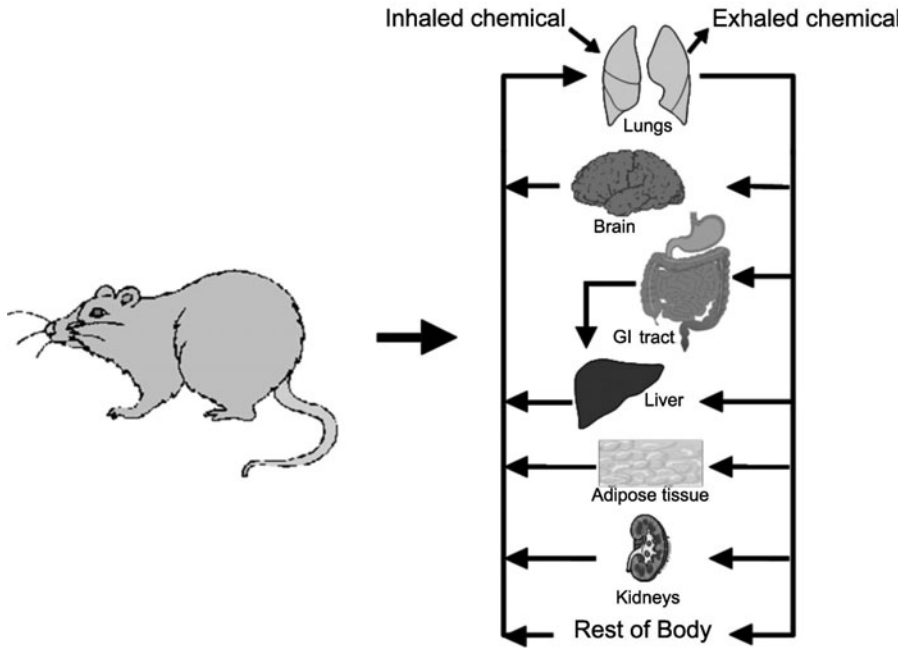

Figure 2. The structure of a PBPK model for a volatile organic chemical in the rat.

Table 1. Individual or groups of tissues frequently represented by compartments in PBPK models

Model Compartments	Tissues
Liver	Liver
Adipose tissue	Perirenal fat
	Epidymal fat
	Omental fat
	Subcutaneous fat
Slowly perfused tissues	Muscle
	Skin
Richly perfused tissues	Adrenal
	Kidney
	Thyroid
	Brain
	Lung
	Heart
	Testis
	Hepatoportal system

Model Equations

PBPK models consist of a set of differential equations based on physiological clearance (CL), in terms of L blood/hr. The various clearance terms represent the influx, efflux, metabolism and excretion processes. The rate of change in the amount of chemical during a given time interval (dA_t/dt) is then computed as follows:

$$\frac{dA_t}{dt} = (CL_{influx} \times C_a) - (CL_{efflux} \times C_{vt}) - (CL_{metabolic} \times C_a) - (CL_{renal} \times C_a) \quad (1)$$

where C_a = chemical concentration in arterial blood and C_{vt} = chemical concentraton in venous blood leaving, the concentrations of which are at equilibrium with concentrations in tissue t.

Equation (1) considers the tissue as a single homogenous compartment. Whereas this is adequate for low molecular weight compounds, it is often necessary to describe the uptake of high molecular substances via the vascular and intracellular compartments of the tissue separately.[38] Tissue distribution is typically modeled as flow-limited, where the concentration of agent in venous blood leaving the tissue is assumed to be in equilibrium with the concentration of agent in the tissue.

Table 2 presents the forms of equations frequently used in PBPK models for describing tissue influx, tissue efflux, renal clearance, as well as metabolic clearance.[37] Even though the venous equilibration model for hepatic metabolism has often been used in PBPK models, other types of physiological descriptions (i.e., parallel tube model, distributed sinusoidal perfusion model) may be used, depending on the intended use of the resulting PBPK model.[39-40]

Table 2. Examples of equations used in PBPK models for describing rate of change in tissues (i.e., influx-efflux), renal clearance (CL_r) and rate of hepatic metabolism ($\frac{dA_{met}}{dt}$)

Influx and efflux	$V_t \frac{dC_t}{dt} = Q_t \left(C_a - C_{vt} \right)$
Renal clearance	$CL_r = \frac{U_s \times V_u}{C_a}$
Metabolism	$\frac{dA_{met}}{dt} = \frac{V_{\max} C_{vt}}{K_m + C_{vt}}$
	$\frac{dA_{met}}{dt} = Q_t \times E \times C_a$

C_a : chemical concentration in arterial blood/plasma
C_t : concentration in tissue "t"
C_{vt} : concentration in venous blood/plasma leaving the tissue "t"
E : hepatic extraction ratio
K_m : Michaelis-Menten affinity constant
Q_t : flow rate to tissue
U_S : concentration of a substance in urine
V_{max} : maximal velocity of enzymatic reaction
V_t : volume of tissue "t"
V_u : urine flow rate

Parameter Estimation

PBPK models consist of a number of input parameters that can be conveniently categorized as physiological, physicochemical or biochemical in nature (Table 3). The physiological parameters frequently required for PBPK modeling include alveolar ventilation rate, cardiac output, tissue blood flow rates and tissue volumes. Table 4 provides reference values suggested by Arms and Travis[41] for adult rats and mice used in toxicity testing. Databases on animal and human physiological parameters in various age groups and strains/races are still evolving.[42-45]

The physicochemical parameters required for PBPK modeling are partition coefficients (PCs), which represent the relative distribution of a chemical between two matrices (i.e., blood and air or tissue and blood) at equilibrium. The blood:air and tissue:blood PCs for a number of chemicals have been determined by using in vivo pharmacokinetic data or in vitro techniques (equilibrium dialysis, ultrafiltration, vial equilibration).[37] Table 5 lists the various in silico methods that have become available for estimating the PCs for specific sub-groups of chemicals or drugs. A number of these animal-replacement methods use data on properties specific to chemicals, as well as characteristics specific to an individual or a population (examples are given in refs. 46-51). These in silico approaches account for the mechanistic determinants of tissue:blood PCs, which together with the volume

Table 3. Input parameters for a basic PBPK model

Type of Parameters	Specific Parameters
Physiological	Tissue volume Tissue blood flow Alveolar ventilation Cardiac output Glomerular filtration rate
Biochemical	Maximum velocity of metabolism Michaelis affinity constant Rate of absorption Binding affinity constant
Physicochemical	Blood:air partition coefficient Tissue:blood partition coefficient

Table 4. Reference physiological valuers for adult rats and mice. Based on Arms and Travis.[41]

	Weight (g)		Flow (mL/min)	
Compartments	Rats	Mice	Rats	Mice
Liver	10.0	1.4	20.8	4.3
Fat	17.5	2.5	7.5	1.5
Slowly perfused tissues	187.5	17.5	12.5	2.6
Richly perfused tissues	12.5	1.3	42.3	8.7
Whole body	250.0	25.0	83.0	17.0

of tissues and blood facilitate the computation of the volume of distribution (Vd), as shown below:[23]

$$Vd = V_b + \Sigma P_{tb} * V_t \quad (2)$$

where V_b = blood volume, V_t = volume of tissues and P_{tb} = tissue:blood PCs.

When uncomplicated by species differences in protein binding, simple allometric scaling of Vd determined in test species can produce reasonable estimates of Vd in humans. However, when such data are not available, or when interspecies difference in protein binding is significant, data on the fraction unbound would be essential to predict Vd, as well as PCs essential for PBPK modeling.[52-53]

The biochemical parameters required for PBPK modeling frequently include absorption rate constants, maximal velocity for metabolism (V_{max}), Michaelis constant (K_m), binding association constant and urinary/biliary excretion rate. These parameters have often been determined on the basis of time-course data collected in vivo or in vitro; data analysis to estimate specific parameter(s) is then conducted by using the portion of the time-course curve that is most sensitive to one or two dominant factors.[54-56]

The rate of oral absorption has been determined in vivo on the basis of kinetic data on the exhaled breath or blood concentrations of administered chemicals. Based on knowledge

Table 5. In silico approaches and their applicability to specific chemical classes for estimating partition coefficients

Chemical Class	Approach	References
Empirical Approaches		
Basic organic chemicals	Relationship of Pt:p with Log P	109
Weakly basic drugs	Relationship of Pt:p with Log P and phosphatidylserine tissue content	110
Volatile organic chemicals and drugs	QSAR relationships of PCs (Brain:air, brain:blood, blood:air, brain:air, brain:blood, muscle:air, muscle:blood, skin:plasma, skin:blood, liver:air, liver:blood, lung:air, lung:blood) using various molecular descriptors	111-117
Histamine receptor H 2 antagonists	Relationship between brain:blood and octanol:water, cyclohexane:water, molecular mass and water accessible volume	118
Histamine receptor H 2 antagonists	QSAR relationship between P brain:blood and free energy of salvation	119
Volatile organic chemicals	Relationship between Pt:b and log P using tissue and blood composition data.	48
Barbituric acids	Relationship Kpu with Log P.	120-121
Structurally diverse compounds	QSAR relationship between P brain:blood with several topological and constitutional descriptors of molecules.	122
Drugs	Use of muscle:plasma as surrogate for the estimation of Pt:p of other tissues except fat.	53
Acid and basic drugs	Use of muscle:plasma as surrogate for the estimation of Pt:p of other tissues except fat.	123

continued on following page

of the determinants (i.e., lipophilicity, pKa, solubility, particle size, permeability, as well as, if applicable, release kinetics and dissolution kinetics), mathematical models and algorithms have been developed to simulate the rate of absorption in animals and humans.[57-58] These types of models have more generally been used in pharmaceutical research, where estimation of rate of absorption is important in determining the passage from preclinical to clinical Phase 1 research. Often with environmental contaminants, the gastrointestinal absorption rates (i.e., first order rate constants) have been estimated on the basis of in vivo data,[37] whereas a number of in vitro systems (reconstituted enzyme preparations, subcellular fractions, postmitochondrial preparations, isolated cells, tissue slices and isolated perfused organs) have been used for the estimation of metabolic rate constants.[59-73] In this regard, several studies involving the use of microsomal protein, postmitochondrial fractions or freshly isolated hepatocytes, have demonstrated the feasibility of incorporating metabolic rate constants directly within PBPK models for low molecular weight organic chemicals.[13,74-76] In general, the K_m values obtained in vitro

Table 5. Continued

Chemical Class	Approach	References
Mechanistic Approaches		
Volatile organic chemicals	Estimation of Pt:b from Log P and tissue composition data (neutral lipids, phospholipids and water)	49
Volatile organic chemicals	Estimation of Pt:a and Pb:a from molecular structure and tissue composition data	50-51
Volatile organic chemicals	Estimation of Pb:a from Log P, tissue composition data and association binding constant for hemoglobin	50, 51, 99
Highly lipophilic chemicals	Estimation of adipose:plasma from tissue composition data only	124
Various Drugs	Estimation of Pt:p from log P, fraction unbound in plasma and tissue composition data	123
Various Drugs	Estimation of Pt:p from log P, fraction unbound in plasma and tissue composition data	125
Moderate to strong basic drugs	Estimation of Kpu from log P, pKa, fraction unbound in plasma and tissue composition and pH data and electrostatic interactions with acidic phospholipids	126
Acidic, very weak basic, neutral and zwitterionic drugs.	Estimation of Kpu from log P, pKa, fraction unbound in plasma, blood:plasma partitioning, tissue composition, pH, albumin and lipoprotein concentration data	127

Pt:p = tissue:plasma partition coefficient; Log P = n-octanol:water partition coefficient; Pt:b = tissue:blood partition coefficient; Kpu = tissue-to-plasma water partition coefficient

have been used directly, but V_{max} obtained in vitro has been scaled to the whole organism based on the mass recovery of the particular fraction, as follows:[37]

$$V_{max\ (in\ vivo)} = V_{max\ (in\ vitro)} \times C_{prot} \times F_{tiss} \quad (3)$$

where $V_{max\ (in\ vivo)}$ = maximal velocity of metabolism in vivo (mg/min per kg body weight), $V_{max\ (in\ vitro)}$ = maximal velocity of metabolism in vitro (mg/min/mg microsomal protein), C_{prot} = concentration of microsomal protein (mg/g tissue) and F_{tiss} refers to the fractional volume of the metabolizing tissue (e.g., g liver/kg body weight).

The generalizability of in vitro to in vivo extrapolation and animal-replacement algorithms is fairly limited, because the critical determinants in each of these cases are likely to vary as a function of the metabolic reactions (Phase I versus Phase II), metabolizing enzymes and physicochemical properties of the substrates. In fact, mechanistic animal-replacement approaches for predicting the numerical values of V_{max} and K_m of Phase I and Phase II metabolism of chemicals are not yet available. Some semi-empirical approaches relating the molecular structure information to metabolic rate constants have been developed.[77] A pragmatic animal-replacement approach focuses on the generation of "envelope" of simulations representing a plausible internal dose, by specifying complete or negligible hepatic extraction in PBPK models.[78] This approach is particularly useful for forecasting the possible internal dose of chemicals that are not rapidly cleared at the portal

of entry, thus making easier the construction of PBPK models to facilitate the planning of the exposure scenario (e.g., number of doses, dosing duration) for in vivo toxicology studies. Such screening level approaches to PBPK parameter estimation might help to determine the extent of improvement in model predictions that can be obtained while investing time and energy to refine or estimate specific input parameters for PBPK models.

The rate constants of chemical reaction with hemoglobin, tissue proteins, etc., determined in vitro or in vivo, have been incorporated into the PBPK model to make predictions of these phenomena in vivo.[79-80] The feasibility of incorporating in vitro data on receptor binding and DNA binding properties of chemicals within PBPK models for simulating in vivo behavior, has also been demonstrated.[81-82]

MODEL EVALUATION

Once the model is constructed, parameterized and written in a simulation/programming language, it is essential to evaluate the usefulness of the model for the intended applications. All mathematical models of complex reality have potentially built-in uncertainty or errors related to model structure and model parameters.[83] The adequacy of the model structure, as well as the parameter values, is often inferred by comparing the model simulations with experimental data that had not been used for estimating the parameters. This process has been referred to as "validation". even though the use of the term "evaluation" is being increasingly preferred by PBPK modelers.[84-85] Model evaluation is more global and consists not only of comparing model simulations with experimental data, but also conducting sensitivity, uncertainty and variability analyses for assessing the adequacy of the input parameters and structure.

Regardless of the terminology (i.e., validation versus evaluation), the intent is essentially to assess whether:

A. the major determinants of the system behavior are adequately captured by the model; and
B. the input parameters adequately represent the species or population and the chemical for specific exposure conditions.

The choice of method(s) for comparing model simulations with data (i.e., visual inspection, discrepancy indices, statistical tests including residual analysis) depends upon the purpose for which the model is to be used.[86-88] Even though quantitative tests of goodness-of-fit are useful, it is equally important to consider the ability of the model to provide an accurate prediction of the general trend of the time-course data (i.e., bumps, valleys).[21,89]

Following the satisfactory evaluation of a PBPK model, it is used for conducting extrapolations and computations of internal dose for improving the dose–response relationship in the context of toxicity testing and risk assessment.

MODEL APPLICATION

The principal application of PBPK models is to predict the target tissue dose of the toxic parent chemical or its metabolite. By using the tissue dose of the toxic moiety of

a chemical (or its surrogate) in risk assessment calculations, a better basis is provided for relating to the observed toxic effects than is the use of the external or exposure concentrations of the parent chemical.[9,15,90] A critical aspect in this regard relates to model selection, i.e., selecting a PBPK model that can adequately address a particular issue associated with an assessment. This process would require the consideration of the following aspects:[91-92]

- The species for which the model has been constructed versus the species used in toxicity tests or dose–response study chosen for an assessment
- The lifestage(s) for which the PBPK model has been parameterized and evaluated versus the lifestage for which the critical toxicity benchmark (e.g., the NOAEL) has been developed
- The exposure route used in the critical toxicity test versus exposure route(s) described in the model, as well as those that are of relevance to the assessment
- The exposure duration(s) for which the model has been tested versus the duration of the critical toxicity test
- The maximal dose for which the model performance has been evaluated versus the doses used in toxicity test or dose-response study
- The plausible measures of internal dose ("dose metric") based on the current state of knowledge on the mode of action of the chemical versus the capability of the PBPK model to simulate these various dose metrics
- The nature of parameter specification in the model (i.e., point estimate, distributions) versus the intended end-use of the model (e.g., estimation of an inter-individual variability factor)

Because PBPK models facilitate the prediction of target tissue dose for various exposure scenarios, routes, doses and species,[15,21] they can help reduce the uncertainty associated with the conventional extrapolation approaches and assessment factors employed in cancer and noncancer risk assessments, as well as improving the interpretation of the outcomes of toxicity tests.

Toxicity Testing

Animal tests generally focus on characterizing the pharmacokinetics, mode of action or toxicity associated with various dose levels, exposure routes and scenarios. Specifically, pharmacokinetic studies focus on determining the time-course of parent chemical, metabolite(s) or biomarkers in the exposed organism. In the design of such studies, it is critical to determine the time-points for sacrifice or sampling, so that animal use can be efficiently minimized. In this regard, one of the applications of PBPK models is to forecast the blood and tissue concentrations in the exposed animal as a function of time, such that appropriate sampling times can be chosen (Fig. 3). Such judicious use of PBPK models will facilitate the efficient determination of sacrifice/sampling times at which the chemical concentrations would still be above the limit of detection (LOD) of the analytical method, as well as be adequately representative of critical portions of the time-course curve to facilitate the calculation of dose metrics (e.g., AUC as a measure of internal exposure). When limited in vivo data are available, PBPK models can be particularly useful to predict kinetics in intact animals on the basis of in vitro data on metabolic rates and PCs.[93-97] Similarly, in silico approaches can also be used in generating initial estimates of chemical-specific parameters for constructing PBPK

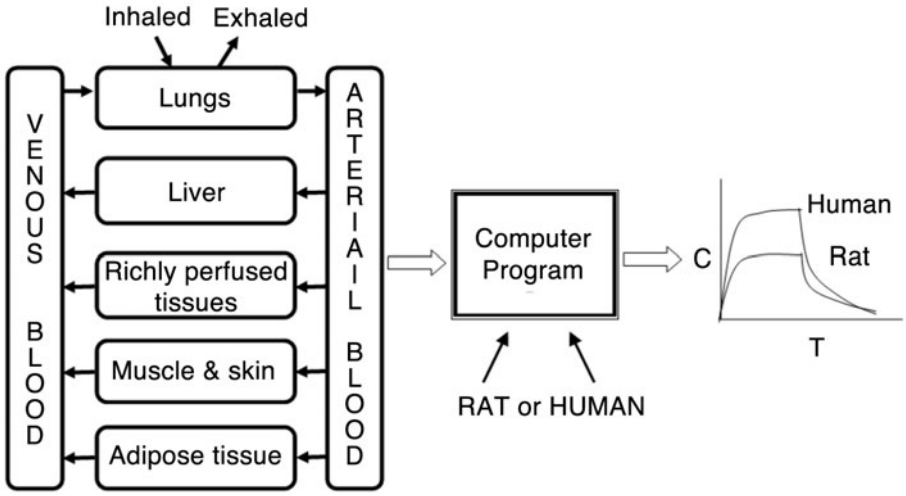

Figure 3. Illustration of the use of PBPK model for prediction of the time-course (C vs T) of tissue dose of a chemical in exposed animals and humans.

models to simulate the time-course of the blood or tissue concentrations of a chemical and its metabolite.[98-99]

In the context of toxicity tests focused on the characterization of the dose–reponse behavior of chemicals or identification of organ-specific effects, the PBPK models are of use in the study design and/or interpretation of results. Pharmacokinetic models and data are particularly useful for study design—specifically for determining the dose levels, as well as frequency, interval and duration of exposure. For example, a PBPK model can be used for determining the exposure conditions that are ideal for maintaining a certain level of internal dose (e.g., over a threshold level) and to choose dose levels that cover a range of conditions (e.g., first order, saturable). A PBPK model can also be used for determining the toxicologically-equivalent doses of systemically-acting chemicals for different exposure routes (Fig. 4). When PBPK models are integrated with biologically-based pharmacodynamic (PD) models, they allow not only the time-course of internal dose in exposed animals to be predicted, but also the toxicological responses, based on an understanding of the mode(s) of action.[14] The PBPK/PD models are also powerful tools for integrating the data on absorption, metabolism, protein binding, receptor interaction and other relevant mechanistic data obtained in vitro with animal physiology, for providing simulations of toxicity outcome in intact animals.[95,100] Even though there has been only limited progress in developing integrated PBPK/PD models for predicting toxicity profiles in silico, there are ample examples of the application of PBPK models in cancer and noncancer risk assessments.[101]

Cancer Risk Assessment

The risk assessment process for genotoxic and epigenetic carcinogens often requires the conduct of high-dose to low-dose, route to route and interspecies extrapolations. Instead

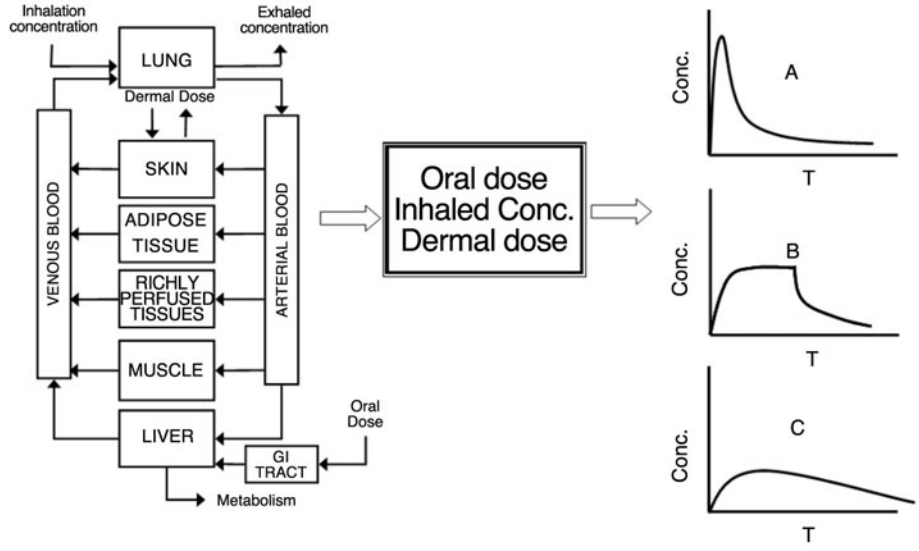

Figure 4. Illustration of the use of PBPK model to predict the concentration of the toxic moiety of chemical in animals exposed via the oral (A) or inhalation (B) routes or via dermal contact (C).

of relying on the conventional approaches based on body weight or body surface area, PBPK models are increasingly used to reduce the scientific uncertainty in the conduct of such extrapolations.[22] Due to their strong biological underpinnings, biokinetic modeling has become the preferred approach for conducting extrapolations of potential internal dose surrogates associated with carcinogenicity.[9,15,17-18] In this regard, extrapolation between laboratory animals and humans is achieved by using species-specific data on input parameters (Fig. 3). Accordingly, physiological parameters (breathing rate, cardiac output, tissue volumes, blood flows, glomerular filtration rate) are obtained for the species of interest or are computed on the basis of body surface scaling. The maximal velocity of metabolism is also scaled on the basis of body surface or body weight in data-poor situations, whereas tissue solubility and the Michaelis constant are most often considered to be species-invariant.[37] The ability of PBPK models to simulate the target tissue dose facilitates the enhancement of the scientific basis of cancer risk assessments. The initial application of PBPK models in cancer risk assessment was demonstrated with dichloromethane (DCM).[9,103] The PBPK model-based cancer risk assessment for this chemical predicted human low-dose risk, about 100- to 200-fold less than that predicted by the conventional approach based on linear extrapolation of high dose to low dose behavior and interspecies dose conversion based on body surface scaling.[104-105] Following the DCM example, there have been several reports of the use of PBPK models in the prediction of the dose metric for enhancing the scientific basis of cancer risk assessment for environmental agents (e.g., vinyl chloride, chloroform, methyl chloroform, 1,4-dioxane, trichloroethylene, acrylonitrile and methyl methacrylate). The vinyl chloride cancer assessment illustrates the unique usefulness of PBPK models, not only for the conduct of high dose to low and interspecies extrapolations, but also for the route-to-route extrapolation. Impressively, the PBPK model-based risk estimates

facilitated the demonstration of the similarity of the range of risk estimates obtained from epidemiological studies and animal bioassays.[106]

Noncancer Risk Assessment

Risk assessments for systemically-acting noncarcinogens have conventionally been based on the knowledge of the point of departure (e.g., NOAEL, LOAEL (lowest observed adverse effect level), BMD (benchmark dose) and the application of uncertainty factors. These factors account for interspecies differences and intraspecies variability in pharmacokinetics and pharmacodynamics, as well as address uncertainty associated with duration extrapolation, data base completeness and data quality.[107]

The application of PBPK models in noncancer risk assessment relies on the availability of sufficient information about the mode of action to define a reasonable internal dose surrogate that is relevant to toxicity. The adverse interaction between chemical agents and living systems is best addressed on a tissue basis, or even on a cellular or subcellular basis. This involves three equally important issues.

First, it requires a knowledge of the most sensitive endpoint, the species that demonstrates that endpoint and the exposure concentration or dose at which no toxicity is observed (NOAEL) in that species. The toxic endpoint of concern needs to be evaluated for relevance—for example, the importance of male rat-specific $\alpha 2\mu$ globulin-mediated nephrotoxicity to human risk assessment is likely to be minimal. In this scenario, the NOAEL will represent a point of departure (the dose–response point that marks the beginning of the low-dose no-effect level or the lower bound of the observed affect).

The second important issue for the use of PBPK models is an understanding of the dose metric, reflective of the effective (risk-relevant) internal dose of the parent chemical or metabolite that is associated with that most sensitive endpoint. The appropriate dose metric is then compared between humans and the most sensitive species by using a PBPK model, since human studies are rarely able to determine tissue-specific dose or toxicity due to ethical concerns.

The final aim is to come full circle and calculate a human equivalent exposure. This would be in the form of a human equivalent concentration (HEC) for inhaled toxicants and a human equivalent dose (HED) for orally-encountered toxicants. Humans encountering these concentrations would develop the same level of the dose metric (e.g., area under the curve [AUC] or maximal concentration [C_{MAX}]) as in the animals exposed to the dose or concentration representing the point of departure (the NOAEL or BMD_L. Generally, once a nonlethal exposure has reached a duration where systemic toxicity is observed, time-normalized dose metrics such as the AUC will represent a dose metric that is more representative of risk. C_{MAX} values are often useful in establishing the dose–response relationship for acute toxicities and are dependent upon dosing rate, such that the high concentration bolus doses commonly encountered in animal experiments will lead to higher peak concentrations than the multi-exposure (divided-dose) scenarios most often encountered by humans.

The role of PBPK models in noncancer risk assessments, particularly for characterizing the magnitude of the pharmacokinetic component of the interspecies uncertainty factor and the intraspecies variability factor, has been summarized by Dewoskin et al.[101] In internal dose-based assessments, the remaining uncertainty relates

to pharmacodynamics, i.e., the response of the tissues to the exposure.[17-18] An example of a noncancer risk assessment that serves to illustrate the use of PBPK models would be ethylene glycol monobutyl ether.[18] Here, the dose metric, $Cmax_{metabolite}$ associated with the point of departure (i.e., $LOAEL_{animal}$) in the animal study was determined by using an animal PBPK model. Subsequently, a human PBPK model was used to determine the oral dose associated with the same level of the dose metric. The resulting human-equivalent dose (7.6 mg/kg/d) was then divided by the appropriate uncertainty factors (10 for human interindividual differences in pharmacokinetics and pharmacodynamics; 3 to account for the LOAEL to NOAEL extrapolation) for deriving the reference dose for humans (0.3 mg/kg/d).[18,108]

CONCLUSION

PBPK modeling offers a scientifically-defensible framework for integrating mechanistic data relating to ADME for predicting dose to target tissues during toxicity tests in animals. A major advantage of these kinds of models relates to their ability to forecast the impact of specific mechanistic processes and determinants on the tissue dose. For example, one can conduct simulations of tissue dose to address the question of "what if …." with regard to variable factors such as the maximal rate of metabolism, the Michaelis constant, etc. In this regard, they provide a basis for integrating in vitro data and making predictions of the tissue dosimetry in the whole animal, thus reducing and/or refining the use of animals in pharmacokinetic and toxicity studies.

In vitro and in silico methods offer valuable alternatives to develop values for physicochemical parameters (e.g., tissue PCs) and biochemical rate constants for use in developing PBPK models. As opposed to in vivo methods, these alternatives offer the advantage that intact animals need not be exposed to test agents and they can be applied to human tissues obtained from organ donors. When the test agent is costly and/or potentially toxic, reducing animal use and avoiding human exposure can have obvious benefits. The reliability of risk values developed following advanced pharmacokinetic studies is largely determined by the choice of test system, so the practitioner should make well-informed choices among the various alternatives.

Effort should be made to assess confidence in the PBPK model for specific applications in toxicity testing and risk assessment. In this regard, PBPK models can support the choice of certain range of doses, such that they are within the linear phase of metabolism, or range of exposure scenarios that lead to steady-state conditions. Similarly, PBPK models can be used to guide dose selection for conducting toxicity test by different routes of exposure. In this case, the models would be used to determine the exposure dose for a new exposure route (e.g., dermal), based on information available for another route (i.e., inhalation) on the basis of equivalent tissue dose. These biologically-based models are dynamic constructs that can be adapted to reflect the exposure conditions of interest to the investigator(s) and updated as new information on mechanistic and molecular determinants becomes available.

In summary, the role of PBPK modeling in improving the exposure–dose–response relationship reflects the use of a systems approach to solving complex problems in experimental toxicology and risk assessment and as such it will be central to the success of the new toxicity paradigms.

DISCLAIMER

This manuscript presents the collective views of the authors. Views and opinions expressed do not necessarily reflect those of their respective employers. The views and opinions herein may not represent the views and policies of the U.S. Environmental Protection Agency.

NOTE ADDED AFTER PROOFS

Since this chapter was drafted, a valuable guidance document has been finalized by the World Health Organization's International Programme on Chemical Safety. *Principles of Characterizing and Applying PBPK Models in Risk Assessment* (WHO/IPCS, 2010)[128] offers the reader important insight into a careful evaluation process for PBPK models of potential use in health risk assessment. This document should be consulted by readers who are interested in more in-depth coverage of this topic.

REFERENCES

1. WHO. Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits. Environmental Health Criteria 170, International Programme on Chemical Safety. Geneva: World Health Organization, 1994.
2. Health Canada. Human health risk assessment for priority substances. Ottawa: Environmental Health Directorate, Health Canada, 1994.
3. Younes M, Sonich-Mullin C, Meek ME et al. Risk: assessment and management. In: Herzstein JA, Bunn WB, Fleming LE, eds. International Occupational and Environmental Medicine. St Louis: Mosby, 1998:62-74.
4. Ritter L, Totman C, Krishnan K et al. Deriving incertainty factors for threshold chemical contaminants in drinking water. J Toxicol Environ Health B 2007; 10:527-557.
5. Sonich-Mullin C, Fielder R, Wiltse J et al. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharm 2001; 34:46-52.
6. Boobis AR, Cohen SM, Dellarco V et al. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit Rev Toxicol 2006; 36:781-92.
7. Boobis AR, Doe JE, Heinrich-Hirsch B et al. IPCS framework for analysing the relevance of a noncancer mode of action for humans. Crit Rev Toxicol 2008; 38:87-96.
8. NRC Risk Assessment in the Federal Government: Managing the Process. Washington: National Academy Press, 1983.
9. Andersen ME, Clewell HJ, Gargas ML et al. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 1987; 87:185-205.
10. Jarabek AM. Interspecies extrapolation based on mechanistic determinants of chemical disposition. Human Ecological Risk Assessment 1995; 1:641-662.
11. Jarabek AM. Inhalation RfC methodology: dosimetric adjusments and dose-response estimation of noncancer toxicity in the upper respiratory tract. Inhal Toxicol 1994; 6:301-325.
12. Monro A. Drug toxicokinetics: Scope and limitations that arise from species differences in pharmacodynamic and carcinogenic responses. J Pharm Biopharm 1994; 22:41-57.
13. Andersen ME, Krishnan K. Relating in vitro to in vivo exposures with physiologically based tissue dosimetry and tissue response models. In: Salem H, ed. Animal Test Alternatives: Refinement, Reduction, Replacement. New York: Marcel Dekker, 1995:9-26.
14. Andersen ME. Toxicokinetic modeling and its applications in chemical risk assessment. Toxicol Lett 2003; 138:9-27.
15. Clewell HJ, III, Andersen ME, Barton HA et al. A consistent approach for the application of pharmacokinetic modeling in cancer and noncancer risk assessment. Environ Health Persp 2002; 110:85-93.
16. Reddy MB, Yang RSH, Clewell HJ, Andersen ME, eds. Physiologically Based Pharmacokinetic Modeling: Science and Application. Hoboken: John Wiley and Sons, 2005:420.

17. EPA. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report) EPA/600/R-05/043A. Washington, DC: US Environmental Protection Agency, 2006.
18. EPA. Toxicological review of ethylene glycol monobutyl ether. In support of summary information on the IRIS. Washington, D.C.: US Environmental Protection Agency, 2006: Available at: http://www.epa.gov/iris/toxreviews/0500tr.pdf.
19. Lipscomb JC, Ohanian EV, eds. Toxicokinetics and Risk Assessment. New York: Informa Healthcare, 2006:361.
20. Bischoff KB. Physiogically-based pharmacokinetic modeling. Drinking Water and Health 1987; 8:36-64.
21. Clewell HJ 3rd, Andersen ME. Dose, species and route extrapolation using physiologically-based pharmacokinetic models. Drinking Water and Health 1987; 8:159-182.
22. Krishnan K, Johanson G. Physiologically based pharmacokinetic and toxicokinetic models in cancer risk assessment. J Environ Sci Health 2005; 23(C):31-53.
23. Gibaldi M, Perrier D. Pharmacokinetics. New York: Marcel Dekker, 1982.
24. Wagner JG. Fundamentals of Clinical Pharmacokinetics. Hamilton, Illinois: Drug International, 1975
25. Renwick AG. Pharmacokinetics in toxicology. In: Hayes WA, ed. Principles and Methods of Toxicology. New York: Raven Press, 1994:101-147.
26. Clewell HJ 3rd, Andersen ME. Risk assessment extrapolations and physiological modeling. Toxicol Ind Health 1985; 1(4):111-131.
27. Haggard HW. The absorption, distribution and elimination of ethyl ether. Analysis of the mechanism of the absorption and elimination of such a gas or vapor as ethyl ether. J Biol Chem 1924; 59:753-770.
28. Kety SS. The theory and application of the exchange of inert gas at the lungs. Pharmacol Rev 1951; 3:1-41.
29. Fiserova-Bergerova V. Mathematical modeling of inhalation exposure. J Combust Toxicol 1975; 32:201-210.
30. Fiserova-Bergerova V. Toxicokinetics of organic solvents. Scand J Work Environ Health 1985; 11(Suppl 1-7):7-21.
31. Bischoff KB, Dedrick RL, Zakharo DS et al. Methotrexate pharmacokinetics. J Pharm Sci 1971; 60:1128-1133.
32. Mapleson WW. An electric analog for uptake and elimination in man. J Appl Physiol 1963; 18:197-204.
33. Riggs DS. The Mathematical Approach to Physiological Problems: A Critical Primer. Cambridge: MIT Press, 1970.
34. Teorell T. Kinetics of distribution of substances administered to the body. I. The extravascular modes of administration. Arch Int Pharmacodyn 1937; 57:205-225.
35. Teorell T. Kinetics of distribution of substances administered to the body. II. The intravascular modes of administration. Arch Int Pharmacodyn 1937; 57:226-240.
36. Clewell RA, Clewell HJ 3rd. Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Reg Toxicol Pharmacol 2008; 50(1):129-43.
37. Krishnan K, Andersen ME. Physiologically-based pharmacokinetic and toxicokinetic modeling. In: Wallace Hayes A, ed. Principles and Methods in Toxicology. New York: Taylor and Francis Inc., 2007:232-291.
38. Gerlowski LE, Jain RK. Physiologically based pharmacokinetic modeling: Principles and applications. J Pharml Sci 1983; 72:1103-1127.
39. Robinson PJ. Effect of microcirculatory heterogeneity in the determination of pharmacokinetic parameters: implications for risk assessment. Drug Metab Rev 1991; 23:43-64.
40. Robinson PJ. Physiologically-based liver modeling and risk assessment. Risk Anal 1992; 12:139-148.
41. Arms AD, Travis CC. Reference Physiological Parameters in Pharmacokinetic Modeling. Office of Health and Environmental Assessment NTIS PB 88-196019. Washington D.C.: US Environmental Protection Agency, 1988.
42. Brown RP, Delp MD, Lindstedt SL et al. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Indust Health 1997; 13:407-484.
43. Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharmac Res 1993; 10:1093-1095.
44. Gentry PR, Haber LT, McDonald TB et al. Data for physiologically based pharmacokinetic modeling in neonatal animals: physiological parameters in mice and Sprague-Dawley rats. J Child Health 2004; 2(3-4):363-411.
45. Thompson C, Johns D, Sonawane B et al. Database for physiologically based pharmacokinetic modeling: physiological data for healthy and health-impaired elderly. J Toxicol Environ Health B 2009; 12:1-24.
46. Balaz S, Lukacova V. A model-based dependence of the human tissue/blood partition coefficients of chemicals on lipophilicity and tissue composition. QSAR 2000; 18:361-368.
47. Béliveau M, Lipscomb J, Tardif R et al. Quantitative structure-property relationships for interspecies extrapolation of the inhalation pharmaconkinetics of organic chemicals. Chem Res Toxicol 2005; 18:475-485.
48. DeJongh J, Verhaar HJM, Hermens JLM. A quantitative property-property relationship (QPPR) approach to estimate in vitro tissue-blood partition coefficients of organic chemical sin rats and humans. Arch Toxicol 1997; 72:17-25.

49. Poulin P, Krishnan K. A biologically-based algorithm for predicting human tissue:blood partition coefficients of organic chemicals. Human Exp Toxicol 1995; 14:273-280.
50. Poulin P, Krishnan K. A mechanistic algorithm for predicting blood: air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin. Toxicol Appl Pharmacol 1996; 136:131-137.
51. Poulin P, Krishnan K. A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. Toxicol Appl Pharmacol 1996; 136:126-130.
52. Obach RS, Baxter JG, Liston TE et al. The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. J Pharmacol Exp Ther 1997; 283:46-58.
53. Poulin P, Theil FP. A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. J Pharm Sci 2000; 89:16-35.
54. Gargas ML, Burgess RJ, Voisard DE et al. Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 1989; 98:87-99.
55. Gargas ML. An exhaled breath chamber system for assessing rates of metabolism and rates of gastrointestinal absorption with volatile chemicals. J Am Coll Toxicol 1990; 9:447-453.
56. Gargas ML, Clewell HJ, Andersen ME et al. Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes and chloroethylenes in the rat. Inhalation Toxicol 1990; 2:295-319.
57. Dokoumetzidis A, Kalantzi L, Fotaki N et al. Predictive models for oral drug absorption: from in silico methods to integrated dynamical models. Expert Opin Drug Metab Toxicol 2007; 3(4):491-505.
58. Fagerholm U. Prediction of human pharmacokinetics—gastrointestinal absorption. J Pharm Pharmacol 2007; 59(7):905-16.
59. Dedrick RL, Forrester DD, Ho DHW et al. In vitro-in vivo correlation of drug metabolism: deamination of 1-β-D-arabinosyl cytosine. Biochem Pharmacol 1972; 21:1-16.
60. Nakajima T, Sato A. Enhanced activity of liver drug-metabolizing enzymes for aromatic and chlorinated hydrocarbons following food deprivation. Toxicol Appl Pharmacol 1979; 50:549-556.
61. Sato A, Nakajima T. A vial equilibration method to evaluate the drug metabolizing enzyme activity for volatile hydrocarbons. Toxicol Appl Pharmacol 1979; 47:41-46.
62. Hilderbrand RL, Andersen ME, Jensen LJ. Prediction of in vivo kinetic constants for metabolism of inhaled vapors from kinetic constants measured in vitro. Fundam Appl Toxicol 1981; 1:403-409.
63. Lin JH, Sugiyama Y, Awazu S et al. Physiological pharmacokinetics of ethoxybenzamine based on biochemical data obtained in vitro as well as on physiological data. J Pharmacokin Biopharm 1982; 10:649-661.
64. Mortensen B, Nilsen OG. Allometric species comparison of toluene and n-hexane metabolism: prediction of hepatic clearance in man from experiments with rodent liver S9 in headspace vial equilibration system. Pharmacol Toxicol 1988; 82:183-188.
65. Reitz RH, Mandrela AL, Guengerich FP et al. In vitro metabolism of methylene chloride in human and animal tissues: Use in physiologically-based pharmacokinetic models. Toxicol Appl Pharmacol 1989; 97:230-246.
66. Reitz RH, Mendrala AL, Park CN et al. Incorporation of in vitro enzyme data into the physiologically-based pharmacokinetic (PBPK) model for methylene chloride: implications for risk assessment. Toxicol Lett 1988; 43:97-116.
67. Gearheart JM, Jepson GW, Clewell HJ et al. A physiologically-based model for the in vivo inhibition of acetylcholinesterase by diisopropylfluorophosphate. Toxicol Appl Pharmacol 1990; 106:295-310.
68. DeJongh J, Blaauboer BJ. In vitro-based and in vivo-based simulations of benzene uptake and metabolism in rats. Altern Lab Anim—ATLA 1996; 24:179-190.
69. DeJongh J, Blaauboer BJ. Simulation of toluene kinetics in the rat by a physiologically based pharmacokinetic model with application of biotransformation parameters derived independently in vitro and in vivo. Fundam Appl Toxicol 1996; 32:260-268.
70. DeJongh J, Blaauboer BJ. Evaluation of in vitro-based simulations of toluene uptake and metabolism in rats. Toxicol In Vitro 1997; 11:485-489.
71. Iwatsubo T, Suzuki H, Sugiyama Y et al. Prediction of species differences (rats, dogs, humans) in the in vivo metabolic clearance of YM796 by the liver from in vitro data. J Pharmacol Exp Ther 1997; 283:462-469.
72. Mortensen B, Lokken T, Zahlsen K et al. Comparison and in vivo relevance of two different in vitro headspace metabolic systems: liver S9 and liver slices. Pharmacol Toxicol 1997; 81:35-41.
73. Lipscomb JC, Barton HA, Tornero-Velez R et al. The metabolic rate constants and specific activity of human and rat hepatic cytochrome P-450 2E1 toward toluene and chloroform. J Toxicol Environ Health 2004; 67(A):537-553.
74. Krishnan K, Gargas ML, Andersen ME et al. In vitro toxicology and risk assessment. Altern Meth Toxicol 1993; 9:185-203.
75. Lipscomb JC, Fisher JW, Confer PD et al. In vitro to in vivo extrapolation for trichloroethylene metabolism in humans. Toxicol Appl Pharmacol 1998; 152:376-387.

76. Poet TS, Wu H, English JC et al. Metabolic rate constants for hydroquinone in F344 rat and human liver isolated hepatocytes: application to a PBPK model. Toxicol Sci 2004; 82:9-25.
77. Béliveau M, Krishnan K. In silico approaches for developing physiologically based pharmacokinetic (PBPK) models. In: Salem H, Katz S, eds. Alternative Toxicological Methods. Boca Raton: CRC press, 2003:479-532.
78. Poulin P, Krishnan K. Molecular structure based prediction of the toxicokinetics of inhaled vapors in humans. Int J Toxicol 1999; 18:7-18.
79. Frederick CB, Potter DW, Chang-Mateu MI et al. A physiologically-based pharmacokinetic and pharmacodynamic model to describe the oral dosing of rats with ethyl acrylate and its implications for risk assessment. Toxicol Appl Pharmacol 1992; 114:246-260.
80. Krishnan K, Gargas ML, Fennell TR et al. A physiologically-based description of ethylene oxide dosimetry in the rat. Toxicol Ind Health 1992; 8:121-140.
81. Farris FF, Dedrick RL, King FG et al. Cisplatin pharmacokinetics: applications of a physiological model. Toxicol Lett 43:117-137.
82. Terasaki T, Iga T, Sugiyama Y et al. Nuclear binding as a determinant of tissue distribution of adriomycin, daunomycin, adriamycinol, daunorubicinol and actinomycin D. J Pharmacodyn 1984; 7:269-277.
83. WHO. Principles of characterizing and applying human exposure models. International Programme on Chemical Safety. Geneva: World Health Organization, 2004.
84. Cobelli C, Carson ER, Finkelstein L et al. Validation of simple and complex models in physiology and medicine. Am J Physiol 1984; 246:R259-66.
85. Barton HA, Chiu WA, Setzer RW et al. Characterizing uncertainty and variability in physiologically-based pharmacokinetic models: state of the science and needs for research and implementation. Toxicol Sci 2007; 99(2):395-402.
86. Haddad S, Gad SC, Tardif R et al. Satistical approaches for the validation of physiologically-based pharmacokinetic (PBPK) model. Toxicologist 1995; 15:48.
87. Iyengar S, Rao M. Statistical techniques in modeling of complex systems: single and multiresponse models. IEEE Transactions on Systems, Man and Cybernetics 1983; SMC-13:175-188.
88. Krishnan K, Haddad S, Pelekis M et al. A simple index for representing the discrepancy between simulations of physiological pharmacokinetic models and experimental data. Toxicol Ind Health 1995; 11(4):413-22.
89. Anderson MW, Eling TE, Lutz RJ et al. The construction of a pharmacokinetic model for the disposition of PCBs in the rat. Clin Pharmacol Ther 1977; 22:765-773.
90. Benignus VA, Boyes WK, Bushnell PJ et al. A dosimetric analysis of behavioral effects of acute toluene exposure in rats and humans. Toxicol Sci 1998; 43:186-195.
91. Chiu WA, Barton HA, Dewoskin RS et al. Evaluation of physiologically based pharmacokinetic models for use in risk assessment. J Appl Toxicol 2007; 27:218-237.
92. Clark LH. Framework for evaluation of physiologically-based pharmacokinetic models for use in safety or risk assessment. Risk Anal 2004; 24(6):1697-1717.
93. Van Ommen B, de Jongh J, van de Sandt J et al. Computer-aided biokinetic modelling combined with in vitro data. Toxicol In Vitro 1995; 9:537-542.
94. Quick DJ, Shuler ML. Use of in vitro data for construction of a physiologically based pharmacokinetic model for naphthalene in rats and mice to probe species differences. Biotechnol Prog 2000; 15:540-555.
95. MacGregor JT, Collins JM, Sugiyama Y et al. In vitro human tissue models in risk assessment: report of a consensus-building workshop. Toxicol Sci 2001; 59(1):17-36.
96. Hissink EM, Bogaards JJP, Freidig AP et al. The use of in vitro metabolic parameters and physiologically based pharmacokinetic (PBPK) modeling to explore the risk assessment of trichloroethylene. Environ Toxicol Pharmacol 2002; 11:259-271.
97. Kamgang F, Peyret T, Krishnan K et al. An intergrated QSPR-PBPK modeling approach for the in vitro-in vivo extrapolation of pharmacokinetics in rats. SAR and QSAR in Environ Res 2008; 19(7-8):1-12.
98. Yamaguchi T, Yabuki M, Saito S et al. Research to develop a predicting system of mammalian subacute toxicity (3) construction of a predictive toxicokinetics model. Chemosphere 1996; 33:2441-2468.
99. Beliveau M, Krishnan K. Estimation of rat blood:air partition coefficients of volatile organic chemicals using reconstituted mixtures of blood components. Toxicol Lett 2000; 116:183-188.
100. Blaauboer BJ. The integration of data on physio-chemical properties, in vitro-derived toxicity data and physiologicaly based kinetic and dynamic as modelling a tool in hazard and risk assessment. A commentary. Toxicol Lett 2004; 138:161-171.
101. DeWoskin RS, Lipscomb JC, Thompson C et al. Pharmacokinetic/physiologically based pharmacokinetic models in integrated risk information system assessments. In: Lipscomb JC, Ohanian EV, eds. Toxicokinetics and Risk Assessment. New York: Informa Healthcare, 2007:301-348.
102. Leung HW, Paustenbach DJ. Physiologically based pharmacokinetic and pharmacodynamic modeling in health risk assessment and characterization of hazardous substances. Toxicol Lett 1995; 79(1-3):55-65.

103. Andersen ME, Clewell HJ, III, Gargas ML et al. Physiologically-based pharmacokinetic modeling with dichloromethane, its metabolite carbon monoxide and blood carboxyhemoglobin in rats and humans. Toxicol Appl Pharmacol 1991; 108:14-27.
104. Singh DV, Spitzer HL, White, PD et al. Addendum to the health risk assessment for dichloromethane. Updated carcinogenicity assessment for dichloromethane. EPA 600/8-82/004F. Washington, DC: US Environmental Protection Agency, 1987.
105. Krishnan K, Andersen ME. Physiological modeling and cancer risk assessment. In: Rescingo A, Thakkur A, eds. New Trends in Pharmacokinetics. New York: Plenum Press, 1991:335-354.
106. Clewell HJ, Gentry RP, Gearhart JM et al. Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. Sci Total Environ 2001; 274:37-66.
107. Dourson ML, Felter SP, Robinson D et al. Evolution of science-based uncertainty factors in noncancer risk assessment. Reg Toxicol Pharm 1996; 24:108-120.
108. Corley RA, Mandrela AL, Smith FA et al. Development of a physiologically-based pharmacokinetic model for chloroform. Toxicol Appl Pharmacol 1990; 103:512-527.
109. Yokogawa K, Nakashima E, Ishizaki J et al. Relationships in the structure-tissue distribution of basic drugs in the rabbit. Pharm Res 1990; 7:691-696.
110. Yata N, Toyoda T, Murakami T et al. Phosphatidylserine as a determinant for the tissue distribution of weakly basic drugs in rats. Pharm Res 1990; 7:1019-1025.
111. Abraham MH, Weathersby PK. Hydrogen bonding. 30. Solubility of gases and vapors in biological liquids and tissues. J Pharm Sci 1994; 83:1450-1456.
112. Abraham MH, Ibrahim A, Acree WE Jr et al. Air to blood distribution of volatile organic compounds: a linear free energy analysis. Chem Res Toxicol 2005; 18:904-911.
113. Abraham, MH, Ibrahim A, Acree WE Jr et al. Air to brain, blood to brain and plasma to brain distribution of volatile organic compounds: linear free energy analyses. Eur J Med Chem 2006; 41:494-502.
114. Abraham MH, Ibrahim A, Acree WE Jr et al. Air to muscle and blood/plasma to muscle distribution of volatile organic compounds and drugs: linear free energy analyses. Chem Res Toxicol 2006; 19:801-808.
115. Abraham MH, Ibrahim A. Blood or plasma to skin distribution of drugs: a linear free energy analysis. Int J Pharm 2007; 329:129-134.
116. Abraham MH, Ibrahim A, Acree WE Jr et al. Air to liver partition coefficients for volatile organic compounds and blood to liver partition coefficients for volatile organic compounds and drugs. Eur J Med Chem 2007; 42:743-751.
117. Abraham MH, Ibrahim A, Acree WE Jr et al. Air to lung partition coefficients for volatile organic compounds and blood to lung partition coefficients for volatile organic compounds and drugs. Eur J Med Chem 2008; 43:478-485.
118. Kaliszan R, Nasal A, Turowski M et al. Quantitative structure-retention relationships in the examination of the topography of the binding site of antihistamine drugs on alpha 1-acid glycoprotein. J Chromatogr A 1996; 722:25-32.
119. Lombardo F, Blake JF, Curatolo WJ et al. Computation of brain-blood partitioning of organic solutes via free energy calculations. J Med Chem 1996; 39:4750-4755.
120. Blakey GE, Nestorov IA, Arundel PA et al. Quantitative structure-pharmacokinetics relationships: I. Development of a whole-body physiologically based model to characterize changes in pharmacokinetics across a homologous series of barbiturates in the rat. J Pharmacokinet Biopharm 1997; 25:277-312.
121. Nestorov I, Aarons L, Rowland M et al. Quantitative structure-pharmacokinetics relationships: II. A mechanistically based model to evaluate the relationship between tissue distribution parameters and compound lipophilicity. J Pharmacokinet Biopharm 1998; 26:521-545.
122. Luco JM. Prediction of the brain-blood distribution of a large set of drugs from structurally derived descriptors using partial least-squares (PLS) modeling. J Chem Inf Comput Sci 1999; 39:396-404.
123. Bjorkman S. Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: theophylline and midazolam as model drugs. Br J Clin Pharmacol 2005; 59:691-704.
124. Haddad S, Poulin P, Krishnan K et al. Ratio of lipid content in adipose tissues and blood as the sole determinant of the adipose tissue: blood partition coefficients of highly lipophilic organic chemicals. Chemosphere 2000; 40:839-843.
125. Berezhkovskiy LM. Determination of volume of distribution at steady state with complete consideration of the kinetics of protein and tissue binding in linear pharmacokinetics. J Pharm Sci 2004; 93(2):364-74.
126. Rodgers T, Leahy D, Rowland M et al. Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci 2005; 94:1259-1276.
127. Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci 2006; 95:1238-1257.
128. WHO/IPCS. Principles of Characterizing and Applying PBPK Models in Risk Assessment. Geneva: World Health Organization, 2010. Available at: http:www.who.int/ipcs/methods/harmonization/areas/pbpk_models.pdf.