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

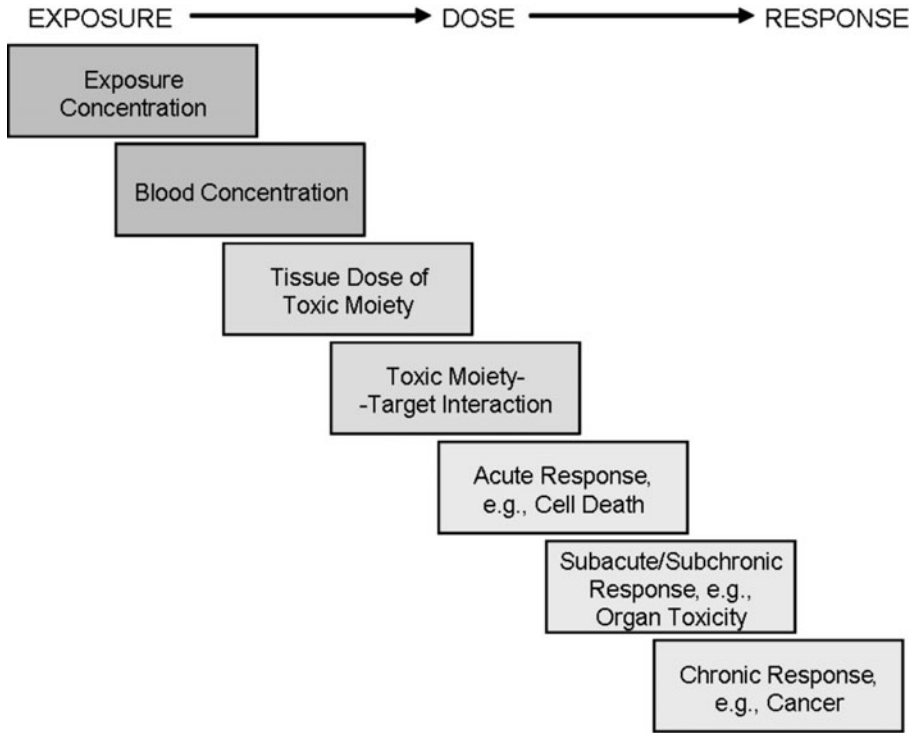


**Figure 1.** The exposure–dose–paradigm. Based on references 9 and 13.

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.

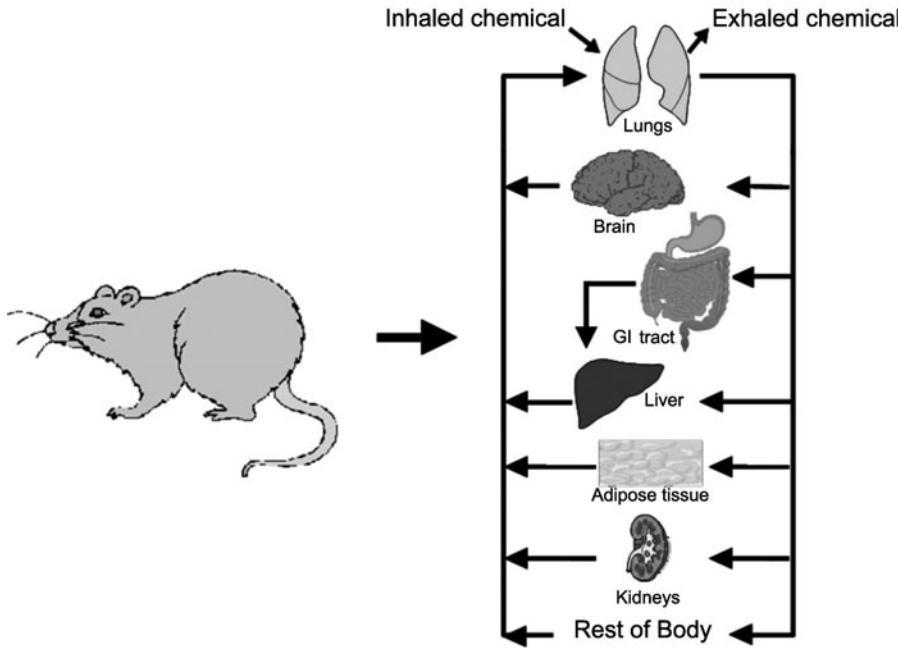


**Figure 2.** The structure of a PBPK model for a volatile organic chemical in the rat.

**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<br>Tissue blood flow<br>Alveolar ventilation<br>Cardiac output<br>Glomerular filtration rate |
| Biochemical | Maximum velocity of metabolism<br>Michaelis affinity constant<br>Rate of absorption<br>Binding affinity constant |
| Physicochemical | Blood:air partition coefficient<br>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

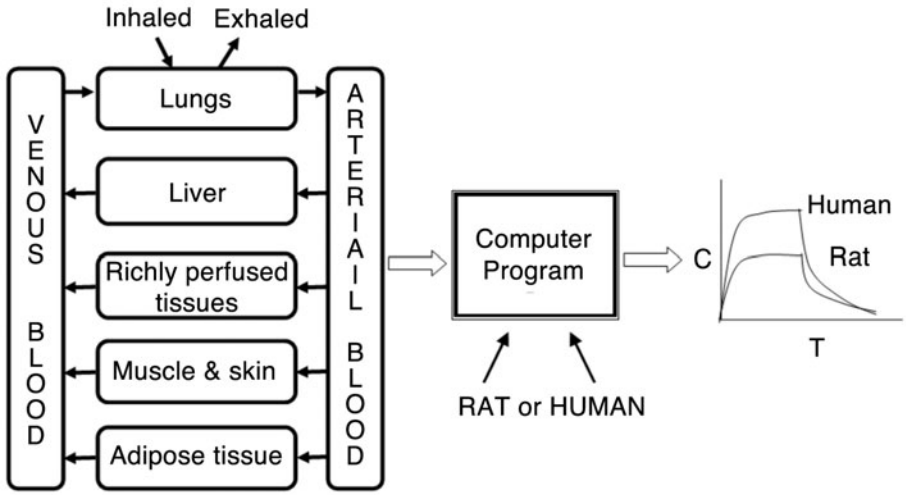


**Figure 3.** Illustration of the use of PBPK model for prediction of the time-course (C vs T) of tissue dose of a chemical in exposed animals and humans.

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

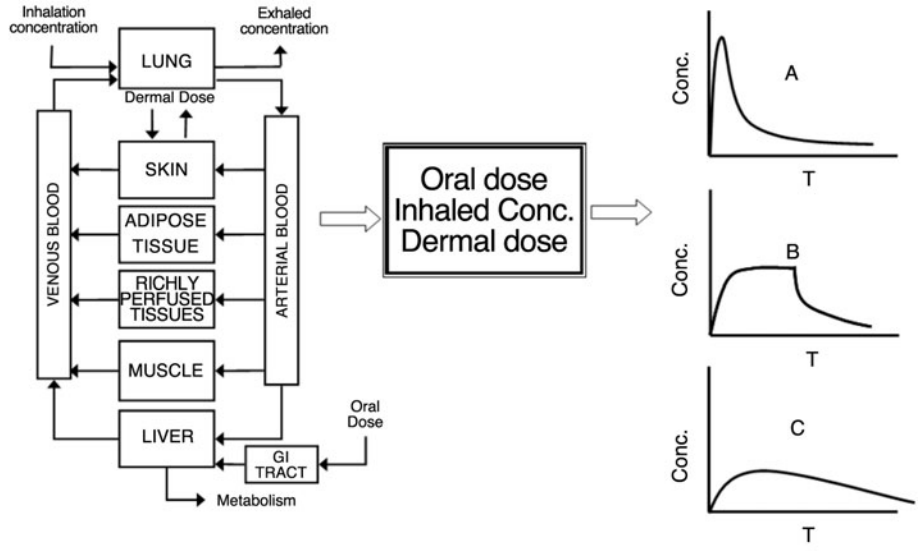


**Figure 4.** Illustration of the use of PBPK model to predict the concentration of the toxic moiety of chemical in animals exposed via the oral (A) or inhalation (B) routes or via dermal contact (C).

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.

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