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Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment

National Center for Environmental Assessment
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LIST OF ABBREVIATIONS AND ACRONYMS

ADME	Absorption, distribution, metabolism, and excretion
AUC	Area under the curve
BBDR	Biologically based dose-response (model)
BMC	Benchmark concentration
BMD	Benchmark dose
Cmax	Maximal concentration
CFD	Computational fluid dynamic(s)
CSF	Cancer slope factor
DAF	Dosimetric adjustment factor
HEC	Human equivalent concentration
IUR	Inhalation unit risk
LOAEL	Lowest-observed-adverse-effect level
MCMC	Markov chain Monte Carlo
MOA	Mode of action
NOAEL	No-observed-adverse-effect level
PBPK	Physiologically based pharmacokinetic
POD	Point of departure
QSAR	Quantitative structure-activity relationship
RfC	Reference concentration
RfD	Reference dose
UF	Uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor

PREFACE

Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment addresses the application and evaluation of PBPK models for risk assessment purposes. PBPK models represent an important class of dosimetry models that are useful for predicting internal dose at target organs for risk assessment applications. This report is primarily meant to serve as a learning tool for U.S. Environmental Protection Agency (EPA) scientists and risk assessors who may be less familiar with PBPK modeling. In addition, it can be informative to PBPK modelers within and outside EPA because it provides an overview of the types of data and models that EPA requires for consideration of a model for use in risk assessment. A draft of this document underwent a public comment period and external peer review in 2005, and this final report incorporates many of the relevant comments and suggestions received in response to the draft report.

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EXECUTIVE SUMMARY

Physiologically based pharmacokinetic (PBPK) models represent an important class of dosimetry models that are useful for predicting internal dose at target organs for risk assessment applications. *Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment* addresses the following questions: Why are risk assessors interested in using PBPK models? How are PBPK models evaluated for use in a risk assessment? What are the questions or data gaps in a risk assessment that can be addressed by PBPK models? However, this document is not meant to serve as formal U.S. Environmental Protection Agency (EPA) guidance.

The text is organized into four chapters. Chapter 1 outlines the scope of the document, the intended audience, and the topics covered in the remaining chapters. Chapter 2 presents the rationale for using PBPK models in risk assessment and the pharmacokinetic data and models needed to derive a reference dose (RfD), a reference concentration (RfC), and unit risk estimates in cancer risk assessment (e.g., cancer slope factor). Chapter 3 describes how models are evaluated, the main model characteristics to review, and the on-going development of acceptance criteria for model use in risk assessment. Chapter 4 discusses applications of PBPK model simulations within the current EPA risk assessment framework. The appendix contains a comprehensive list of publications, current as of the end of 2005, relating to PBPK modeling and its use in health risk assessment.

PBPK models consist of a series of mathematical representations of biological tissues and physiological processes in the body that simulate the absorption, distribution, metabolism, and excretion of chemicals that enter the body. PBPK models are designed to estimate an internal dose of a proposed toxic moiety to a target tissue(s) or some appropriate surrogate dose metric for a target tissue dose. The choice of an internal dose metric is based on an understanding of the chemical's mode of action. The internal dose metric (sometimes called the biologically effective dose) replaces the administered dose in the derivation of the quantitative dose-response relationship, with the intent of reducing the uncertainty inherent in risk assessments based on an applied dose. This reduction in uncertainty and the improved scientific basis for the dose-response value are the main advantages of PBPK models and the reasons for the growing interest in their use. PBPK models also can simulate an internal dose from exposure conditions of interest where no data are available, i.e., they can extrapolate to conditions beyond those of the

data set used to develop the model. An important and active area of research is the characterization of the uncertainty in risk assessments based on PBPK model results compared with the uncertainty in results based on the administered dose.

PBPK models exist for a wide range of chemicals with varying properties. The vast majority of PBPK modeling efforts to date have focused on chemicals that distribute systemically within the body and cause systemic effects, although the models' applicability for describing the pharmacokinetics of other chemicals, such as reactive gases, has been successfully demonstrated. Because this document is intended to describe some of the basic principles of PBPK modeling and its use in risk assessment, it primarily draws upon the experience and literature concerning chemicals with systemic distributions.

Examples of PBPK model applications in risk assessments include interspecies extrapolation of the dose-response relationship (based on estimates of the internal dose), route-to-route extrapolation, estimation of response from varying exposure condition, estimation of human variability (within the whole population or subpopulations), and high-to-low dose extrapolation. PBPK models used in risk assessments would, at a minimum (1) contain a compartment that is either identified with the target tissue, contains the target tissue, or is identified as a surrogate for the target tissue; (2) have defensible physiological parameter values that are within the known plausible range; and (3) have undergone a thorough evaluation for their structure, implementation, and predictive capability.

Evaluation of PBPK models intended for risk assessments includes a review of the model purpose, model structure, mathematical representation, parameter estimation (calibration), and computer implementation. Criteria for acceptance of a PBPK model for use in risk assessment include the following: (1) the model represents the species and life stage of relevance to a particular risk assessment, (2) it has been evaluated and peer-reviewed for the adequacy of its structure and parameters, and (3) it provides adequate simulations of the concentration of the toxic moiety (parent chemical or metabolite) in the target organ (or a surrogate compartment) following the relevant route(s) of exposure and over the time-course for which the chemical is present in that tissue.

When a PBPK model is available for the appropriate test species, it is used to estimate the value of internal dose metrics, which are then used to derive a given point of departure (e.g., no-observed-adverse-effect level, lowest-observed-adverse-effect level, benchmark dose, benchmark concentration) for use in dose-response analyses for toxicity endpoints, including

cancer, chronic toxicity, and other toxicity endpoints. Some risk assessment applications can be accomplished using only a model for the test species, e.g., prediction of the toxicity in that species by another route of exposure for purposes of route extrapolation. For most applications, a human version of the PBPK model is also developed to estimate an administered dose to a human that would result in the equivalent internal dose in a human that led to the observed toxicity in a test species or, less frequently, the biologically effective dose from a human clinical or epidemiology study. PBPK model analysis is accepted as a scientifically sound approach to estimating the internal dose of a chemical at a target site and as a means to evaluate and describe the uncertainty in risk assessments.

1. INTRODUCTION

1.1. SCOPE OF THE DOCUMENT

The objective of this document is to provide a description of approaches for using physiologically based pharmacokinetic (PBPK) data and models in human health risk assessment. Its primary focus is on the evaluation and use of PBPK models for predicting internal dose at target organs in risk assessment applications. Many of the past efforts on PBPK modeling have focused on water-insoluble gases that cause systemic toxicity (i.e., producing effects remote from the site of exposure) and on some nonvolatile organics. This document primarily draws on the experience and literature resulting from these efforts. These approaches can also be applied to agents such as reactive gases and particulate matter where the target organ is the respiratory tract, generally in conjunction with specialized respiratory tract modeling (e.g., computational fluid dynamic [CFD] modeling). Guidance concerning alternative approaches to dosimetry modeling should also be consulted for determining a reference concentration (RfC) value (U.S. EPA, 1994). The discussions herein are conceptually applicable, in a broad sense, to many kinds of dosimetry models and a wide range of substances.

In developing this document, it was assumed that risk assessors are familiar with some basic concepts of pharmacokinetics and that model developers are familiar with some of the basic concepts of risk assessment; therefore, the document serves as an overview of PBPK modeling and its application in risk assessment. Appropriate references to secondary review articles and reports from which additional information can be obtained are provided.

Finally, it is important to realize that the application of PBPK models in risk assessment is evolving. Thus, this document does not specify (or recommend) when the effort to construct and apply PBPK modeling is justified; rather, it highlights some of the benefits of PBPK modeling in risk assessment.

1.2. INTENDED AUDIENCE

The document was prepared with two primary audiences in mind: (1) risk assessors who need to know about the potential applications of PBPK models in risk assessments, and (2) PBPK model developers who need to better understand how their efforts can help improve health risk assessment.

1.3. ORGANIZATION OF THE DOCUMENT

The remaining three chapters form the core of this document. They describe what risk assessors need in terms of pharmacokinetic data, and why (Chapter 2); how to evaluate PBPK models for use in risk assessments (Chapter 3); and how to use PBPK models in risk assessments to address specific areas of uncertainty (Chapter 4).

Chapter 2 addresses data needs in terms of reference dose (RfD) and RfC derivation as well as predictive estimates in cancer risk assessment. It also contains a brief discussion on the minimal data requirements for constructing PBPK models, as well as the use of pharmacokinetic data and PBPK models to improve exposure assessments.

Chapter 3 presents an approach and some criteria for evaluating PBPK models intended for use in risk assessments that will facilitate the assessor's decision regarding whether or not an available model is adequate and scientifically defensible for use in reducing uncertainties in a given risk assessment. The PBPK modeling issues are considered under each of the following topic areas: model structure, mathematical description, parameter estimation (calibration), computer implementation, and evaluation. Current criteria as well as accepted methods are identified and then assembled to facilitate the identification of PBPK models that meet the requirement for use in risk assessment.

Chapter 4 discusses how PBPK models and data can be applied within the current U.S. Environmental Protection Agency (EPA, or the Agency) risk assessment framework to address specific areas of uncertainty. The following types of PBPK model applications in risk assessment are presented in this chapter: high-dose to low-dose, interspecies, intraspecies, route-to-route, and scenario extrapolations; mixture risk assessment; and linkage with pharmacodynamic models. This chapter also highlights how PBPK models are used in cancer and noncancer assessments.

Finally, the appendix provides a list of publications relating to PBPK modeling and its use in health risk assessment.

2. PHARMACOKINETIC DATA AND MODEL NEEDS IN RISK ASSESSMENT

2.1. PHARMACOKINETICS AND DOSIMETRY MODELING

Pharmacokinetics (*pharmakon* + *kinetics*; *pharmakon* (Greek) = drugs and poisons; *kinetics* = change as a function of time) involves the study of the time course of the parent chemical or metabolite concentrations or amounts in biological fluids, tissues, and excreta and the construction of mathematical models to interpret such data (Wagner, 1981). The time course of the concentration of a chemical or its metabolite in biota is determined by the rate and extent of absorption, distribution, metabolism, and excretion (ADME). The pharmacokinetics or ADME of a substance determines the *delivered dose* or the amount of chemical available for interaction in the tissues. Relating adverse response observed in biota to an appropriate measure of delivered dose (e.g., concentration of the toxic chemical in the target tissue) rather than administered dose or exposure concentration is likely to improve the characterization of many dose-response relationships (see Section 2.2.).

A range of modeling approaches is used to characterize exposures and the resulting delivered doses. The variety of approaches reflects differences in chemical and physical characteristics (e.g., stable or reactive gases, particulate matter, lipophilic organics, water-soluble compounds), differences in pharmacokinetic properties, and the ability of compounds to cause contact site or systemic toxic effects (U.S. EPA, 2004, 1994; Andersen and Jarabek, 2001; Overton, 2001).

Exposure to many drugs and toxicants occurs via the oral route and causes systemic effects, and many simple (e.g., one- and two-compartment) pharmacokinetic models have been used to analyze the pharmacokinetics of such exposures (Renwick, 2001; O'Flaherty, 1981). Generally, these compartment models contain a central compartment that represents the whole body (or plasma) where distribution occurs nearly instantaneously (one-compartment model) or an additional compartment (two-compartment model) where the distribution is affected by additional processes such as metabolism or sequestration into fat. Compartment models help characterize a chemical's kinetic behavior, and they are useful in deriving values for a chemical or drug's distribution in the body or clearance from the blood (i.e., half-life).

The values derived from a compartment model analysis, however, apply only to the conditions of the study from which the experimental data were obtained. To better represent the biological determinants of a chemical's disposition in the body and predict the internal dose that

would result from different exposure regimens (including hypothetical exposures where no data are available), models have evolved with multiple compartments and mathematical descriptions of the real physiological processes and tissues most likely to affect chemical disposition (e.g., absorption from the gut or lung, cardiac output, metabolism in the liver, renal clearance). These models are called physiologically based pharmacokinetic (PBPK) models, and they are the focus of this document.

The need to predict behaviors of volatile anesthetics, including compounds now used exclusively as industrial chemicals, was a driving force for the development of PBPK models (Krishnan and Andersen, 2001). The general principles developed in these early PBPK modeling efforts for systemically distributed compounds are also applicable to other compounds. For example, the respiratory tract is a frequent site of both exposure and toxicity, and it has been a particular focus for a range of modeling approaches, including those developed to simulate the kinetics of gases of various reactivities and solubilities, as well as particulate matter (see U.S. EPA, 1994). More recently, the kinetics of reactive gases and particulate matter within the respiratory tract are simulated with advanced approaches such as two-dimensional and three-dimensional CFD modeling (U.S. EPA, 2004; Martonen et al., 2001; Overton, 2001; Kimbell et al., 1993).

The role of metabolism is a significant factor in the development of PBPK models. Saturable metabolism results in nonlinear relationships between the level of administered dose and the levels of the internal dose for a parent or metabolite. In combination with other physiological and chemical events, the resulting administered dose-response relationship can quickly become difficult to resolve with simple analytical tools. PBPK models provide an excellent means to account for multiple process interactions and nonlinearities and to provide insight into the whether the parent chemical or the metabolite is the main form (or toxic moiety) leading to adverse effects.

Metabolism has also played an important role in the development of models for respiratory tract toxicities. Nasal or other respiratory tract toxicities following exposure to many volatile organic compounds has often been linked to the generation of a toxic metabolite in respiratory tract tissues. To simulate the kinetics and resulting toxicities for these compounds, CFD models have been coupled with PBPK models. The CFD models describe the deposition of the chemical in different regions of the nose, and the PBPK model then simulates the tissue absorption, metabolic, and clearance processes (Frederick et al., 2002; Bogdanffy et al., 2001).

Other complex kinetic events in the respiratory tract are also being modeled, including the kinetics for compounds that are relatively water soluble and that exhibit fractional absorption, or the so-called "wash-in, wash-out" effect (Perkins et al., 1995; Medinsky et al., 1993; Johanson, 1991).

Although the approaches detailed in the earlier RfC methodology (U.S. EPA, 1994) do not address many of these more recent advances, there is recognition of the need for additional approaches that address these and other challenging aspects of respiratory tract dosimetry.

The relevant modeling approach, therefore, depends on the physical and chemical characteristics of the material, the method and route of exposure or delivery, and the toxicities under consideration. All of these modeling approaches attempt to describe the dose delivered to the relevant areas of the body, whether that is a region of the respiratory tract or skin or systemic delivery through the blood supply to target organs. These approaches permit estimation of some measure of delivered dose for improved understanding of the dose-response relationship.

2.2. DOSE-RESPONSE AND MEASURES OF DELIVERED DOSE

Dose-response relationships that appear unclear or confusing at the administered dose level can become more understandable when expressed on the basis of internal dose of the chemical. Figure 2-1 depicts the case of a hypothetical chemical for which the correlation between dose and response is weak or complex (Panel A). However, once the relationship is based on internal dose, there emerges a clear and direct relationship between dose and response (Panels B and C). The major advantage of constructing dose-response relationships on the basis of internal or delivered dose is that it can provide a stronger biological basis for conducting extrapolations and for comparing responses across studies, species, routes, and dose levels (Melnick and Kohn, 2000; Benignus et al., 1998; Aylward et al., 1996; Andersen et al., 1987; Clewell and Andersen, 1985).

Relating blood and tissue concentrations with response in exposed organisms has long been recognized in pharmacology (e.g., Wagner, 1981). In pharmacokinetics, the target tissue dose that most closely relates to an adverse response is often referred to as the internal "dose metric" (Andersen and Dennison, 2001). Dose metrics used in risk assessment applications, ideally, reflect the biologically active form of the chemical (parent chemical, metabolites, or adducts), its level (concentration or amount), duration of internal exposure (instantaneous, daily, lifetime, or a specific developmental period), and intensity (peak, average, or integral), as well as

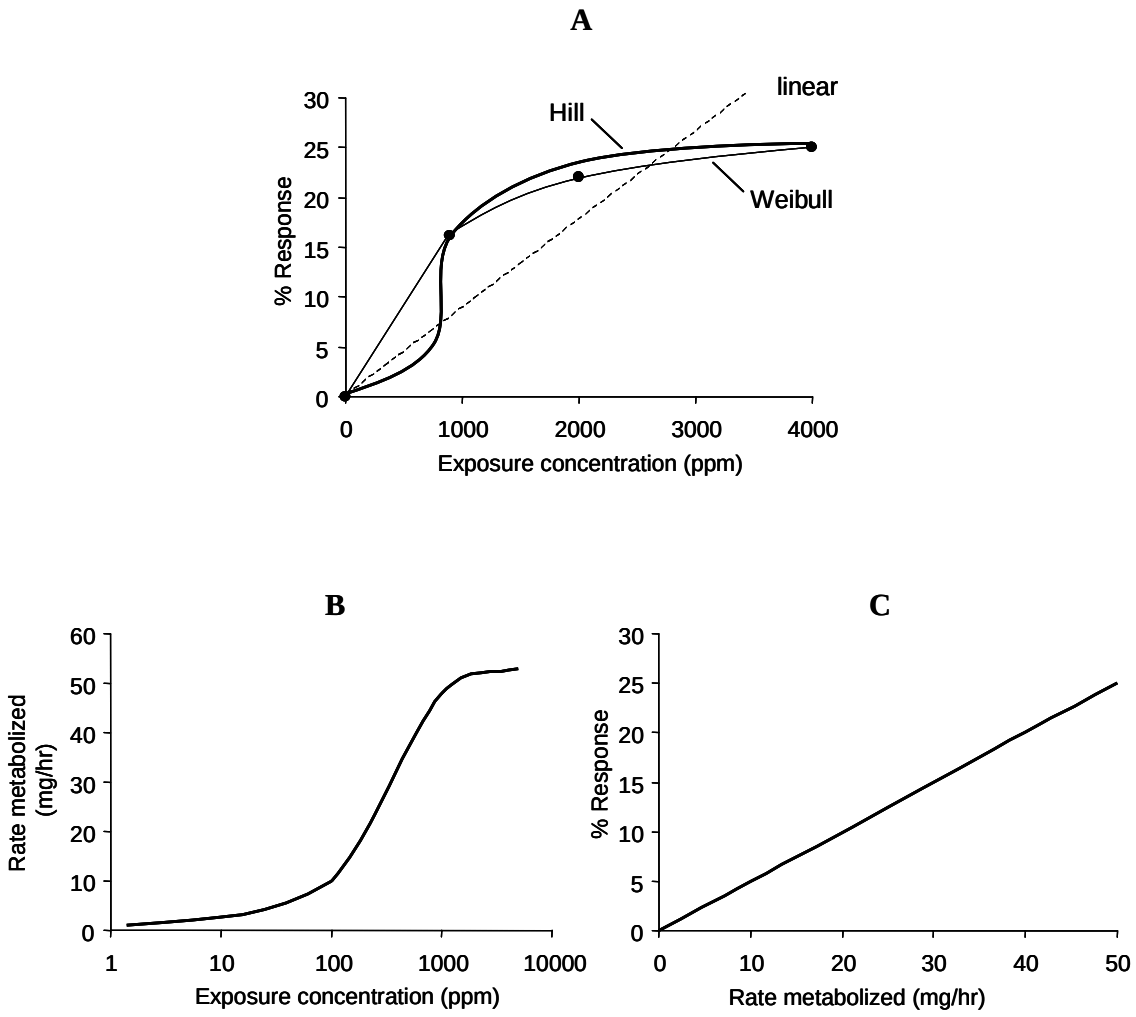


Figure 2-1. Relationship between the exposure concentration and adverse response for a hypothetical chemical. Panel A depicts the case of a chemical for which the correlation between dose and response is weak or complex, along with equally plausible curve fits (linear, Hill, and Weibull). This dose-response relationship is improved when it is based on an appropriate measure of internal dose (Panels B and C).

the appropriate biological matrix (e.g., blood, target tissue, surrogate tissues). For assessment of health risks related to lifetime exposure of systemically acting chemicals, in the absence of mode of action (MOA) information to the contrary, the integrated concentration of the toxic form of chemical expressed as the daily average (i.e., average daily area under the concentration vs. time curve, or area under the curve [AUC]) in target tissue has been considered to be an appropriate dose metric (Clewell et al., 2002a; Voisin et al., 1990; Collins, 1987).

When the toxicant is not the parent chemical but a reactive intermediate, the amount of metabolite produced per unit time or the amount of metabolite in target tissue over a period of time (e.g., mg metabolite/L tissue during 24 hr) has been used as the dose metric (Andersen and Dennison, 2001). For developmental effects, the dose surrogate is defined in the context of the window of exposure during a particular gestational event (e.g., Luecke et al., 1997; Welsch et al., 1995).

Box 2-1. Examples of dose metrics useful for exploring dose-response relationships

Parent chemical
- Peak concentration
- Average concentration
- Amount or quantity
- AUC (integral)

Metabolite
- Peak concentration
- Average concentration
- Amount or quantity
- Rate of production
- Cumulative rate of formed/time/L tissue
- AUC (integral)

Miscellaneous
- Receptor occupancy (extent/duration)
- Macromolecular adduct levels
- Depletion of cofactors

Even though the AUC and rate of metabolite formation are among the most commonly investigated dose metrics, other surrogates of tissue exposure may also be appropriate for risk assessment purposes, depending on the chemical and the MOA (Clewell et al., 2002a). Dose metrics that may be used to derive dose-response relationships for risk assessment are listed in Box 2-1; evaluation of dose metrics for use in risk assessment is further discussed in Section 4.2. Finally, it should be noted that PBPK models can also be useful for hypothesis testing, particularly with regard to choosing among potential dose metrics. This is discussed further in Chapters 3 and 4, and particularly in Section 4.5.5.

2.3. PHARMACOKINETIC DATA NEEDS IN RISK ASSESSMENT

The quantitative dose-response assessment portion of the risk assessment process can be used to determine a point of departure (POD) for one or more of the most sensitive critical effects. The POD is the dose-response point that marks the beginning of a low-dose extrapolation, and it can be the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL) for an observed incidence or the lower bound on dose for an estimated incidence or change in response level from a benchmark dose (BMD) analysis. The quantitative characterization relates the administered dose to the observed responses in laboratory or field studies. In some cases, data are available to relate an internal dose at a target

tissue to the response, but generally the internal dose-response relationship is derived from a model analysis. An understanding of the MOA leading to the most sensitive endpoint is used to determine the most appropriate dose measure for deriving a POD. Noncancer and nonlinear cancer assessments derive a POD for use in risk assessment based on the available data. This process often requires conduct of interspecies, high-dose to low-dose, duration, and/or exposure route extrapolations of the dose-response from available data to the likely human exposure conditions and most sensitive human subpopulations.

These extrapolations assume that when the value of the internal dose metric is identical in two situations (rat vs. human, oral vs. inhalation, 6-hr exposure vs. 24-hr exposure), the two administered doses are pharmacokinetically equivalent. For example, exposure of rats to 50 ppm toluene for 6 hr and of humans to 17.7 ppm toluene for 24 hr yields the same blood AUC (3.8 mg/L/hr), implying that these exposure scenarios in rats and humans are pharmacokinetically equivalent (Figure 2-2). The example in Figure 2-2 demonstrates that pharmacokinetic equivalence is not always linear equivalence; if one assumes that all aspects of the pharmacokinetics of toluene in humans is identical to those in rats, an equivalent 24-hr exposure would be 12.5 ppm (i.e., 6 hr × 50 ppm is equivalent to 24 hr × 12.5 ppm), not 17.7 ppm.

Knowledge of the MOA supports the choice of the dose metric. For example, the most appropriate dose metric to characterize the dose-response relationship for reactive gases that cause contact site toxicity is the total amount of chemical in the target tissue over time, whereas for the anesthetic effects of a volatile organic, the current (or peak) concentration in the blood is the most appropriate dose metric. For the latter, the acute effects are more (or entirely) dependent on concentration rather than on total amount over time, so extrapolations are best conducted using that dose metric.

PBPK models are often intended to estimate target tissue dose in species and under exposure conditions for which little or no data exist. Thus, if a complete pharmacokinetic data set were available, then there would be no need to develop a PBPK model. Such an optimal pharmacokinetic data set for risk assessment would consist of the time-course data on the most appropriate dose metric associated with exposure scenarios and doses used in the critical studies chosen for the assessment (e.g., animal bioassays or human clinical and epidemiological studies) and relevant human exposure conditions. An example of such a dose metric is the concentration of a toxic metabolite in target tissue over a 24-hr period in the test species and in humans. This information would be obtained for the window of exposure, route and scenario of exposure

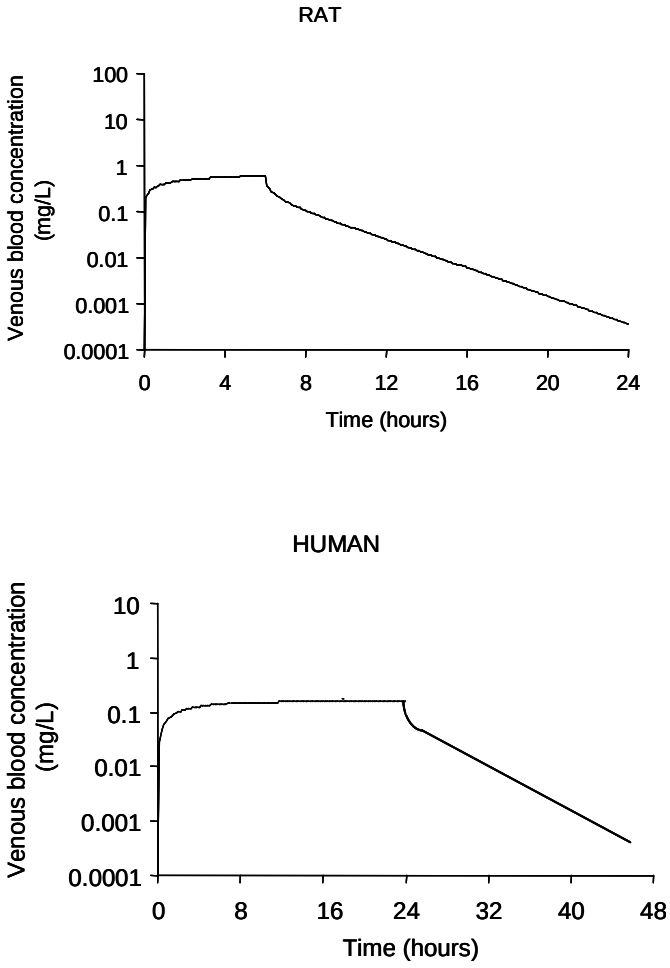


Figure 2-2. Rat-human extrapolation of exposure concentrations of toluene based on equivalent dose metrics (AUC [area under the curve] of toluene in blood, 3.8 mg/L/hr). Rat exposures are for 6 hr to 50 ppm; human exposures are for 24 hr to 17.7 ppm. Both exposures yield the same AUC, as determined using species-specific PBPK models published by Tardif et al. (1997). Similar exercises can be done to determine the exposure concentrations that yield equivalent peak concentrations (Cmax) in rats and humans.

associated with the critical study as well as for the window of susceptibility, appropriate route, and exposure scenarios in humans.

In almost all cases, however, the optimal data set is not available, and often the available animal pharmacokinetic data may be limited. In the absence of experimental kinetic data on the biologically active form of a chemical in target tissues, data on blood concentration of the parent

chemical, urinary metabolite levels, or fraction absorbed may be used as a surrogate for the tissue levels. These and other subsets of pharmacokinetic data can be used to develop a PBPK model to estimate the level of the toxic moiety of interest, and the uncertainty in those estimates can be formally characterized.

2.4. PHARMACOKINETIC MODELS IN RISK ASSESSMENT

2.4.1. Regulatory Needs and Considerations

Regulatory agencies such as EPA derive dose-response values based on the current understanding of a dose-response relationship. Reference values correspond to an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The reference values developed at EPA include RfC for chronic inhalation exposures and RfD for chronic oral exposures. For chronic oral and inhalation cancer risk assessments with an unknown or a linear MOA (e.g., mutagenic carcinogens), EPA develops unit risk estimates, including the cancer slope factor (CSF) for oral exposures and the inhalation unit risk (IUR). The underlying assumption in these derivations is that the exposure concentration (or applied dose) of a parent chemical results in an internal exposure of the putative toxic form of the chemical in a target organ that will be less than or equal to a level that is not associated with significant adverse responses during a lifetime (reference value) or that yields a likely risk at or below the estimated lifetime risk (unit risk).

Even though a key factor in the induction of adverse effects is the presence of the toxic form of a chemical in the target organ, it is rare that data are available on the time course of the toxic moiety in the target tissue(s) in humans. Even in animal studies, it is more practical to obtain measures of blood, plasma, and urinary concentrations of toxic chemicals and their metabolites than the actual toxic moiety level in the relevant tissue. Pharmacokinetic models are therefore used to estimate the tissue concentration of toxic substances.

Among the compartmental pharmacokinetic models, PBPK models are the most appropriate and useful for conducting the extrapolations needed to derive reference values because they model the underlying physiological and chemical processes that determine chemical disposition, and they can be used to predict target organ concentrations for hypothetical exposures (Krishnan and Andersen, 2001; Andersen, 1995; Leung, 1991; Rowland, 1985; Himmelstein and Lutz, 1979). By simulating the kinetics and dose metric of chemicals, PBPK

models can reduce the uncertainty related to interspecies, intraspecies, route-to-route, duration, and high-dose to low-dose extrapolations needed to derive RfC, RfD, and cancer unit risk estimates. The following sections discuss how the PBPK models are used in health risk assessment. Figure 2-3 provides an overview of the development and use of PBPK models for risk assessment; it should be noted that model development can occur within academic, industry, and governmental bodies, and often involves collaboration or sharing of information.

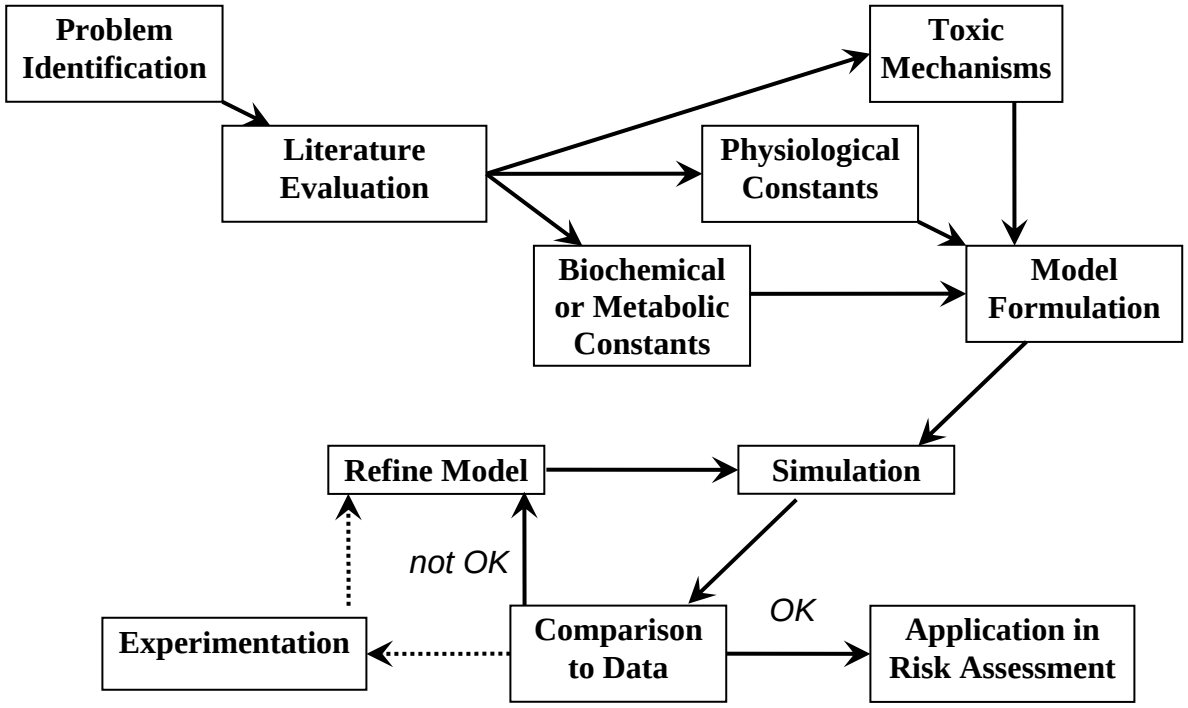


Figure 2-3. Basic flowchart of PBPK model development.

2.4.2. Use of PBPK Models in Dose-Response Assessment

PBPK models are useful for performing various forms of extrapolations where the data necessary for predicting risks to humans are not available and cannot easily (or ethically) be obtained. The primary advantage gained by using PBPK models in risk assessment is their ability to relate toxicity responses in a test species to humans and outcomes observed in smaller populations to likely outcomes in the general population. Thus, foremost among the extrapolations afforded by PBPK models are inter- and intra-species extrapolations.

In risk assessments based on nonlinear MOAs (e.g., most noncancer assessments), RfC and RfD values are derived from PODs (i.e., NOAEL, LOAEL, or benchmark concentration [BMC]) to derive a human equivalent concentration (HEC) or dose. In RfC derivation, pharmacokinetic adjustments, called dosimetric adjustment factors (DAFs), are applied to account for species differences in chemical disposition. These factors are dependent on the nature of the inhaled toxicant and MOA, as well as the endpoint (local effects vs. systemic effects). Dosimetry data in the test animals and humans (e.g., deposition data, region-specific dosimetry, blood concentration of systemic toxicants), if available, can help estimate the DAF. In the absence of such data, knowledge of critical parameters or mathematical models in the test species and humans can be useful in estimating the DAF. Similar methods are employed in RfD derivation.

An alternative to the use of DAFs is to employ models to make interspecies extrapolations. A variety of computational tools are available for determining the uptake and deposition of gases and particulates in nasal pathways and the respiratory tract (U.S. EPA, 2004; Bogdanffy and Sarangapani, 2003; Hanna and Lou, 2001; Tran et al., 1999; Bush et al., 1998; Asgharian et al., 1995; Jarabek, 1994; Kimbell et al., 1993). Although PBPK models have most frequently been applied to systemically acting gases and vapors, they have also been applied, in conjunction with other models (e.g., CFD), to more locally acting gases. Another limitation to DAFs is that they do not account for metabolism, so PBPK modeling approaches would clearly be preferable for metabolized compounds if adequate data are available.

PBPK models are also useful for incorporating variability in chemical disposition into a risk assessment. There are numerous determinants of a chemical's disposition in the body (e.g., protein levels, enzyme activity levels, tissue volumes, breathing rates, cell proliferation rates) (e.g., Dorne et al., 2002, 2001a, b; Walton et al., 2001). Focusing on individual determinants of disposition is useful for understanding their mechanistic basis and potential impacts on dosimetry and response, but such an approach can easily lead to unrealistic estimates of total variability of a chemical as well as toxic response (Lipscomb, 2004) because the magnitude of variability associated with such individual determinants may be neither cumulative nor additive. The net impact of various determinants on chemical disposition is more properly evaluated by integrating the available information with a PBPK or biologically based dose-response (BBDR) model.

Two additional forms of extrapolation amenable to PBPK modeling include route and duration adjustments. The PODs in critical toxicity studies are not always obtained for exposure

scenarios of interest to risk assessment. Ideally, the POD used in the RfC process would be the inhalation route-specific NOAEL, LOAEL, or BMC. Route-to-route extrapolation, however, can be conducted on the basis of equivalent potential doses when information on the POD is available only for a noninhalation route of exposure (e.g., oral route) (Pauluhn, 2003). Historically, simplistic assumptions were used to convert the NOAEL (mg/kg/day) associated with an oral exposure route to equivalent inhaled concentration, based on breathing rate and body weight of the test species. Such simplistic approaches, however, incorrectly assume that the rates of ADME and tissue dosimetry of chemicals are the same for a given total dose, regardless of the exposure route and intake rate. PBPK modeling is useful for conducting route-to-route extrapolation on the basis of equivalent delivered dose from PODs identified from the NOAEL, LOAEL, or BMC (e.g., oral to inhalation).

As mentioned above, RfC and RfD values are intended for continuous exposure of human populations, such that the POD used in an RfC derivation (for example) would correspond to 24 hr/day exposures (U.S. EPA, 1994). Because the PODs are frequently obtained from animal exposures or occupational exposures that occur for 6 to 8 hr/day, 5 days/wk, adjustment to a continuous 24-hr exposure is conducted on the basis of hours per day and days per week (i.e., $6/24 \times 5/7$), which essentially results in a lower concentration for continuous exposures (U.S. EPA, 2002). Depending on the dose metric identified or hypothesized to be the most appropriate for the chemical and endpoint, the duration-adjusted exposure values can be obtained with PBPK models (U.S. EPA, 2002; Jarabek, 1994). This approach is based on the expectation that the pharmacodynamic aspect does not change between the various durations of within-day exposures (<24 hr).

As discussed in detail in Chapter 4, PBPK models can also be used to convert a POD in a critical cancer study to an appropriate dose metric. Here again, simpler approaches such as body weight scaling can be replaced with PBPK models capable of inter- and intra-species extrapolation as well as route-to-route extrapolation. These models can also facilitate high-dose to low-dose extrapolation by converting exposure concentrations in critical studies into predicted dose metric values.

2.4.3. Use of Pharmacokinetic Data and Models in Exposure Assessment

The conventional approach to exposure assessment involves the calculation of applied dose for each route of exposure based on information about the concentration of the chemical in

the medium, frequency and duration of exposure, rate of contact with the medium, and body weight of the individual (Paustenbach, 2000). With increased data availability, however, absorbed dose can be calculated (U.S. EPA, 1992). In order to calculate absorbed dose, pharmacokinetic data such as time-course data on concentration or total quantity in alveolar air, urine, or blood are required (Paustenbach, 2000). Estimating a delivered dose from biomarker data, absorbed dose, or applied dose, in fact, may not be straightforward. PBPK models provide a means to improve these estimates and to fully utilize available data.

PBPK models can be used in conjunction with an exposure assessment to improve the quantitative characterization of the dose-response relationship and the overall risk assessment. PBPK models can be used to identify and evaluate the relationship between an applied dose and biomonitoring or biomarker data, or between an applied dose, biomarker level, and internal target tissue dose (e.g., Timchalk et al., 2004, 2001; Csanady et al., 1996; Fennell et al., 1992; Krishnan et al., 1992). PBPK models have also been used to establish biological exposure indices (e.g., breath, blood, or breath concentrations) to protect workers from harmful exposures to solvents (Droz et al., 1999; Thomas et al., 1996a; Kumagai and Matsunaga, 1995; Leung, 1992; Perbellini et al., 1990) or in epidemiology studies to reconstruct human exposures over time (Canuel et al., 2000; Roy and Georgopoulos, 1998; Vinegar et al., 1990). Comprehensive PBPK models are being developed that provide estimates of an internal tissue dose from multiroute (oral, inhalation, dermal) or multichemical exposures (Levesque et al., 2002; Liao et al., 2002; Corley et al., 2000; Rao and Ginsberg, 1997; Roy et al., 1996; Georgopoulos et al., 1994). The net tissue dose associated with a multiroute (aggregate) and/or multichemical (cumulative) exposure are especially useful for advancing risk assessment beyond the one chemical, one exposure route paradigm.

2.4.4. Pharmacokinetic Models in Risk Assessment: Summary

Adverse tissue responses are more directly and closely related to the internal target tissue dose of the toxic moiety than to the concentration of the parent chemical in the environment. Therefore, the scientific basis of, and confidence in, risk assessments are enhanced when they are supported by estimates of the internal tissue dose. Data for the internal tissue dose levels, however, are generally not available, and the relationship between external and internal dose may not be easily resolved. PBPK models provide a means of estimating the internal dose for many different exposure regimens based on what is known about the physiology of the test

species and humans and the chemical of interest. PBPK models reduce the uncertainty in dose-response and exposure assessment and fully utilize the available data.

In the context of dose-response assessment, PBPK models have application in:

1. Interspecies extrapolation of pharmacokinetically equivalent doses (RfD, RfC, CSF, and IUR),
2. Estimation of the pharmacokinetic component variability (RfC and RfD derivation),
3. Route-to-route extrapolation of the POD (RfC, RfD, CSF, and IUR),
4. Duration adjustment of the POD (RfC and RfD derivation), and
5. High-dose to low-dose extrapolation (CSF and IUR).

In the context of exposure assessment, PBPK models are useful for

1. Converting applied dose into tissue dose,
2. Calculating tissue dose associated with multiroute and multimedia exposures, and
3. Relating biomarker data to tissue dose and potential dose by exposure reconstruction.

PBPK models vary in their quality, transparency, and predictive capability, and they must undergo a rigorous evaluation if the model is to be used in a risk assessment. The process and criteria for evaluating a PBPK model are the subject of the next chapter.

3. EVALUATION OF PBPK MODELS INTENDED FOR USE IN RISK ASSESSMENT

PBPK models intended for risk assessment applications should be evaluated for quality and transparency. There are no published criteria or well-defined standards for evaluating PBPK models; however, several publications have addressed good modeling practices and approaches for evaluating and documenting biological models intended for risk or safety assessments (Clark et al., 2004; Andersen et al., 1995; Yates, 1978). Evaluation of PBPK models intended for risk assessment applications includes considerations for model purpose, model structure, mathematical representation, parameter estimation, computer implementation, and predictive capacity as well as sensitivity, variability, and uncertainty analyses. Each of these issues is discussed in detail in the following sections. Although these considerations are provided in separate sections, it is important to realize that model evaluation, from development through to application, can be an iterative process.

3.1. MODEL PURPOSE

Not all PBPK models are developed to support risk assessments. Some are developed to be used as research tools for testing biological hypotheses or for guiding improved experimental design. The purpose for which a PBPK model is developed influences its structure, level of detail, and model parameterization (e.g., species). Thus, the structure of a PBPK model designed for use in research may not serve the intent and purposes of one applied in risk assessment, and the complexities and capabilities of PBPK models vary according to their intended use. PBPK models are generally developed to accomplish one or more of three objectives:

- To integrate diverse sets of pharmacokinetic data on a particular chemical;
- To investigate the pharmacokinetic basis of the toxicity of a chemical that appears to be complex at the administered dose level; and/or
- To predict tissue dosimetry for situations other than those directly measured in animals or humans (i.e., extrapolation).

All PBPK models are simplified representations of biological systems of varying complexities and are designed to predict the behavior and outcome of biological processes affecting the chemical pharmacokinetics in an in vitro or an in vivo system. Some PBPK models

are designed specifically to integrate diverse data sets in efforts to uncover mechanistic determinants of the pharmacokinetics of a specific chemical and to aid in the interpretation of a chemical's mode(s) of action (Haddad et al., 1998; Clewell and Andersen, 1987). However, such PBPK models are not always intended for use in predicting the pharmacokinetic behavior of a chemical under exposure conditions and species in animal toxicity studies that might be important to risk assessment. Models designed for such purposes must integrate diverse sets of pharmacokinetic data and also must be capable of predicting available in vivo pharmacokinetic data sets. Models with defined "predictive capabilities" are valuable for risk assessment because they provide confidence that such models can also predict the in vivo pharmacokinetics for chemicals under exposure conditions where little or no such data exist (e.g., the animals in the toxicity study or the humans in risk assessment scenarios). Thus, models with this form of predictive capacity afford risk assessors the ability to extrapolate across species, dose levels, and exposure scenarios. For more on model purpose, the reader is referred to Clark et al. (2004).

For application in risk assessment, the preferred PBPK model is one that is capable of predicting the pharmacokinetics and tissue dose of the potential toxic moiety of a chemical under conditions applicable to critical studies in animals or humans and human environmental exposures.

3.2. MODEL STRUCTURE

The structure of a PBPK model in large part depends on the purpose for which the model is developed and the philosophy of the modeler. There is virtually no limit to the number and complexity of compartments in a model intended to describe molecular/cellular events (see Figure 3-1). Parsimony in selecting model structures, however, is an important and guiding principle in developing models for use in risk assessments. The complexity of PBPK models used in risk assessment is often constrained by limited data available to calibrate and test the model and the need for risk assessors to defend the model assumptions and the values derived from model simulations.

The simplest conceptual model represents the organism as a one-compartment system. One- and two-compartment kinetic models are useful in characterizing the toxicokinetics of a chemical for any given data set, but they are not useful for extrapolating beyond the data used to develop the model. PBPK models differ from one- or two-compartment models by representing many more physiological, physicochemical, and biochemical processes in the species of interest,

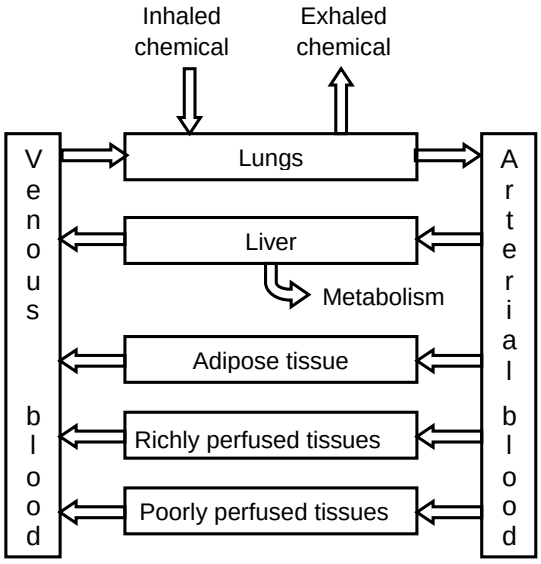
and they can be used to predict internal dose levels for hypothetical exposure regimens based on what is known about the toxicokinetics of a chemical. In most PBPK models, tissues are represented by specific compartments, each with a unique set of physiological (i.e., blood flows) and physicochemical (i.e., partition coefficients) parameters. Target tissues are generally represented individually (e.g., brain) and nontarget tissues are lumped together (e.g., slowly perfused tissues). Depending on the available data, PBPK models intended for risk assessment applications would, preferably, include the target organ as one of the compartments. More often, a PBPK model would be capable of estimating blood concentration, which is often used as a surrogate for tissue concentrations. Major portals of entry (e.g., lung, gastrointestinal tract), storage organs (e.g., adipose tissue), metabolism/transformation sites (e.g., liver, kidney) as well as elimination routes (e.g., renal, pulmonary, fecal) would be included if at all possible.

It is often acceptable to mathematically describe ADME of chemicals in PBPK models in lumped or whole-body surrogate compartments without a highly resolved physically representation in all of the tissues where these processes occur (Krishnan and Andersen, 2001), provided that this lack of physical representation does not interfere with a model's use as an extrapolation tool. When data are available to support more complex representations, the PBPK model can be elaborated to represent more complex mechanistic and biological interactions. For example, the liver can be divided into separate compartments depending on the localization of enzymatic activity. Figure 3-1 provides examples of PBPK model structures that have been commonly used to simulate the kinetics of volatile and nonvolatile substances. Note that these models facilitate the simulation of the concentration of chemicals or their metabolites in the target organ or a surrogate tissue (usually blood).

Frequently, compartments in PBPK models are assumed to be homogenously and completely mixed reactors. This means that the concentration of the chemical anywhere in the tissue is the same and related by the partition coefficient to the concentration of the chemical in venous blood. This assumption is typically used unless there are data to support more elaborated descriptions, such as diffusion-limited compartments.

Ideally, the structure of a PBPK model intended for risk assessment applications would contain the target organ (or a surrogate tissue) as well as compartments representing tissues of unique physiological and biochemical relevance to the pharmacokinetics of the chemical in question.

A



B

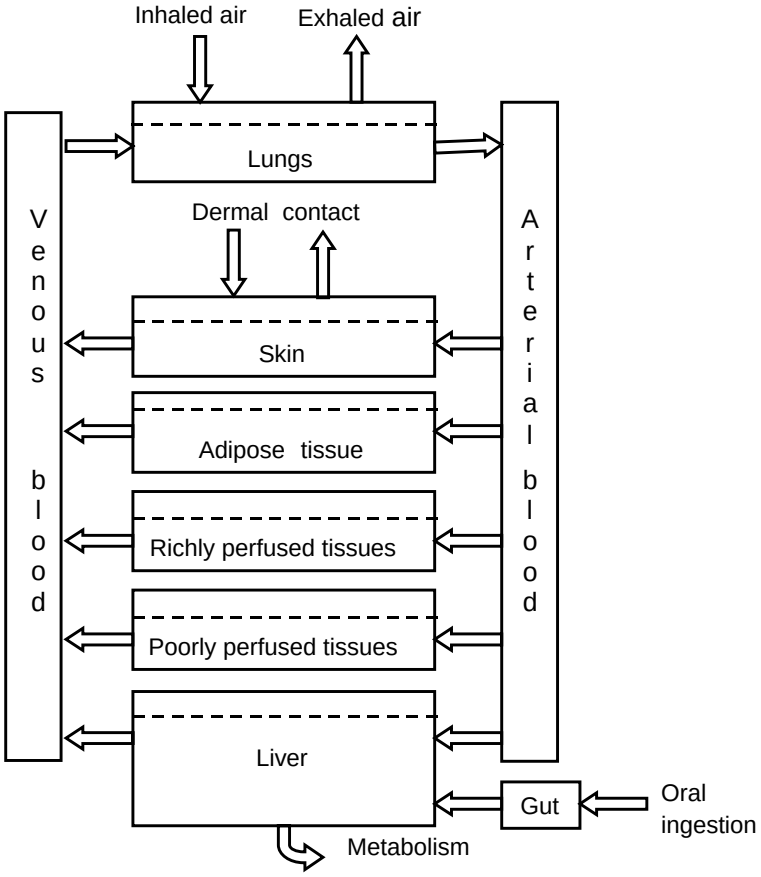


Figure 3-1. Sample PBPK model structures. (A) A four-compartment model for simulating perfusion-limited tissue uptake of inhaled chemical. Gas exchange through lungs is indicated with arrows, and metabolism is described in liver. (B) A model for simulating diffusion-limited tissue uptake and multi-route exposures. Dotted lines represent the separation of cellular matrix and tissue blood components.

3.3. MATHEMATICAL REPRESENTATION

Once the qualitative aspects of the model structure are deemed acceptable, the next step in the evaluation is mathematical representation. Here the focus is on the adequacy of the number and form of mathematical equations used to represent the tissues and processes in the real system being modeled. Model code, rationale, and supporting documentation should be readily available to the reviewer. In PBPK modeling, each tissue compartment is generally described with a mass-balance ordinary differential equation that describes changes in the amount of chemical in the tissue over time. These changes result from chemical distribution in and out of the tissue and clearance processes (e.g., metabolism or excretion) in the tissue. For chemicals distributing to tissues by passive processes, the tissue:blood partition coefficients describe the relationship between tissue and blood concentrations. Descriptions of this blood flow-limited uptake have been used successfully in many of the past efforts in PBPK modeling that dealt with small-molecular-weight organics. For other compounds, including some with high-molecular-weight or significant protein binding, membrane diffusion can be the rate-limiting process; for these chemicals, uptake is described with differential equations for the tissue blood and cellular matrix subcompartments (Krishnan and Andersen, 2001; Andersen, 1995; Leung, 1991; Rowland, 1985). Tables 3-1 and 3-2 provide examples of commonly used mathematical representations for tissue compartments and physiological processes that determine chemical disposition. Note that mass balance differential equations have units of mass per time (e.g., mg/hr) or sometimes concentration per time (e.g., mg/L/hr).

The rates of metabolism in PBPK models have typically been described as first-order, saturable Michaelis-Menten (i.e., shifting from first order to zero order) or second-order processes. At low concentrations, metabolic clearance frequently appears linear, or first order, with respect to plasma concentration. At higher concentrations, metabolic clearance can become saturated and a constant amount of chemical is metabolized per unit time (i.e., zero-order kinetics). Second-order processes and requisite equations are more complex and may be based on a reversible equilibrium relationship (e.g., macromolecular binding) or the concentrations of chemical and cofactors required for metabolism (e.g., conjugation reactions). In each case, the reason for using a particular description should be clearly provided. PBPK models using particular mathematical descriptions of tissue uptake, metabolism, and protein binding without any justification cannot be used confidently for risk assessment applications. For example, if enzyme-mediated metabolism is described as a first-order process in a PBPK model, the

Table 3-1. Equations of a four-compartment PBPK model to simulate the inhalation exposure of volatile organic compounds

Tissue compartments	Equations[a]
Arterial blood[b]	$Ca = \frac{Q_c \times Cv + Q_p \times Cinh}{Q_c + \frac{Q_p}{P_b}}$
Liver	$\frac{dA_l}{dt} = Q_l \times \left(Ca - Cv_l\right) - \frac{V_{max} \times Cv_l}{Km + Cv_l}$ $A_l = \frac{dA_l}{dt} \times dt + A_l$ $C_l = \frac{A_l}{V_l}$ $Cv_l = \frac{C_l}{P_l}$
Fat	$\frac{dA_f}{dt} = Qf \times \left(Ca - Cv_f\right)$ $A_f = \frac{dA_f}{dt} \times dt + A_f$ $C_f = \frac{A_f}{V_f}$ $Cv_f = \frac{C_f}{P_f}$
Richly perfused tissues	$\frac{dA_r}{dt} = Qr \times \left(Ca - Cv_r\right)$ $A_r = \frac{dA_r}{dt} \times dt + A_r$ $C_r = \frac{A_r}{V_r}$ $Cv_r = \frac{C_r}{P_r}$
Poorly perfused tissues	$\frac{dA_s}{dt} = Qs \times \left(Ca - Cv_s\right)$ $A_s = \frac{dA_s}{dt} \times dt + A_s$ $C_s = \frac{A_s}{V_s}$ $Cv_s = \frac{C_s}{P_s}$

Table 3-1. Equations of a four-compartment PBPK model to simulate the inhalation exposure of volatile organic compounds (continued)

Tissue compartments	Equations[a]
Venous blood	$Cv = \frac{Ql \times Cv_l + Qf \times Cv_f + Qr \times Cv_r + Qs \times Cv_s}{Qc}$
Alveolar air	$Calv = \frac{Ca}{P_b}$

[a] Equations are from Ramsey and Andersen (1984).
[b] The steady-state arterial blood equation in this example is used for chemicals that reach rapid equilibrium in blood, such as highly fat-soluble volatile chemicals. In other cases, a detailed mass-balance equation for the arterial blood may be needed.

A = amount (mg)
a = arterial blood
alv = alveolar air
b = blood:air
C = concentration (mg/L or mmol/L)
c = cardiac output
f = fat
inh = inhaled air
Km = Michaelis-Menten affinity constant (mg/L)
l = liver
P = partition coefficient
p = pulmonary ventilation
Q = flow rate (L/hr^{-1})
r = richly perfused tissues
s = slowly perfused tissues
t = tissue:blood
V = volume (L)
v = mixed venous blood
vf = venous blood leaving fat
vl = venous blood leaving liver
V_{max} = maximal velocity of enzymatic reaction (mg/hr^{-1})
vr = venous blood leaving richly perfused tissue
vs = venous blood leaving poorly perfused tissue

Table 3-2. Equations used for describing diffusion-limited uptake in PBPK models

Subcompartments	Equations
Tissue blood	$V_{t1}\frac{dC_1}{dt} = Q_t \times (C_{in} - C_{out}) - [PA] \times \frac{(C_1 - C_2)}{Pt}$
Cellular matrix	$V_{t2}\frac{dC_2}{dt} = [PA] \times \frac{(C_1 - C_2)}{Pt}$

A = amount (mg)
C = concentration (mg/L or mmol/L)
in = inflow
out = outflow
PA = permeability-area coefficient
Q = flow rate (L/hr^{-1})
t1 = tissue blood
t2 = cellular matrix
V = volume (L)
Pt = tissue:blood partition coefficient

scientific rationale for employing such a description is needed before the model can be used for purposes of extrapolation and prediction. Because PBPK models are simplified representations of the real systems, the full details and actual complexity of the physiological and biochemical processes are not incorporated in the equations used. Depending on the level of detail required and the objective of the modeling effort, appropriate descriptions of the biochemical processes can be included in these models.

For use in risk assessment, the equations chosen to describe ADME should be scientifically supported and, where possible, documented.

3.4. PARAMETER ESTIMATION

Chemical-specific and species-specific parameter values are required to solve the equations constituting a PBPK model. Typically, PBPK models require the numerical values of physiological parameters such as alveolar ventilation rate, cardiac output, tissue blood flow rates, and tissue volumes. They also incorporate absorption rate parameters (e.g., for dermal or oral uptake), clearance parameters related to metabolism, or renal and biliary excretion pathways, as well as tissue distribution parameters such as partition coefficients (blood:air, skin:water, skin:air, and tissue:blood), protein binding characteristics, or transporter activities. Additional parameters (e.g., tissue DNA levels, hematocrit, number and concentration of binding proteins) may be required in some cases. For many other purposes, knowledge of the average value or the range of plausible values of model parameters is sufficient; however, for estimating interindividual differences in tissue dosimetry, knowledge of the distributions of input parameters is essential.

3.4.1. Physiological Parameters

The physiological parameters used in PBPK models should either correspond to those obtained in the experimental pharmacokinetic study or be within the range of plausible values for the species and life stage. Peer-reviewed compilations of ranges and reference values of physiological parameters for adult animals and humans are available (Brown et al., 1997; Davies and Morris, 1997; Fiserova-Bergerova, 1995; Leggett and Williams, 1991; Arms and Travis, 1988) (Tables 3-3 through 3-6), yet no such compilations exist with respect to physiological parameters for specific subgroups of populations (e.g., developing and lactating animals,

Table 3-3. Commonly used physiological parameters for mice, rats, and humans

Physiological parameters[a]	Mouse	Rat	Human
Body weight (BW) (kg)	0.025	0.25	70
Tissue volume (fraction of BW)			
Liver	0.055	0.04	0.026
Fat	0.1	0.07	0.19
Organs	0.05	0.05	0.05
Muscle and skin	0.7	0.75	0.62
Cardiac output (Q_c) (L/min)	0.017	0.083	6.2
Tissue perfusion (fraction of Q_c)			
Liver	0.25	0.25	0.26
Fat	0.09	0.09	0.05
Organs	0.51	0.51	0.44
Muscle and skin	0.15	0.15	0.25
Minute volume (L/min)	0.037	0.174	7.5
Alveolar ventilation (L/min)	0.025	0.117	5

[a]Many PBPK models often lump certain tissues together into single compartments such as rapidly/richly and slowly/poorly perfused compartments.

Source: Adapted from Travis and Hattemer-Frey (1991).

Table 3-4. Range of values of the volume and perfusion of select tissues in the mouse

Tissue	Volume (% body weight)	Regional blood flow (% cardiac output)
Adipose	5–14[a]	
Brain	1.35–2.03	3.1–3.5
Heart	0.4–0.6	5.9–7.2
Kidneys	1.35–1.88	7–11.1
Liver	4.19–7.98	
Lungs	0.66–0.86	
Muscle	35.8–39.9	12.2–19.6
Skin	15.9–20.8	3.3–8.3

[a] Varies proportionately with body weight.

Source: Adapted from Brown et al. (1997).

Table 3-5. Range of values of the volume and perfusion of select tissues in the rat

Tissue	Volume (% body weight)	Regional blood flow (% cardiac output)
Adipose	4.6–12[a]	
Brain	0.38–0.83	1.5–2.6
Heart	0.27–0.4	4.5–5.1
Kidneys	0.49–0.91	9.5–19
Liver	2.14–5.16	13.1–22.1
Lungs (upper respiratory)	0.37–0.61	11.1–17.8
Muscle	35.4–45.5	
Skin	15.8–23.6	

[a] Varies proportionately with body weight.

Source: Adapted from Brown et al. (1997).

Table 3-6. Range of values of perfusion of select tissues in humans

Tissue	Regional blood flow (% cardiac output)
Adipose	3.7–11.8[a]
Brain	8.6–20.4
Heart	3.8–8
Kidneys	12.2–22.9
Liver	11–34.2
Muscle	5.7–42.2
Skin	3.3–8.6

[a] Varies proportionately with body weight.

Source: Adapted from Brown et al. (1997).

pregnant women, children). Physiological parameters for specific subgroups is an area of active research, and there are some published references for some parameter values (Gentry et al., 2004; Pelekis et al., 2003; Hattis et al., 2003; Price et al., 2003a, b; Haddad et al., 2001a; Schoeffner et al., 1999; Luecke et al., 1994).

In PBPK models for organic chemicals, the sum total of the volumes of compartments corresponding to soft tissues is smaller than the body weight; sometimes 85% of the body weight (100% [body weight] – 15% [estimate of nonperfused portion based on the weight of skeletal/structural components as percent body weight]) is used (Brown et al., 1997). Even though the tissue volumes (expressed in liters) are needed for PBPK modeling, tissue weights (kg) are usually used with the assumption of unit density (L = kg). This assumption is a reasonable approximation because tissue densities typically range from 0.9 kg/L for fat to 1.06 kg/L for muscle (Mendez and Keys, 1960).

The tissue blood flow rates in the model should add up to cardiac output. Maintaining mass balance in PBPK models requires that the sum of all flows to the compartments be equal to the cardiac output, although other errors in model equations can result in a lack of mass balance. The ratio of cardiac output to alveolar ventilation rate is roughly 1 in a resting individual but decreases with activity (Astrand and Rodahl, 1970). The specification of cardiac output independent of the value of ventilation rate is unacceptable, particularly if the ratio (ventilation:perfusion) is not in the normal physiological range. Frequently in PBPK models, ventilation rate, cardiac output, and tissue perfusion rates and tissue volumes are specified for the individual animal or human being simulated.

The values of common physiological parameters for a test species or human vary depending on body weight. To simplify the recalibration of certain parameter values when running the model for a different species, all tissue volumes are expressed as fractions of body weight such that, for any given body weight, the volumes in liters can be readily calculated by multiplying the body weight by the corresponding fractional value. Similarly, because the cardiac output and alveolar ventilation rate are related to basal metabolic rates and/or body surface rather than to body weight, these models can be specified as a power function of body weight, with the exponent varying from 0.67 to 0.75 (e.g., Tardif et al., 1997; Andersen et al., 1987). These scaling functions are based on cross-species analyses for adult animals and may not be appropriate for all life stages.

An acceptable PBPK model would contain tissue volumes, flow rates, and ventilation:perfusion ratios that are within physiological limits. The sum total of the tissue volumes should not exceed the body weight, and the sum total of tissue blood flow rates should equal cardiac output.

3.4.2. Partition Coefficients

Tissue distribution is dependent on a variety of processes, including passive diffusion, active transport, and cellular concentrations of lipid and binding proteins, among others. Partition coefficients describe the steady-state concentration in the tissue compared with blood or for volatiles in blood versus air. Calibration of PBPK models for partition coefficients has sometimes been done by fitting model simulations to in vivo data. In such cases, pharmacokinetic data collected following a single bolus dose or repeated doses leading to steady state are analyzed with the PBPK model to estimate the tissue:blood partition coefficients (Gabrielsson and Bondesson, 1987; Gallo et al., 1987; Lam et al., 1982; Chen and Gross, 1979). Steady-state data provide the most straightforward data for model calibration; however, they require correction for tissues in which there are significant specific binding or metabolic processes. In tissues where there is a significant level of metabolism or binding, the calculation of an apparent tissue:blood partition coefficient should account for the amount of chemical consumed by such processes (Chen and Gross, 1979).

The tissue:blood, skin:water, skin:air, and blood:air partition coefficients required for PBPK modeling of volatile organic chemicals are conveniently determined in vitro using the vial equilibration method (Beliveau and Krishnan, 2000; Kaneko et al., 1994; Gargas et al., 1989; Johanson and Dynesius, 1988; Fiserova-Bergerova and Diaz, 1986; Sato and Nakajima, 1979). Tissue:blood partition coefficients for nonvolatile chemicals may be estimated in vitro using radioactive chemicals in ultrafiltration, equilibrium dialysis, or vial equilibration procedures (Murphy et al., 1995; Jepson et al., 1994; Sultatos et al., 1990; Igari et al., 1983; Lin et al., 1982). The partition coefficients estimated by these in vitro methods are acceptable, provided equilibrium is attained during the experimental conditions. A time-course analysis should be conducted to choose an appropriate time point (at which equilibrium is attained) for determining partition coefficients in vitro, and appropriate studies will help demonstrate that metabolism or chemical reactions are not depleting the chemical.

Algorithms based on the consideration of solubility and binding of chemicals in biological matrices have also been developed and applied for predicting tissue:blood, tissue:air, and blood:air partition coefficients of volatile organic chemicals. This approach requires knowledge of tissue and blood composition in terms of lipid and water contents, the octanol:water or oil:water partition coefficients of the chemical, and binding association constants, if applicable (e.g., Poulin and Thiel, 2000; Poulin and Krishnan, 1996a, b, 1995). At

the present time, there is no validated animal-replacement approach for predicting association constants for blood or tissue protein binding of organic chemicals (Poulin and Krishnan, 1996b). The biologically based algorithms as such are useful in providing initial estimates of tissue:blood partition coefficients solely on the basis of the consideration of solubility in water and lipid contents of tissues and blood. A number of other empirical or semiempirical methods relating molecular structure or physicochemical characteristics to tissue:blood and blood:air partition coefficients of chemicals are also available (Beliveau et al., 2005, 2003; Payne and Kenney, 2002; Abraham and Weathersby, 1994). Their use is acceptable, as long as the qualitative and quantitative aspects of the structural features and physicochemical characteristics of the new chemical are within the range of values that were used to calibrate the algorithm.

Partition coefficients required for PBPK models can be obtained using in vitro methods, in vivo data obtained at steady state, or theoretical algorithms within the boundary of valid application.

3.4.3. Biochemical Parameters

Absorption rates, metabolic parameters (e.g., first-order or second-order rate constants, maximal velocity, and Michaelis constant), tissue diffusion constants (for describing diffusion-limited uptake), and transporter activity parameters required for PBPK modeling can be determined by fitting a model to data from in vivo studies. In order to estimate these parameters, pharmacokinetic data (e.g., time course of tissue or blood concentration of parent chemicals, urinary metabolite levels) obtained following a single bolus dose or infusion may be used. For volatile organic chemicals, data from exhaled breath and closed chamber gas uptake studies are frequently used with success (Gargas et al., 1986; Filser and Bolt, 1981, 1979; Andersen et al., 1980). Descriptions of serum protein binding have important impacts on tissue distribution and clearance, with parameters often estimated in vitro (Himmelstein and Lutz, 1979). The rate constants of chemical reaction with hemoglobin and tissue proteins determined in vitro or in vivo have been incorporated into the PBPK model to make predictions of these phenomena in vivo (e.g., Krishnan et al., 1992). Biochemical parameters estimated from in vivo data using a model are dependent on the model structure and values of other parameters (e.g., physiological), so the application of these values in models with different structures or parameter values must be evaluated with care. The use of a Bayesian approach is likely to enhance the precision of parameter estimations from in vivo data by more formally developing and evaluating the

uncertainty and accuracy of parameter values and by incorporating multiple data sets into their derivation (e.g., Vicini et al., 1999).

Appropriate methods for application of in vitro systems (e.g., freshly isolated hepatocytes, microsomes, post-mitochondrial fractions, cytosols) to provide metabolic constants for incorporation into PBPK models continues to be an active area of investigation. These data may be applicable to modeling using the parallelogram approach. For example, chemical-specific in vitro metabolic data from cultured hepatocytes can be scaled to represent in vivo liver clearance using in vitro data such as estimates of the number of hepatocytes present per 1 g of liver tissue and the average liver weight (in grams) of the species and age group of interest. In vitro data for humans can then extrapolated to in vivo by assuming that the same relationship that successfully describes the in vitro to in vivo relationship in animals effectively converts the human in vitro data to the in vivo situation.

There are several examples of successful application based on appropriate in vitro-in vivo scaling methods (Lipscomb et al., 2003, 1998; Hissink et al., 2002; Cole et al., 2001; Mortensen and Nilsen, 1998; Mortensen et al., 1997; De Jongh and Blaauboer, 1997, 1996; Reitz et al., 1996a, 1989; Hwang et al., 1996; Iwatsubo et al., 1996; Kedderis and Held, 1996; Gearhart et al., 1990), although the extrapolation of in vitro data to intact animal is not clear in all cases (e.g., Haddad et al., 1998, 1997). But the in vitro studies are particularly useful for evaluating the extent of metabolism in target tissues, characterizing interindividual differences in metabolism, and conducting animal-human extrapolation of metabolism constants based on a parallelogram approach (Kedderis and Lipscomb, 2001; Thrall et al., 2000; Ploemen et al., 1997; Reitz et al., 1996b; Andersen et al., 1991). Receptor binding and DNA-binding properties of chemicals have also been successfully described with PBPK models on the basis of in vitro-derived data (Farris et al., 1988; Terasaki et al., 1984).

Biochemical parameters for PBPK models can be estimated using in vivo data or on the basis of adequate scaling of in vitro data.

3.5. COMPUTER IMPLEMENTATION

Most PBPK models require the use of numerical simulation methods because they contain differential equations and descriptions of nonlinear processes, making exact solutions difficult or impossible. There are a number of integration algorithms and programming languages currently available for coding and solving PBPK model equations (Table 3-7). Many

Table 3-7. Examples of simulation software used for PBPK modeling

Software	Developer/vendor	Salient features	Examples of application
Fortran compiler with IMSL library packages, C, Pascal, Basic	Many vendors sell the different compiler packages available on the market	Machine language compiler packages that require certain knowledge of computer programming; models can be customized to simulate specific condition	Hoang (1995); Karba et al. (1990)
ACSL, ACSL-Tox , or acslXtreme (Advance Continuous Simulation Language)	The Aegis Technologies Group, Inc., Huntsville, AL	The most commonly used for PBPK modeling in the toxicology community; language designed for modeling and evaluating the performance of continuous systems described by time-dependent, nonlinear differential equations	Thomas et al. (1996a); Dong (1994)
SimuSolv	Dow Chemical Company, Midland, MI (no longer distributed outside the company)	Makes use of ACSL language to write the dynamic nonlinear systems that are translated into FORTRAN at run time	Rey and Havranek (1996); Ramsey and Andersen (1984)
Matlab	The MathWorks, Natick, MA	Mathematical software with matrix-related computations, numerical integration algorithms capable of solving systems of ordinary differential equations, and graphical nonlinear simulation (Simulink)	Easterling et al. (2000)
Microsoft Excel	Microsoft Corporation, Redmond, WA	Neither translation of the model nor the compilation into a program is required, but the user should specify integration algorithm and interval	Haddad et al. (1996); Johanson and Naslund (1988)
ScoP (Simulation Control Program)	Simulation Resources, Inc., Redlands, CA	An interactive control program for constructing models; when used with a C compiler, SCoP greatly simplifies the construction of a simulation program	Menzel et al. (1987)
Stella	Isee Systems, Lebanon, NH (formerly High Performance Systems Inc.)	Macintosh, interactive graphical user interface software; enables the user to generate models with diagrams, where a minimal knowledge of computer programming is required	Hoang (1995)

Table 3-7. Examples of simulation software used for PBPK modeling (continued)

Software	Developer/vendor	Salient features	Examples of application
Mathematica	Wolfram Research, Inc., Champaign, IL	Mathematical software with matrix-related computations; numerical integration algorithms capable of solving systems of ordinary differential equations	Burmaster and Murray (1998)
Berkely Madonna	Robert Macey and George Oster, University of California at Berkeley, CA	A general-purpose differential equation solver program developed on the Berkeley campus under the sponsorship of National Science Foundation and the National Institutes of Health; currently used by academic and commercial institutions for constructing mathematical models for research and teaching	Reddy et al. (2003)
SONCHES (Simulation of Nonlinear Complex Hierarchical Ecological Systems)	Central Institute of Cybernetics and Information Processes, Academy of Sciences of GDR, Berlin	A computer system where connections between various data libraries in the preparation and post-processing of simulation are executed by macro commands	Wünscher et al. (1991)
CMATRIX	Robert Ball and Sorell L. Schwartz, Georgetown University, Washington, DC	A system that allows the user to create compartmental models based on personal biological knowledge, leaving the construction and numerical solution of the differential equations to the software	Ball and Schwartz (1994)
BASICA	California Department of Pesticide Regulation, Sacramento, CA	Numerical integration algorithms developed by the Department for PBPK modeling	Dong (1994)
AVS (Application Visualization System)	Advanced Visual Systems, Inc., Waltham, MA	A visualization software package capable of importing processed resonance images and combining the use of ACSL to create three-dimensional representations of the PBPK of a chemical in an organism	Nichols et al. (1994)
MCSim	Drs. Bois and Maszle	Software that facilitates the conduct of Bayesian analysis with PBPK models but has no graphical interface	Jonsson and Johanson (2003)

of the commercially available software packages routinely make use of integration algorithms to obtain numerical solution to differential equations (Reddy et al., 2003; Easterling et al., 2000; Burmaster and Murray, 1998; Menzel et al., 1987) such that (for most cases) the modeler or the risk assessor needs only to evaluate that an appropriate algorithm was used. However, if a programming language (FORTRAN, BASIC) or spreadsheet (Lotus 1-2-3, QuattroPro, Microsoft Excel) is used for modeling, then the modeler should write the codes for an appropriate numerical integration algorithm (e.g., Euler, Gear, Runge-Kutta routines; predictor-corrector methods). In such cases, the integration algorithm as well as the integration interval, i.e., the time interval at which the calculations of the change in concentration (or amount) of chemical in various compartments are performed, should be specified (e.g., Haddad et al., 1996; Blancato and Saleh, 1994).

The modeler also needs to be aware of the optimization routine offered by software packages, particularly if parameters are to be estimated from experimental data by statistical optimization (Holmes et al., 2000). The personal and portable computers marketed today possess sufficient speed, disk space, and run-time memory required for PBPK modeling and parameter optimization; therefore, this aspect requires no formal evaluation.

The accuracy of computational representation of PBPK models is evaluated by "debugging," which refers to the process of error detection in computer programs. Errors in PBPK model code may result either from typing errors or from illogical mathematical statements. To eliminate these errors, it is essential to carefully verify the model code after entry into the computer. Commercially available simulation software, while converting the model codes written in a source language to machine language, can detect syntax/language errors related to incorrect writing of model codes. However, such error diagnostic features cannot detect errors associated with incorrect mathematical representation of a process, even if the code is written in correct programming language and without typing mistakes.

The modeler who uses a PBPK model in risk assessment is ultimately responsible for ensuring that the code and equations are entered correctly and that the code is subject to routine error diagnostic checks; this may include re-coding the model. Such verification can initially be done by the developer, subsequently by individuals not involved in the model development (e.g., peer-reviewers and co-workers) (Clark et al., 2004), and again when used for risk assessment purposes.

Solution to the differential equations in a PBPK model need not be evaluated if a highly reputable commercial or open source simulation software has been used, although an appropriate algorithm should have been selected. However, it is necessary to ensure that the code and equations are entered correctly and that the code is subject to routine error diagnostic checks. When the modeler writes his/her own program, the appropriateness of the integration algorithm and integration intervals should be justified; similar concerns would exist initially for newly developed commercial or open source software.

3.6. EVALUATION OF PREDICTIVE CAPACITY

The purpose of model evaluation is to assess the adequacy of a model and corresponding parameters to consistently describe the available pharmacokinetic and dose-response data of a chemical-biological system, as well as to characterize the uncertainty associated with the parameter values. In a risk assessment context, this also involves evaluation of the suitability and applicability of the model for regulatory and health protection purposes. Model evaluation includes verification and validation (or calibration). In brief, model validation deals with building the right model, whereas model verification deals with building the model right (Balci, 1997). Model verification includes many of the topics covered in this chapter up to this point. It involves an evaluation of the accuracy with which a chemical-biological system has been transformed into a model specification (e.g., the model diagram or equations) and the accuracy to which such a diagram or set of equations has been coded into an executable computer program. Model validation/calibration, on the other hand, involves substantiating that the model, within its domain of applicability, behaves with satisfactory accuracy.

It is important to correct a common misunderstanding about what "validated model" means. A model that has been calibrated against one data set and adequately simulates a different data set can be said to be "validated," but it is only validated to the extent to which those two data sets accurately represent the larger population, not in any global sense independent of the data used to develop and test the model. PBPK models are used to extrapolate to hypothetical exposure conditions or dosing regimens but, again, these extrapolations are only valid to the extent that the data used to calibrate and test the model are of sufficient quality to support the extrapolations. To avoid giving the impression that a model has been validated to predict outcomes for which it has not been adequately tested, some modelers will use the terms "calibrated" to describe a model containing parameters that have been

optimized to fit one (e.g., an "internal" data set and the term "having predictive utility" to describe a model that adequately reflects another (e.g., an "external") data set. There are, however, some advocates for using "all" of the available data to develop parameter values, and for that approach the calibration and predictive utility distinctions no longer work. This issue of varying methods and terms used to develop and evaluate PBPK models is a reflection of the relative newness of the modeling discipline in risk assessment, and the research community is actively working to advance the methodology.

A potential complexity that may come to light during the model evaluation process is the existence of discrepancies between data sets or even among different measurements within a data set. For example, when one dosimetry study reports that the sum of all urinary metabolites excreted by rats is 20–30% and another study reports 40–50% urinary clearance, no PBPK model may be able to simulate both data sets. When this occurs the modeler will need to evaluate the data sets to identify potential sources of these differences and ultimately may need to use expert judgment to select one over the other or accept the uncertainty implied if both are acceptable. Obvious sources of discrepancies that a model may be able to explain would be due to dose route and dose level. Differences that could be more difficult to explain with a single model (and a single set of parameters) can arise from differences in dose vehicle and animal strain. In the case of strain differences, if the modeler finds that she/he can describe all the data from one strain of rats but not a data set from a different strain, there is at least a reasonable chance that some model parameters (e.g., metabolic rate constants) differ for the second strain. However, if no potential sources of variation can be identified for discrepancies between data sets for the same strain, vehicle, route, and dose range, then any model will fail to fit both sets. In such instances, consideration of the quality of the analysis or other features of the study, or perhaps the one that is most consistent with all other data sets, can inform the decision of which data to utilize.

It is also possible for a data set to be internally inconsistent. For example, when the sum of all excreta does not equal the administered dose, or the sum of metabolites measured in the blood do not equal the total blood level measured using a radiolabel, then there is a lack of mass balance in the pharmacokinetic data. Here too, the modeler must exercise professional judgment in determining how to simulate the data and whether some data can be excluded. For example, a model may be able to describe the data from three doses used in a study but not those from a fourth dose (that is neither the highest nor the lowest dose). In this case, a classical pharmacokinetic analysis may show that even with an unstructured model, the parameters for

that odd dose must be quite distinct from the others, leading the modeler to conclude that there was an error or unreported variation in the dosing or data collection. If a dosing error is suggested, then the modeler may try varying the dose for that data set to see whether the model can then fit the data. In the case of a mass balance discrepancy, the modeler may choose to normalize the data, forcing it into balance, or to introduce a "loss" term such as binding to tissue components if that is consistent with the biochemistry.

In the following sections, it is presumed that all such discrepancies in the data themselves have been dealt with.

3.6.1. Model Verification

As mentioned above, verification of a PBPK model involves evaluation of the biological plausibility of the model structure and parameters, as described in the documentation, and the mathematical correctness of the equations. Although these topics are discussed in previous sections, the issue of verification is being highlighted here in the context of a risk assessor who may have played no part in the initial development of a model. In this context, the risk assessor(s) must, in essence, retrace the model development in order to understand the model well enough for application in regulatory decision making. This includes assessing the model from purpose and structure all the way to examination of the model code in order to ensure that it mathematically implements the model as described in the documentation. This examination includes checking for correctness of statements and functions and correct order of statement execution (for languages that are not self-sorting). Although trivial, checking the mass balance is important when evaluating errors in model structure that could lead to erroneous increases or decreases in the level of the chemical in tissues. Another check on model behavior is to set the exposure level to zero; this is necessary to ensure that the model can accurately represent steady-state levels of the chemical and that the background level does not change with time in the absence of exposure.

Whether a code-based or a graphical-based software interface is used, it is preferable that the language produce, as one output, the set of equations that constitute the PBPK model. Code-based representations ease the task of providing sufficient documentation demonstrating that the model is actually constructed as may be described in prose within a peer-reviewed publication. To facilitate model verification, the model code would be organized and annotated in such a way as to facilitate understanding by individuals (e.g., reviewers) other than the original program

developer. This also affords relatively easy translation into a reviewer's software of choice. In the case of a model intended for use in a risk assessment application, it is imperative to provide documentation of the particular parameter values and simulations that are required to reproduce any validation runs and dose metric calculations. This usually entails the provision of step-by-step directions, either using the language's scripting capability or in separate documentation, that allow reproduction of the validation plots and dose-metric calculations by following specific directions or by invoking specified program blocks.

3.6.2. Model Validation/Calibration

Model evaluation should consider the ability of the model to predict the kinetic behavior of the chemical under conditions that test the principal aspects of the underlying model structure. Ideally, a PBPK model would be compared with data that are informative regarding the parameters to which the dose metric predictions are sensitive (this pre-supposes the use of sensitivity and uncertainty analysis to identify the parameters of concern; see Section 3.7). For example, validation of a human model based solely on parent chemical data would not necessarily provide confidence that the model could be used to predict a metabolite dose metric. The use of parent chemical kinetic data to validate model estimates of metabolism in the human can be highly misleading because it can be the case that the metabolism parameters have little impact on parent chemical concentrations, whereas other uncertain parameters (e.g., ventilation rate, blood flow, fat content) can strongly influence model predictions of parent chemical kinetic behavior. However, even in such cases, sensitivity and uncertainty analyses can help to characterize the confidence (or lack thereof) with which the model makes predictions (see Section 3.7).

The adequacy of model parameter values may be evaluated in different ways; no single method has been accepted or endorsed by the modeling or regulatory community as yet. Statistical methods required for evaluating the adequacy of model parameters are based on comparison of simulations with experimental data and depend on whether the objective is to perform internal evaluation (in which all model parameters are estimated from the same data set), external evaluation (in which different data sets are used for model calibration and testing the predictive capability of the model), or semi-external evaluation (in which some of the model parameters are based on the data set). Although no systematic research effort or guidance is

available in this regard, there is much interest in developing consistent and acceptable evaluation methods, and progress is being made.

To date, PBPK model evaluation generally has not been conducted rigorously from a statistical perspective. Although quantitative tests of goodness of fit often may be a useful aspect of the evaluation process, they generally are not designed to test hypotheses for PBPK models, which can be highly nonlinear and may contain a large number of parameters. None of the classical procedures (e.g., t-, Mann-Whitney, two-sample χ^2, and two-sample Kolmogrov tests) that determine whether the underlying distributions of the two data sets are similar is applicable because the output processes of almost all real-world systems and simulations are nonstationary and autocorrelated. Furthermore, a question exists as to whether the use of statistical hypothesis tests is even appropriate. Since the model is only an approximation of the actual system, a null hypothesis that the system and model are the same is clearly false.

Perhaps a more important consideration may be a model's ability to accurately predict the general behavior of the data in the intended application and whether or not the differences between the system and the model are significant enough to affect conclusions derived from the model. In this regard, Haddad et al. (1995) screened various statistical procedures (correlation, regression, confidence interval approach, lack-of-fit F-test, univariate analysis of variance, and multivariate analysis of variance) for their potential usefulness in evaluating the degree of agreement between PBPK model simulations and experimental data. According to these authors, the multivariate analysis of variance represents the most appropriate classical statistical test for comparing PBPK model predictions with experimental data.

For now, however, the most common (if not the most rigorous) approach to model evaluation has involved visual inspection of the plots of model predictions (usually continuous and represented by solid lines) with experimental values (usually discrete and represented by symbols) against a common independent variable (usually time). The greater the commonality between the predicted and experimental data, the greater the confidence in the model structure and parameters. The correspondence between predictions and experimental data should be not only at the level of individual values (e.g., blood concentration values) but also at the level of the profile (i.e., bumps and valleys in the pharmacokinetic curve). In other words, the shape of the simulated curve should correspond to that of the experimental data and also be one that would result in a plot of the residuals (i.e., difference between the simulation and the data) without a systematic deviation from a scatter around zero.

Figure 3-2 shows several examples of visual evaluations of the adequacy of PBPK model predictions. The models used in panels A and C could be considered adequate because they simulate the behavior of the experimental data even though they do not accurately simulate every single experimental datum. On the other hand, model D would be considered less adequate, and further work would be required to refine the model. Examination of the model simulation in panel B suggests that this model is simulating a bolus exposure, whereas the data set (same in all panels) appears to be from an inhalation study, suggesting that the modeler has chosen either the wrong exposure parameters for the model or the wrong data set for comparison.

This approach to model evaluation says nothing about the adequacy of the model structure or parameters; it only reflects an individual's judgment of how closely the model predicts the observed behavior. Evaluating the adequacy of model structure and equations is fairly straightforward when compared with the evaluation of the model parameter values. For example, inadequacies in PBPK model structures can be inferred simply by observing the simulated and experimental pharmacokinetic profiles (Lilly et al., 1998). If the model cannot fit the pharmacokinetic profiles for *any* realistic parameter values, or it can do so only by using values that are inconsistent with other data, then one can reasonably conclude that the structure is inadequate.

This evaluation of model structure provides the developer an opportunity to think about the need for additional compartments, critical determinants of disposition, or different quantitative descriptions of the phenomena and to improve the capability of the model accordingly. Again, a useful way of comparing the experimental and simulated data is to plot the residuals (i.e., difference between experimental and simulated data) as a function of time or as a function of various controllable variables. If two or more models fit the experimental data equally well, new experiments may be designed to identify the model that more accurately predicts the other attributes of the biological system (Kohn, 1995).

One approach for determining whether the level of complexity (number of parameters) in a model is justified by the data is to use a nested modeling approach, where the model is reduced to a simpler (nested) model when one or more parameters is set to zero or some other "baseline" value (Collins et al., 1999); however, additional changes to the model may be needed in order to maintain mass balance. The increase in goodness-of-fit obtained by allowing those parameters to be varied can then be evaluated statistically using a χ^2 statistic to determine whether the additional degrees of freedom afforded by those parameters are justified (Collins et al., 1999).

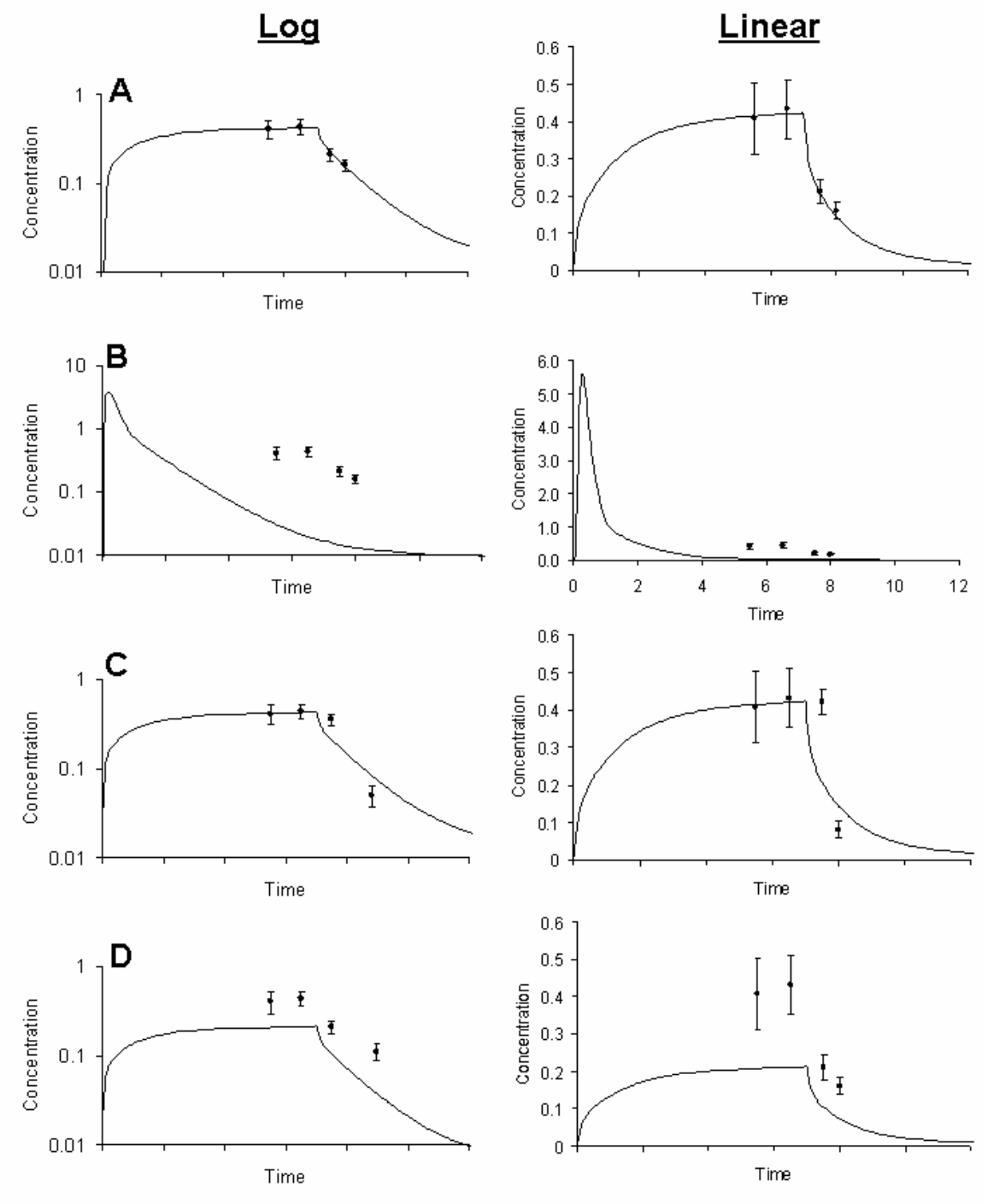


Figure 3-2. Comparison of four PBPK model simulations (left, log scale; right, linear scale). Solid lines are model simulations overlayed with experimental data (symbols). Models A and C appear to simulate the data reasonably well. Model D seems to underpredict all points, suggesting that refinement is necessary. In model B, there appears to be a mismatch between the exposure parameters in the model (apparently bolus) and that of the data set (apparently inhalation).

There is increasing concern about the relevance and usefulness of external evaluation in PBPK modeling. External evaluation requires that some of the available pharmacokinetic data not be used during the model calibration phase, but set aside for evaluating the performance of the model. Not everyone is in agreement with such an approach. Some investigators argue that all the data used for model evaluation can be used to improve the parameter estimates, so that no data are "wasted" toward that end. Such an iterative approach to model evaluation and calibration maximizes the use of the available data. Others, however, argue that this type of modeling can become a form of self-fulfilling prophecy.

The issue of external evaluation is particularly problematic for human data because the actual parameters for each individual in a population might be sufficiently different, such that a model with a single set of parameters may not be reasonably expected to simulate the observed kinetics in all individuals. Therefore, the process of modeling not only can take into account existing information on parameters but also accommodate new information based on fits to additional data sets. In this context, Bayesian analysis utilizing Markov chain Monte Carlo (MCMC) calculations is being increasingly explored for use in PBPK modeling (Jonsson and Johanson, 2002, 2001; Bois, 2000a, b). In the Bayesian approach, the prior information on parameters is updated on the basis of new pharmacokinetic data, such that the resulting posterior estimates consistently describe all data and support better characterization of the uncertainty and distribution in the parameter values. One continuing challenge of the Bayesian approach is how to use important data or biological information that are not easily amenable to incorporation into MCMC calculations, the concern being that leaving such information out gives undue priority to the particular studies that are included. Thus, posterior distributions always need to be scrutinized as to their consistency with existing biological knowledge; for instance, posteriors that are strongly inconsistent with (carefully constructed) priors may indicate problems with model structure or errors or inconsistencies in the data.

Cross-validation is another potentially useful approach for model evaluation (Keys et al., 2003). In the structure-activity relationship modeling arena (Beliveau et al., 2003), cross-validation uses all of the available data sets by repeated subsampling. This type of a "leave-one-out" cross-validation allows the use of available data both for estimation and evaluation of model parameters.

It is likely that no single approach will be sufficient or applicable in all contexts. Each of these approaches has its merits and limitations, and their applicability for PBPK model evaluation depends on the purpose of the model and data availability.

There are other important issues concerning parameters for which values are estimated by optimizing model output to experimental data ("fitting"), including parameter values based on posteriors from a Bayesian analysis. In such instances, the modeler must assess the identifiability of the parameter given the data. The practical reality of modeling biological systems is that, regardless of the complexity of the model, there will always be some level of "model error" in the form of inconsistencies or lack of representation of the real biological system that can result in systematic discrepancies between the model and the experimental data. This type of inherent structural deficiency in all models interacts with deficiencies in the identifiability of the model parameters, potentially leading to misidentification of the parameters. Due to the confounding effects of model error and parameter correlation, it is quite possible for an optimization algorithm to obtain a better fit to a particular data set by changing parameters to values that no longer have any meaningful correspondence to the biological entity the parameter was initially intended to represent. This problem can be ameliorated in Bayesian analyses through appropriate prior distributions. It is usually preferable, however, to repeatedly vary the model parameters manually before performing an optimization to obtain a sense of their identifiably and correlation under various experimental conditions. Some simulation languages aid this process by including routines for calculating parameter covariance or for plotting joint confidence region contours.

Estimates of parameter uncertainty obtained from traditional optimization routines can be viewed as lower-bound estimates of true parameter uncertainty because only a local, linearized variance is typically calculated. In characterizing parameter uncertainty, it is probably more instructive to determine what ranges of parameter values are clearly inconsistent with the data than to accept a local, linearized variance estimate provided by the optimization algorithm. The Bayesian approach, in principle, gives a more global characterization of parameter uncertainty (see Section 3.7.3).

3.6.3. Model Documentation

Adequate documentation is critical in the evaluation of a model. The level of documentation for a PBPK model depends on its intended use. For models developed for

research or hypothesis testing, the documentation need only include sufficient information about the model so that an experienced modeler can accurately reproduce its structure and parameterization. Usually this documentation would include a combination of one or more "box and arrow" model diagrams together with any equations that cannot be unequivocally derived from the diagrams. For simple models, a well-constructed model diagram, together with a table of the input parameter values and their definitions, may be all that an accomplished modeler would need to create the mathematical equations defining a PBPK model.

For models submitted in support of a risk assessment, the level of documentation is considerably greater. These models will be subjected to internal and external peer review, and their structure, supporting data, simulations, and use in the derivation of reference values must be completely transparent and reproducible. In addition to graphical representations of the model, this level of documentation would likely include well-annotated and complete documentation of the model code, all data (fully referenced) that were used to calibrate and/or test the model, a description of the calibration and testing procedures used, fully referenced sources for all parameter values (or the optimization methods, results, and data used in optimizing parameters), sensitivity analysis or other rationale that guided the choice of which parameters were optimized, simulation run conditions, any additional analyses that help characterize or support the quality of the model, and all supporting documentation that would be needed by an experienced modeler to run the model and accurately reproduce any simulations used (or submitted for use) in deriving reference values.

The model diagram should be labeled with the names of the key variables associated with the compartment or process represented by each box and arrow. All tissue compartments, metabolism pathways, routes of exposure, and routes of elimination must be clearly and accurately presented. The model diagram should also clearly differentiate blood flow from other transport (e.g., biliary excretion) or metabolism, and arrows should be used where the direction of transport could be ambiguous.

In general, there would be a one-to-one correspondence of the boxes in the diagram to the mass balance equations (or steady-state approximations) in the model. Similarly, the arrows in the diagram would correspond to the clearance (transport or metabolism processes) in the model. Each of the arrows connecting the boxes in the diagram should correspond to one of the terms in the mass balance equations for both of the compartments it connects, with the direction of the arrow pointing from the compartment in which the term is negative to the compartment in which

it is positive. Arrows connected only to a single compartment, which represent uptake and excretion processes, are interpreted similarly.

Interpretation of the model diagram is supplemented by the definition of the model input parameters in the corresponding table. The definition and units of the parameters can indicate the nature of the process being modeled (e.g., diffusion-limited vs. flow-limited transport, binding vs. partitioning, saturable vs. first-order metabolism). The values used for all model parameters are to be provided, with units. If any of the listed parameter values are based on allometric scaling, the scaling method should be fully described with body weights used to obtain the allometric constant and the power of body weight used in the scaling. Any equations included to supplement the diagram should be dimensionally consistent and in a standard mathematical notation.

Model documentation plays a critical role in effective transfer of complex biological models between model developers and potential users, particularly those who will evaluate the model and implement it in risk assessment applications. Approaches for model documentation are still evolving, but adequate documentation is essential to the transparency and reproducibility of risk assessments. One approach that can be implemented in many modeling programs that have scripting capabilities is to create computer code that reproduces all the key results, although not necessarily the full procedure to obtain these results (e.g., optimization, Monte Carlo analysis, etc., which sometimes involve multiple software). The key results are (1) fits to data used for parameter estimation, (2) calculation of dose metrics for critical studies (e.g., animal toxicology or occupational epidemiological studies) to be used in developing dose-response values (e.g., RfC, RfD, CSF, or unit risk), and (3) calculation of human dose metrics used in developing dose-response values. Such scripts help to quickly recreate critical numerical values used in the risk assessment and facilitate working with a new model for evaluation or application in risk assessment. However, that also means that these scripts need to be evaluated carefully to ensure that they are correct in terms of the computer implementation and the situation they are modeling. Finally, if model development and implementation in risk assessment are carried out by different people, not all of these scripts would be expected upon completion of model development; others would be created during its application in the risk assessment.

PBPK models intended for use in risk assessment should be evaluated to ensure that they provide simulations of pharmacokinetic profiles consistent with the experimental data and that the parameters (point estimates, range of values, or distributions) are appropriate for the

intended application. Scripts facilitate transparency and reproducibility in modeling by recreating fits to kinetic data, estimation of dose metrics in critical studies, and calculation of human dose metrics for development of dose-response values (e.g., RfD, CSFs).

3.7. SENSITIVITY, VARIABILITY, AND UNCERTAINTY ANALYSES

In models of biological systems, estimates of the values of model parameters will always have some variance, due both to biological variation and experimental or model errors. The interest in having a PBPK model that describes a variety of data with a consistent set of parameters prevents a model from providing an optimal fit to all sets of experimental data. For example, a PBPK model of a compound with saturable metabolism is required to reproduce both the high and low concentration behaviors, which appear qualitatively different, using the same parameter values. If one were independently fitting single curves with a model, different parameter values might provide better fits at each concentration, but they would be relatively uninformative for extrapolation.

Where only some aspects of the model can be evaluated, it is particularly important to assess the uncertainty associated with the aspects that are untested. For example, a model of a chemical and its metabolites that is intended for use in cross-species extrapolation to humans would preferably be verified using data in different species, including humans, for both the parent chemical and the metabolites. If only parent chemical data are available in the human, the correspondence of metabolite predictions with data in several animal species could be used as a surrogate, but this deficiency needs to be carefully considered when applying the model to predict human metabolism.

One of the values of biologically based modeling is the identification of specific data that would improve the quantitative prediction of toxicity in humans from animal experiments. The variability, uncertainty, and sensitivity of parameters constituting the PBPK models can also be evaluated, which is desirable for models that are used to derive dose-response values.

3.7.1. Sensitivity Analysis

Sensitivity analysis provides a quantitative evaluation of how parameters in input functions influence the dose metric or outcome. Such an analysis provides insight into how each parameter influences estimates of the dose metric and the subsequent dose-response value, and which parameter(s) have the greatest impact on the dose-response value estimate. Sensitivity

analysis facilitates a focused use of resources for more detailed analysis and for further data gathering to reduce uncertainty and to better characterize pharmacokinetic variability (Clewell et al., 1994; Bois et al., 1991; Hetrick et al., 1991).

Sensitivity analysis for PBPK models typically compares the magnitude of change in output for a defined change in each input parameter. This process of single changes in one parameter while all others are held constant is called "local" parameter sensitivity analysis. This analysis yields sensitivity ratios that correspond to the ratio of change in simulation output (e.g., tissue dose) to change in parameter value. Figure 3-3 depicts hypothetical sensitivity ratios associated with some input parameters of a PBPK model. The greater the absolute value of the sensitivity ratio, the more important the parameter. In this example, the sensitivity ratio for breathing rate is the highest of all input parameters, indicating that it is the most sensitive model parameter for the dose metric. The sensitivity ratio of 2 for breathing rate signifies that a 1% change in the numerical value of this parameter will result in a 2% change in the dose metric.

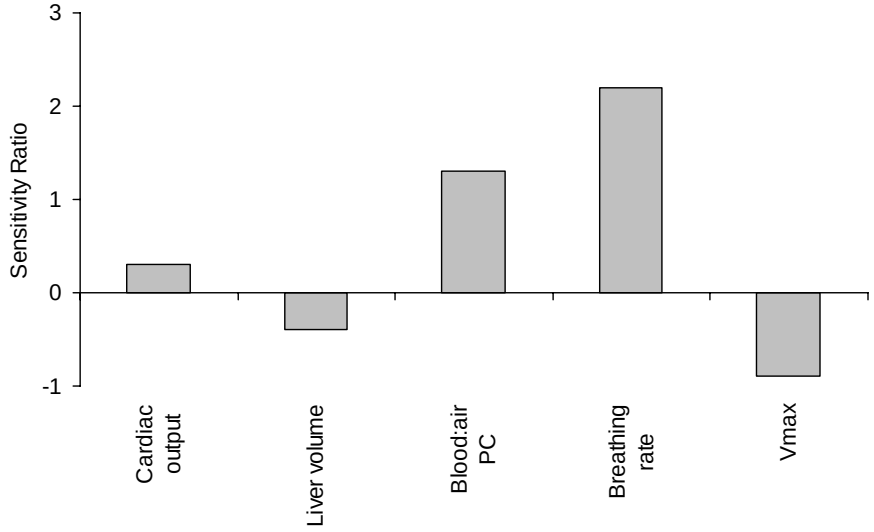


Figure 3-3. Sensitivity ratios associated with certain input parameters of a hypothetical PBPK model.

In practice, sensitivity ratios greater than 1 (in absolute value) are of concern because this results in the amplification of input error (Allen et al., 1996). It is critical that fractional blood flows sum to cardiac output when they are varied in sensitivity analyses or else mass balance will be violated and normalized sensitivity ratios much larger than 1 may be obtained for blood flow parameters.

There are several caveats when conducting local parameter sensitivity analyses (such as described above). One is that they only show the sensitivity of the model predictions to a change in the single parameter *when all other parameters are held constant*. For example, consider the sensitivity to breathing rate depicted in Figure 3-3. What would have happened if one had known, before starting the modeling process, that the breathing rate was 20% higher than the default value actually used? Would the predicted dose metric have then turned out to be 40% higher? Only if none of the other parameters were calibrated to the model data during the modeling process. If in each case (default breathing rate vs. 20% higher) one had started with that value of the breathing rate and then calibrated the V_{max} and other parameters to the data, the result would be different values of V_{max}, etc., that would compensate to some extent for the change in breathing rate.

As a simple analogy, consider the fit of a straight line to some data, where the intercept is a "known" parameter and the slope is fitted. After fitting the line, one might determine that the fit is very sensitive to the intercept by showing that if the intercept is changed while holding the slope constant (i.e., standard sensitivity analysis), the value of the line equation changes a lot. But if one had started with a larger value of the intercept at the beginning of the modeling process, fitting the line to the data would have resulted in a lower value for the slope, such that the value of the line equation would not change as much as when only the intercept is increased. In short, the parameter estimation process leads to certain correlations between the values of parameters that are fixed as inputs and those that are fitted. Thus, standard sensitivity analysis, although very informative about the importance of individual parameters, can overestimate the actual impact of changes in individual parameters because it does not account for correlations.

In the case of Figure 3-3, it might be that starting with a different breathing rate and then calibrating V_{max}, etc., would have yielded almost identical values for the dose metric and that the overall modeling *process* is insensitive to breathing rate, even though the model predictions are sensitive to changes in breathing rate when none of the other parameters are changed. Another example would be that blood concentrations can be highly sensitive to oral absorption rate constants until steady state is achieved; if the risk assessment is estimating steady-state concentrations in humans, estimates of the oral absorption rate constants may be essentially irrelevant. On the other hand, depending on the data at hand, "local" sensitivity analyses may underestimate the impact of changing individual parameters due to the nonlinearity of the model. For instance, if all the data were obtained at doses where metabolism was saturated, then the

model fit will be insensitive to the "Km" over a wide range of values, but for making low-dose predictions, the Km may be a very sensitive parameter. Thus, it is important to carry out sensitivity analyses under conditions reflecting the studies providing data for model calibration (i.e., pharmacokinetic studies), under conditions appropriate for estimating dose metrics in critical studies, and finally under conditions appropriate to the risk assessment. These analyses help identify the key parameters under the conditions relevant for the various steps in a dosimetry-based risk assessment.

3.7.2. Variability Analysis

The focus of a variability analysis is to evaluate the range of values that a parameter expected to be present in individuals may have in a population and the impact of that variability on variability in the dose metric. PBPK models can account for interindividual differences in specific parameters (e.g., enzyme levels, tissue volumes, body weights, workload) and simulate tissue dose variability in populations (Dankovic and Bailer, 1994; Sato et al., 1991). Alternatively, PBPK models can simulate an average individual representing a specific subgroup of the population (e.g., adult women, pregnant women, lactating women, children), and thus evaluate subgroup-specific tissue dose (Corley et al., 2003; Gentry et al., 2003; Price et al., 2003b; Sarangapani et al., 2003; Krishnan and Andersen, 1998; Fisher et al., 1997), although this latter approach would not provide the probability or likelihood of a particular output for a population.

The magnitude of interindividual variability can be characterized using information such as the estimated tissue dose corresponding to the 95th percentile and 50th percentile. To derive this information, Monte Carlo simulations based on distributions of input parameters (physiological parameters, enzyme content/activity with or without the consideration of polymorphism) have frequently been used (Lipscomb et al., 2003; Gentry et al., 2002; Haber et al., 2002; Lipscomb and Kedderis, 2002; Timchalk et al., 2002; Bogaards et al., 2001; El-Masri et al., 1999; Thomas et al., 1996a, b). The Monte Carlo method consists of repeated computations using inputs selected at random from statistical distributions for each parameter to generate a statistical distribution for the output, i.e., dose metric (Figure 3-4). The Monte Carlo approach to variability analysis has been used to evaluate the net impact of the variability of critical biochemical and physiological parameters (e.g., Clewell and Andersen, 1996; Portier and Kaplan, 1989).

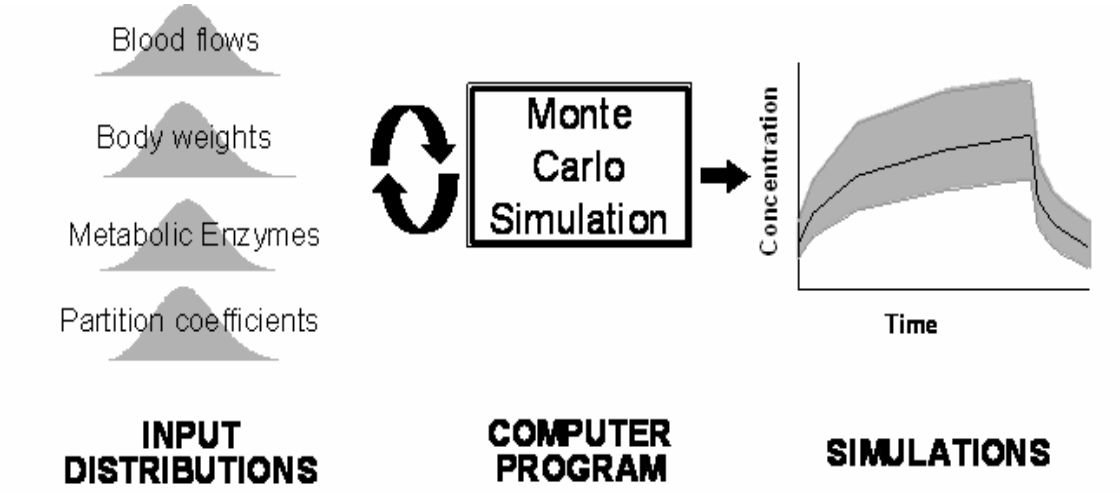


Figure 3-4. Monte Carlo simulation. In this approach, the distribution of internal concentration versus time is simulated by repeatedly (often as many as 10,000 iterations) sampling input values based on the distributions of individual parameters in a population.

When conducting variability analysis, it is important to identify correlations in model parameters. For example, cardiac output (Q_C) and breathing rate (Q_A) are expected to vary in proportion to each other, so using independent distributions that might give a very high value of Q_C with a very low value of Q_A would be unrealistic. On the other hand, one could consider the distributions of Q_C and the distribution of the $f_{AC} = Q_A/Q_C$ and multiply the value selected from the f_{AC} distribution by the value selected from the Q_C distribution to obtain the value of Q_A to be used.

Conceptually, the Bayesian framework is particularly well suited for variability analyses because it allows a "hierarchical" structure in which parameters can be specified at the "population" (e.g., mean and variance, with uncertainty) and "individual" (i.e., drawn from the population) levels (Jonsson and Johanson, 2002, 2001), thereby conducting a simultaneous analysis of variability and uncertainty. Moreover, Bayesian analyses combine prior knowledge about parameters and their variability and uncertainty with data from new experimental studies generating "posterior" distributions of the parameters for both the population and individuals (along with uncertainty) that reflect both preexisting knowledge and the new data (Bernillon and Bois, 2000; Johanson et al., 1999; Bois, 1999). However, care also must be taken in implementing these analyses to account for biologically supported correlations among

parameters, which is as important for Bayesian analyses as it is for "traditional" Monte Carlo simulation. In addition, because the posteriors from Bayesian analyses are calibrated to a particular data set, care must be taken when deciding when and how the posterior distributions for the PBPK parameters are used to make predictions. Consideration must be given as to whether the subject populations in the data sets represent the population(s) of interest. For example, data from subjects in a controlled human exposure may be only representative of a population at rest. Although the Bayesian analysis may correctly estimate a relatively low ventilation rate for this group, that ventilation rate may not be appropriate for the activity level in the general population. Thus, one must chose data sets and parameter values carefully to reflect the population of interest in the risk assessment. In the above example, an additional traditional Monte Carlo simulation could be performed in which some posterior distributions would be replaced with distributions considered more representative of the population of interest.

A variability analysis for a PBPK model is not a prerequisite for its use in risk assessment applications. The assessment of the impact of parameter variability on tissue dose, however, is a prerequisite for a PBPK model intended for use in estimating the interindividual variability (pharmacokinetic component).

3.7.3. Uncertainty Analysis

Uncertainty analysis for PBPK models characterizes the impact that a lack of precise knowledge about the numerical value of a parameter or model structure itself has on estimates of the dose metric. Uncertainty regarding model structure or parameter values may contribute to uncertainty in the predicted dose metric, particularly for low-dose exposure situations (Hattis et al., 1990). Uncertainty analysis is particularly useful when a PBPK model does not adequately simulate the experimental data. Such a situation may arise due to either lack of precise estimates of parameter values or inadequacies in the model structure. In such cases, either a quantitative uncertainty analysis or model-directed mechanistic studies might improve the predictive ability and robustness of the PBPK model (Haddad et al., 1998; Clewell and Andersen, 1987).

Quantitative uncertainty analyses for specific dose metrics (e.g., amount metabolized, tissue concentration of parent chemical at a specific time, cancer estimates) have been conducted using a traditional Monte Carlo approach, a Bayesian MCMC analysis (Elder, 1999; Gelman et al., 1996; Krewski et al., 1995; Farrar et al., 1989), a stochastic response surface method, or a fuzzy simulation approach (Nestorov, 2001; Isukapalli et al., 1998). The latter method is

particularly useful when statistical distributions of parameters cannot be reliably defined and only semiquantitative, qualitative, and vague information is available.

If there is a lack of confidence regarding the numerical value of a parameter (e.g., imprecision due to the method used for parameter estimation), a quantitative analysis of the uncertainty associated with a parameter(s) of the PBPK model will help characterize the impact on the dose metric of interest. Uncertainty analysis will be of limited utility if the available data directly inform the dose metrics of interest, such as the case where a model adequately fits multiple data sets that directly measure the relevant internal dose following exposure by the routes and in species of interest. However, even predictions from such "well-calibrated" models may benefit from uncertainty analysis, particularly if the dose metrics of interest are indirectly inferred (e.g., total metabolism when only blood concentrations are measured). In addition, where possible and relevant, uncertainty analysis can be performed to strengthen credibility of the PBPK model and guide resource allocation for risk assessment-oriented research.

Sensitivity, uncertainty, and variability analyses should be conducted using acceptable statistical methods. EPA has published guiding principles for Monte Carlo analysis (U.S. EPA, 1997), but no such guidance for Bayesian methods has been released, although active research and development are ongoing. When using the Bayesian approach, care should be taken to ensure that the resulting PBPK model simulations respect the following basic conditions:

- The numerical values of physiological parameters (representing prior or posterior distributions) are within known, plausible limits;
- The sum of tissue volumes is lower than the body weight;
- The sum of tissue blood flows is equal to cardiac output;
- The mass balance is respected (chemical absorbed = chemical in body + chemical eliminated); and
- The covariant nature of the parameters is appropriately respected (e.g., the person with lowest breathing rate cannot be the one receiving the highest cardiac output)

While taking advantage of the sophisticated statistical approaches, it is important to ensure that the resulting model and parameters are within plausible range or representative of the reality.

Sensitivity, uncertainty, and variability analyses can help improve the credibility of PBPK models as well as prioritize research needs to improve the model for risk assessment

applications. However, such analyses may not be required for all PBPK models intended for risk assessment applications.

3.8. DEVELOPING PBPK MODELS FOR USE IN RISK ASSESSMENT: STRATEGIES FOR DEALING WITH DATA-POOR SITUATIONS

3.8.1. Minimal Data Needs for Constructing PBPK Models

When an adequately evaluated PBPK model is not available for the species, life stage, and route relevant to a risk assessment application, significant resources may be needed to develop such a model, depending on the chemical, the availability of prior information, and the complexity of disposition mechanisms being modeled. The minimal data required for developing such models for a chemical in any given species are

- Partition coefficients,
- Biochemical constants,
- Route-specific absorption parameters, and
- In vivo pharmacokinetic data for model evaluation.

As outlined in this chapter, the partition coefficients required for PBPK modeling may be estimated using the theoretical algorithms found in the literature. Their use, however, should be limited to the domain of validity and the families of chemicals for which such algorithms have been developed and validated. Biochemical constants such as metabolism rates or binding association constants may be obtained using in vitro systems. Other biochemical parameters may be required, such as renal clearance, which currently can only be determined from in vivo data. Additionally, route-specific absorption parameters such as the rate of oral absorption and the skin permeability constant are required to describe oral absorption and dermal absorption, respectively, prior to achieving steady state. Of these, the skin permeability coefficient can be obtained using available quantitative structure-activity relationships (QSARs). Such absorption parameters are not required for simulating intravenous administration and inhalation exposures. Finally, some in vivo pharmacokinetic data (at a minimum blood concentration time-course data at two dose levels) are required for evaluating the PBPK model for a particular route of exposure.

The minimal data set identified above should be available for the species used in the critical study. Human models, however, may be constructed with knowledge of species-specific

blood solubility/binding characteristics. Other model parameters, including metabolism rates, may be either scaled or kept species-invariant, according to the current state of knowledge (Section 4.5). Of course, the availability of a data set for external evaluation in humans may be a limiting factor. In such cases, surrogate data sets may be used for model evaluation purposes.

3.8.2. Surrogate Data for Interspecies and Interchemical Extrapolations

In the absence of human data for model evaluation purposes, surrogate data have been used successfully, although it must be noted that surrogate data may add additional uncertainty to a risk assessment. A parallelogram approach can be used to generate surrogate data. This approach uses two data sets: one demonstrating the relationship between in vitro and in vivo findings in a test species, and the other demonstrating the relationship between in vitro human and in vitro test species findings; these data are used to predict the in vivo effects in humans. Accordingly, if human data either cannot be collected or is not available for a chemical of interest, it may suffice to evaluate a related chemical for which such data are available. Jarabek et al. (1994) used this parallelogram approach for model development and interspecies extrapolation of the pharmacokinetics of HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane). In this case, the authors developed rat PBPK models for HCFC-123 as well as a structural analog (halothane) by estimating partition coefficients and metabolic constants. Following the evaluation of the rat PBPK model for each of these chemicals, human models were constructed. The adequacy of the human model for halothane was evaluated using available human in vivo data; the model for HCFC-123 was assumed to reasonably simulate the in vivo pharmacokinetics in humans due to the structural and metabolic similarities between the two chemicals, despite the absence of in vivo human HCFC-123 pharmacokinetic data (Williams et al., 1996; Jarabek et al., 1994). This is one practical way of getting around the lack of human data for model evaluation, particularly when external evaluation is intended.

To deal with situations where there is a lack of data to determine PODs for closely related chemicals, a family approach has been suggested. This approach, proposed by Barton et al. (2000), is based on the principle that the acceptable concentrations for related chemicals, particularly metabolites, can be derived using data on the parent chemical. Thus, if the NOAEL for the parent chemical is established, there would also have been internal systemic exposures to its metabolites. By determining the external exposure levels for these compounds that result in the same systemic exposure, the NOAELs for these compounds can be established. The

determination of the internal dose and systemic exposures for the parent chemical and metabolites is accomplished using PBPK models, thus facilitating the derivation and establishment of the RfD/RfC with a poor database.

QSAR approaches are also available for constructing inhalation PBPK models for volatile organic chemicals in the rat (Beliveau et al., 2003). Accordingly, the contributions of various molecular fragments (CH_3, CH_2, CH, C, C=C, H, Cl, Br, F, benzene ring, and H in benzene ring) toward the parameters of PBPK models have been determined. With the knowledge of the number of the fragments occurring in a given molecule, the partition coefficients and the metabolic constants can be obtained and a first-generation PBPK model can be constructed. This QSAR approach is useful to initially develop PBPK models for other chemicals, as long as the number and nature of fragments in the chemical do not differ from the ones in the calibration set used in the study (Beliveau et al., 2003).

3.9. EVALUATION OF PBPK MODELS: SUMMARY

The basic criteria for evaluation of PBPK models intended for risk assessment applications, as outlined in Sections 3.1 through 3.7, are summarized below.

- The PBPK model would predict the pharmacokinetics and tissue dose of the toxic form of a chemical or a surrogate such as parent compound.
- The structure of a PBPK model would contain the target organ or a surrogate tissue, such as blood.
- The equations chosen to describe ADME would be justified on the basis of known mechanisms of such processes for the chemical of interest or by analogy with other chemicals.
- The tissue volumes, flow rates, and ventilation:perfusion ratios specified in the model would be within reasonable physiological limits.
- The power function frequently assumed for scaling of physiological flows on the basis of body weight ranges between 0.67 and 0.75 unless species- or individual-specific data are available.
- Maximal velocities of metabolism may also be scaled on the basis of body weight, typically raised to the 0.75 power, but measured values for specific enzymes in humans do not generally correlate with body weight, so the choice of whether and how to scale metabolism is at the discretion of the modeler.

- Partition coefficients required for PBPK models can be obtained from steady-state in vivo or in vitro data or theoretical algorithms in the application domain.

- Biochemical parameters for PBPK models can be estimated using in vivo data or valid in vitro methods.

- A PBPK model is frequently implemented using commercially available software requiring that the model code (but not the coding of integration algorithms) be checked. If the modeler chooses to write his/her own program, then the appropriateness of the integration algorithm and integration interval should be justified. The PBPK model code is checked for accuracy of units, mass balance, blood flow balance, and behavior at zero dose.

- Evaluation of the PBPK model structure and parameters should be conducted to ensure that the model adequately predicts the pharmacokinetic behavior (i.e., bumps and valleys in the concentration vs. time plot) of the chemical and that the parameters (point estimates, range of values or distributions) consistently describe available data.

- A model used in a risk assessment would be accompanied by sufficient documentation to support an independent evaluation and reconstruction of the model and simulation results. Scripts facilitate transparency and reproducibility in modeling by providing computer code to recreate fits to kinetic data, estimation of dose metrics in critical studies, and calculation of human dose metrics for development of dose-response values (e.g., RfD, CSFs). A more rigorous verification that may be considered by the risk assessor is to independently re-code the model to ensure that the documentation is thorough and that there are no bugs in the code.

- Sensitivity, variability, and uncertainty analyses can help improve the credibility of PBPK models by identifying the parameters that have the greatest impact on a model output. In addition, these analyses are useful in prioritizing research needs to improve a model for risk assessment application. Such analyses, however, may not be required for all PBPK models intended for risk assessment applications.

4. APPLICATION OF PBPK MODELS IN RISK ASSESSMENT

4.1. CHOOSING PBPK MODELS APPROPRIATE FOR USE IN RISK ASSESSMENT

PBPK models are most often used in risk assessments to simulate tissue and blood concentrations of a toxic moiety (parent chemical or metabolite) resulting from the dosing regimens in the animal toxicity or human studies that are the basis for deriving dose-response values (e.g., RfC, RfD, CSFs). Specifically, the model would be able to simulate the dose metrics in the test species and/or in humans for the exposure route and exposure scenario of relevance. For most applications in risk assessment, a PBPK model

- Would have been developed or calibrated for the species and life stages of relevance to the risk assessment,
- Would be structured and adequately parameterized to simulate uptake via routes associated with human exposures as well as the critical study chosen for the assessment,
- Would be able to provide predictions of the time-course of concentration of the toxic moiety or appropriate surrogate (parent chemical or metabolite) in the target organ of interest or a suitable surrogate compartment, and
- Must have been peer-reviewed and evaluated for its quality and predictive capability.

Figure 4-1 depicts how the above criteria can be applied for selecting appropriate PBPK models. Basically, a peer-reviewed PBPK model for the relevant species and life stage consisting of parameters for simulating relevant routes of exposure and potentially relevant dose metrics is appropriate for use in risk assessment.

The first criterion, though appearing self-evident, is quite fundamental, because the models available in the literature sometimes were not parameterized for the specific species and life stage used in the critical toxicological study forming the basis of a risk assessment. For example, PBPK models for volatile organic compounds may have been developed in rats, yet one of the critical studies in the assessment is in mice. When the PBPK model has not been developed for the species or life stage used in a critical study, additional work may be needed to further elaborate the model.

PBPK models chosen for risk assessment applications would be able to provide simulations of the tissue dose of the toxic moiety or an appropriate dose metric for exposure scenarios and routes associated with the critical study as well as human exposures. Finally,

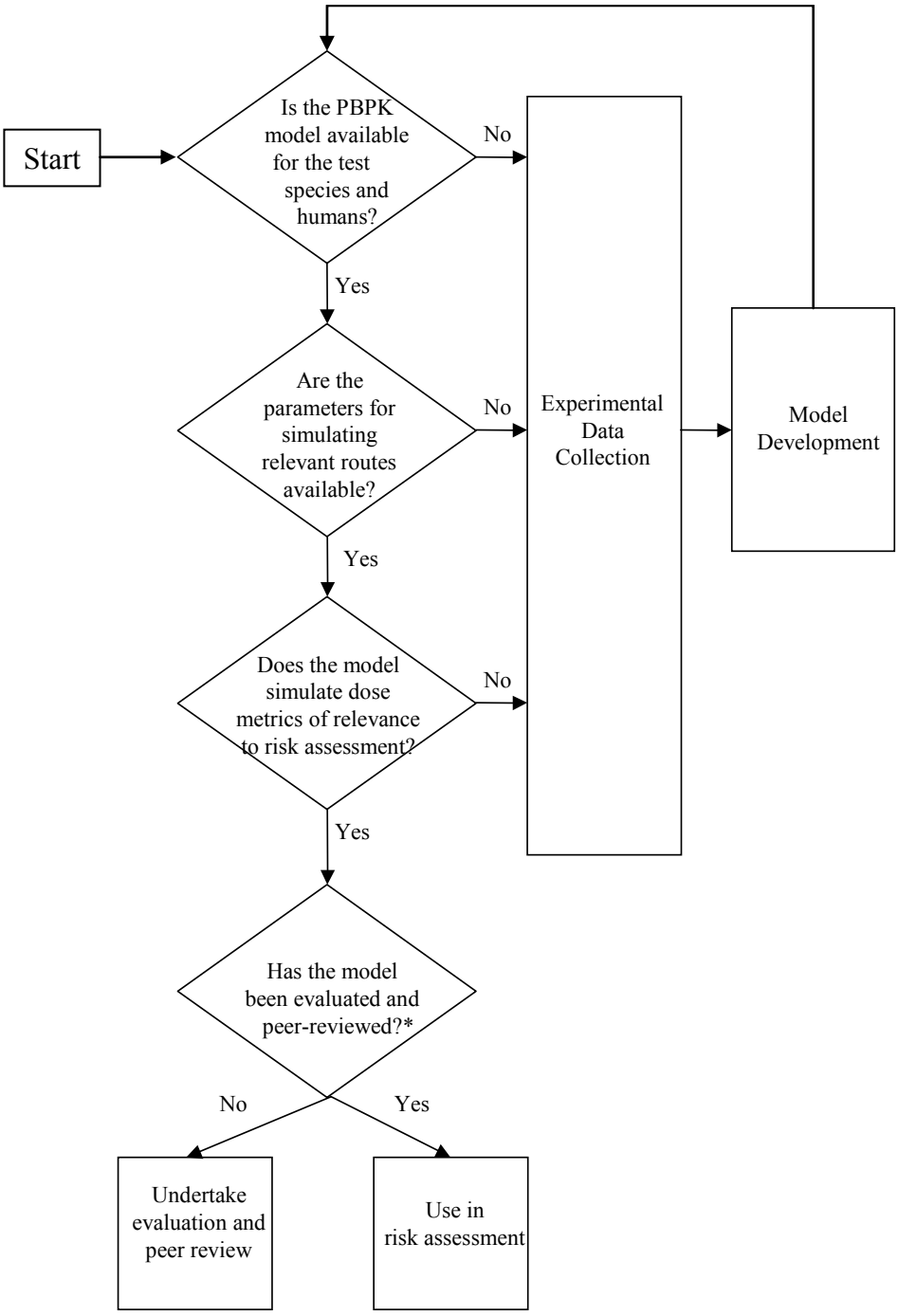


Figure 4-1. Flowchart for selecting PBPK models appropriate for use in risk assessment. * In this context, the model should be evaluated as described in Chapter 3.

the PBPK model for the relevant species and life stage and exposures corresponding to the critical study and risk assessment needs should be peer-reviewed; if it has not been, then efforts may be directed towards such a review (see Chapter 3).

Peer-reviewed PBPK models that facilitate the prediction of dose metrics for a chemical through relevant routes of exposure for the life stage and species used in critical studies are a prerequisite for their use in risk assessments. Most risk assessment applications also require a model parameterized for humans if the critical studies were in animals.

4.2. EVALUATION OF DOSE METRICS FOR PBPK MODEL-BASED ASSESSMENTS

When using PBPK models in risk assessment (RfD, RfC, and unit risk estimates), the basic data needed are

1. POD and critical effect from one or more key studies,
2. Peer-reviewed PBPK model for the relevant test species and humans, and
3. Dose metric appropriate for the risk assessment and supported by the MOA (if known).

The methods and challenges associated with the identification of critical effects and PODs for an assessment remain the same regardless of whether one uses PBPK models or not. The approaches for identifying PODs can be found elsewhere (U.S. EPA, 2005a, 1994). The criteria and issues associated with the selection of PBPK models useful for risk assessment were considered in the previous section. It is worth noting that although a human model is typically needed, route extrapolation and some other limited applications can be undertaken with only a model for the relevant test species. The third data need noted above, i.e., the identification of the appropriate dose metric, is a key aspect determining the use of PBPK models in risk assessment.

The dose metric, or the appropriate form of chemical most closely associated with the toxicity, varies from chemical to chemical, depending on the MOA and critical effect. It has two basic properties: the moiety and the measure thereof. The dose metric for PBPK-based risk assessment is chosen following the identification of the potential toxic moiety and evaluation of the relationship with the endpoint of concern. A useful framework for evaluating hypothesized MOAs is included in *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b). Although the framework specifically deals with carcinogens, the concepts are broadly applicable to noncancer MOAs. The framework provides useful discussion related to evaluating multiple MOAs (particularly over dose ranges) and for assessing relevance to humans. Furthermore, available data on closely related chemicals may be used to infer the likely toxic moiety.

Similarly, the toxicity data for various exposure routes and modes of administration may be compared to infer the potential toxic moiety (IPCS, 2005).

After the potential toxic moiety has been identified, the appropriate measure of tissue exposure to the toxic moiety can be selected (Figure 4-2). For example, peak concentration has been related to some neurotoxic effects of solvents (e.g., MacDonald et al., 2002; Benignus et al., 1998; Pierce et al., 1998; Bushnell, 1997), such as the correlation of concentration of trichloroethylene at the time of testing with observed effects on behavioural and visual functions (Boyes et al., 2000). For tetrachlorodibenzodioxin, tissue concentrations of the chemical measured during a critical period of gestation have been reported to predict the intensity of developmental responses (Hurst et al., 2000). The gender-specific genotoxic effects of benzene in mice are related to differences in the rate of oxidative metabolism (Kenyon et al., 1996).

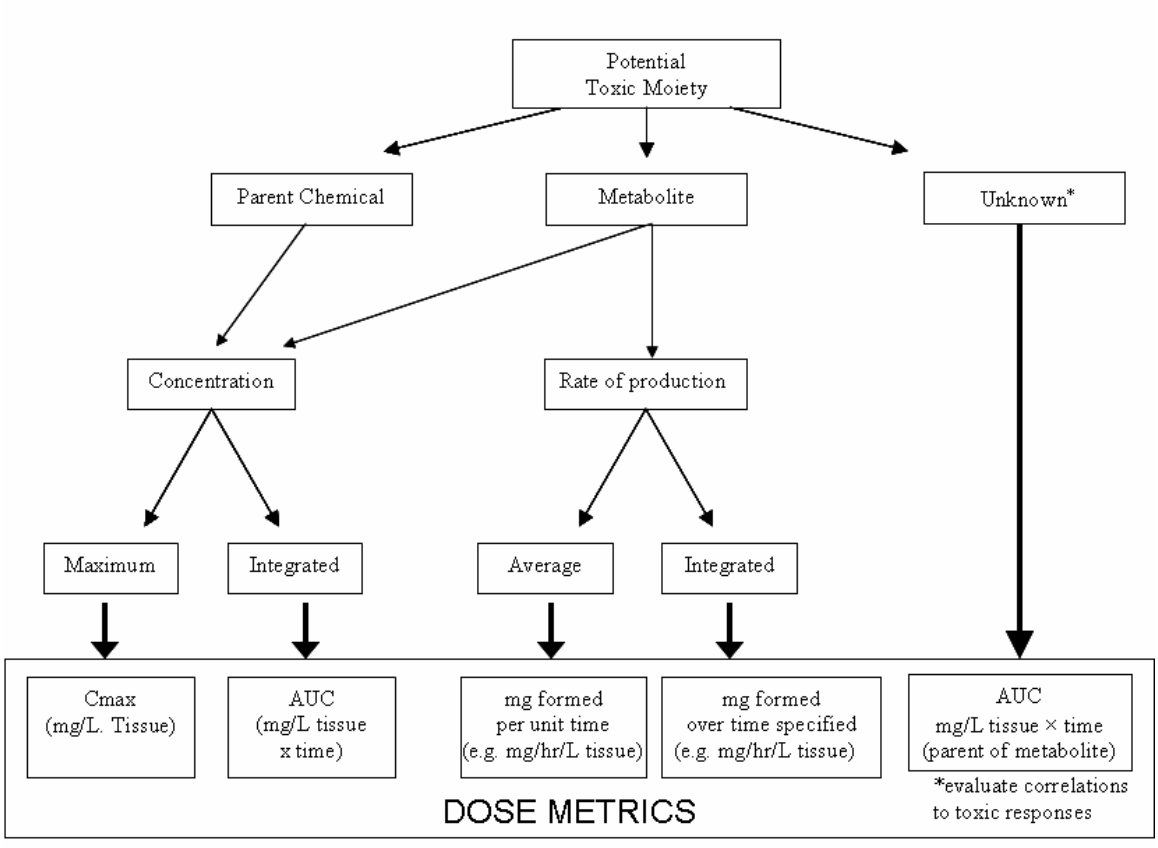


Figure 4-2. Examples of measure of tissue exposure to toxic moiety for risk assessment applications.

For chronic effects of chemicals, the integrated concentration of the toxic form of chemical in target tissue over time (i.e., AUC), typically determined as the daily average, is often considered a reasonable dose metric (Clewell et al., 2002a; Voisin et al., 1990; Andersen et al., 1987; Collins, 1987). For carcinogens producing reactive intermediates, the amount of metabolite produced per unit time and the amount of metabolite in target tissue over a period of time (e.g., mg metabolite/L tissue during 24 hr) have been used as dose metrics (Andersen and Dennison, 2001; Andersen et al., 1987). For developmental effects, the dose surrogate is defined in the context of the window of exposure for a particular gestational event (e.g., Welsch et al., 1995). Although the AUC and rate of metabolite formation figure among the most commonly investigated dose metrics, other surrogates of tissue exposure may also be appropriate for risk assessment purposes, depending on the chemical and its MOA (e.g., maximal concentration [Cmax] of the toxic moiety, duration and extent of receptor occupancy, macromolecular adduct formation, or depletion of glutathione) (Clewell et al., 2002a). Table 4-1 lists the dose metrics used in a number of PBPK-based cancer and noncancer risk assessments described in the peer-reviewed literature.

When the appropriate dose metric cannot readily be identified, evaluation of the relationship with the endpoint of concern can be undertaken with each of the dose metrics to identify the one that exhibits the best association (e.g., Andersen et al., 1987; Kirman et al., 2000). This becomes particularly important when there are limited or confusing data on the plausible MOA of the chemical. At a minimum, the appropriate dose metric can be identified as the one that demonstrates a consistent relationship with positive and negative responses observed at various dose levels, routes, and scenarios in a given species. In other words, the level of the dose metric would be lower for exposure conditions that elicit no effect and higher for conditions that elicit toxic responses, regardless of the route and exposure scenario (Clewell et al., 2002a).

Where there is an inadequate basis for prioritizing one dose metric over another, some suggest using the most conservative one (the dose metric estimating the highest risk or lowest acceptable exposure level) to be health protective (Clewell et al., 2002a). The use of appropriate dose metric can help to reconcile route and species differences in cancer responses, provided there are no pharmacodynamic differences. There has been at least one instance in which PBPK model-derived dose measures could not reconcile rat and mouse kidney tumor data (Smith et al., 1995), indicating the significant role of factors other than the target tissue exposure to toxic moiety.

Table 4-1. Dose metrics used in PBPK model-based cancer and noncancer risk assessments

Chemical	Endpoint	Dose metric	Reference
Acrylonitrile	Brain tumors	Peak metabolite concentration in target tissue	Kirman et al. (2000)
Bromotrifluoromethane	Cardiac sensitization	Concentration of parent chemical at the end of exposure	Vinegar and Jepson (1996)
Butoxyethanol (2-)	Forestomach lesions and tumors	Concentration of butoxyethanol/ butoxy acetic acid in forestomach	Poet et al. (2003)
Chloroform	Liver cancer	Amount of metabolites covalently bound to biological macromolecules L liver per day; % cell kill/day	Reitz et al. (1990a)
	Hepatic effects and kidney tumor	Maximal rate of metabolism per unit kidney cortex volume	Meek et al. (2002)
Chloropentafluorobenzene	Liver toxicity	AUC of parent chemical in liver	Clewell and Jarnot (1994)
1,4-Dioxane	Liver tumors	Time-weighted average concentration in liver over lifetime	Leung and Paustenbach (1990)
		Liver AUC	Reitz et al. (1990b)
Ethyl acrylate	Forestomach tumors	Tissue-specific glutathione depletion	Frederick et al. (1992)
Ethylene glycol ethers	Developmental toxicity	Peak concentration and average daily AUC of the alkoxyacetic acid (metabolite) in blood	Sweeney et al. (2001)
Formaldehyde	Cancer	DNA-protein crosslinks	Schlosser et al. (2003); Casanova et al. (1996)
Heptafluoropropane	Cardiac sensitization	Concentration of parent chemical at the end of exposure	Vinegar and Jepson (1996)
Isopropanol	Neurobehavioral effects	Peak blood concentration	Gentry et al. (2002)
	Developmental/ reproductive effects	AUC of isopropanol and its metabolite (acetone)	Gentry et al. (2002)
Methoxyacetic acid	Developmental effects	AUC of parent chemical (gestational day 11)	Clarke et al. (1993, 1992)
		Maximal concentration of parent chemical (gestational day 8)	Welsch et al. (1995)
Methyl chloroform	Hepatic effects	Area under the liver concentration vs. time curve	Reitz et al. (1988a)
Methyl mercury	Neurological effects	Fetal brain concentrations	Gearhart et al. (1995)

Table 4-1. Dose metrics used in PBPK model-based cancer and noncancer risk assessments (continued)

Chemical	Endpoint	Dose metric	Reference
Methyl methacrylate	Nasal lesions	Amount metabolized/time/volume nasal tissue	Andersen et al. (2002, 1999)
Methylene chloride	Cancer	Rate of glutathione transferase metabolites produced/L liver/time	Andersen et al. (1987)
Styrene	Lung tumors (mouse)	Steady-state concentration of ring oxidation metabolite mediated by CYP2F	Cruzen et al. (2002)
Tetrachlorodibenzodioxin	Biochemical responses	Body burden	Kim et al. (2002)
	Cancer risk	Time-weighted receptor occupancy Up/down regulation of receptor occupancy Fraction of cells induced	Andersen et al. (1993) Portier et al. (1993) Conolly and Andersen (1997)
Toluene	Behavioral effects	Brain concentrations at the time of testing	Van Asperen et al. (2003)
Trichloroethylene	Renal toxicity	Metabolite production/L kidney/day	Barton and Clewell (2000)
	Neurotoxicity	Blood concentration of metabolite (trichloroethanol)	Barton and Clewell (2000)
	Cancer (liver lung and kidney)	Amount metabolized/kg/day; AUC for trichloroacetic acid or dichloroacetic acid/L plasma; production of thioacetylating intermediate from dichlorovinylcysteine in kidney	Clewell et al. (2000); Fisher and Allen (1993)
Vinyl acetate	Olfactory degeneration and tumor development	Intracellular pH change associated with the production of acetic acid; proton concentration in olfactory tissue	Bogdanffy et al. (2001, 1999)
Vinyl chloride	Angiosarcoma	mg metabolized/L liver; mg metabolite produced/L liver/day	Clewell et al. (2001); Reitz et al. (1996b)

AUC = area under the curve

An important consideration in risk assessments conducted with a PBPK model is that the critical study (i.e., the study upon which the RfC, RfD, or CSF is based) cannot always be

selected on the basis of administered dose or exposure concentration. This is because the relationship of the HEC or human equivalent dose to the administered animal dose depends on the selected dose metric, which may vary from one endpoint to another and with the nature of the exposure (species, route of administration, vehicle, duration, etc.). Instead, the pharmacokinetic model is used to calculate the appropriate dose metrics for each of the endpoints of concern in each study (Barton and Clewell, 2000). To calculate the dose metrics, the model parameters are set to those for the species in the toxicity study, whether an experimental animal study or a human study. In the case of developmental studies, it may be necessary to estimate parameters for a pregnant female or neonate rather than for an average adult, and physiological and biochemical parameters may have to be time dependent. To the extent possible, it is best to use study-specific data on animal strain, body weights, age, and activity when selecting parameters for the model. The experimental parameters in the model are then set to reproduce the exposure scenario performed in the study, and the model is run for a sufficient period of time to characterize the animal exposure to the chemical and, if necessary, its metabolites.

There are often a number of options regarding the way in which the model can be run to characterize the dose metric (Clewell et al., 2002a). The choices made will depend on the dose metric(s) selected (e.g., peak vs. average), the nature of the chemical (e.g., volatile vs. persistent), and the nature of the risk assessment (acute vs. chronic, cancer vs. noncancer). Frequently, an average daily dose metric such as the average daily AUC is estimated (note that the average daily AUC is the same metric as the time-weighted average concentration, differing by only a factor of 24 if the daily AUC was expressed in terms of hours). In general, the averaging period in the case of cancer is typically taken to be the lifetime, whereas the averaging period in the case of noncancer risk assessment is usually considered to be the duration of the exposure or, perhaps, a critical window of exposure.

For short-term exposures, the model must be run for an appropriate period, which depends on the dose metric being used and the timing of the measurement of toxicity in relation to the period of exposure. For short exposure, this is easily done by running the model for the total duration of the exposure (or exposures, for repeated exposure studies) to obtain dose metrics. If the animals were held for a postexposure period before toxicity was evaluated, the model must be run either until the end of the postexposure period or for a sufficient duration to ensure that the parent chemical or metabolite, depending on dose metric, has been completely cleared from the body. On the other hand, if toxicological evaluations, e.g., neurological tests,

were performed during or immediately at the end of the exposure period, then the dose metric might be determined at the time of evaluation. The resulting dose metric obtained for the total duration of the exposure (including any postexposure period) may need to be divided by the number of days over which the experiment was conducted to derive the average daily value for an integrated measure such as AUC.

The same approach (running the model for the total duration of the study) can be used to calculate dose metrics for longer-term exposures. This approach would typically be necessary for models that describe changes in physiology or biochemistry during different life stages (e.g., children, elderly). An alternative approach, which is often attractive for modeling of chronic exposures with time-invariant model parameters, is to estimate the steady-state dose metric.

There are two principal methods for calculating a steady-state estimate. In the first, the model is run until steady state is reached and then the dose metric is calculated by subtraction. For example, in the case of a chronic oral or inhalation exposure conducted 5 days per week, the model can be run consecutively for 1 week, 2 weeks, 3 weeks, and so on. To calculate the average daily AUC for a given week, the value at the end of the previous week is subtracted from the value at the end of the current week and the result is divided by 7. This process is repeated until the change in the dose metric from one week to the next is insignificant. For continuous exposures, the comparison can be made on a daily basis rather than weekly.

The other method for estimating the steady-state dose metric is to estimate it from a single-day exposure. The model is run for a single-day exposure plus an adequate postexposure period to capture clearance of the parent compound or relevant metabolite. This value of the single-day dose metric is then modified by the necessary factors to obtain an average daily value (e.g., by multiplying by five-sevenths in the case of the 5-day-per-week exposure just described). This method is faster, but is only approximate if the system is not linear. Typically, it is sufficiently accurate for estimating average daily AUC when exposures are below the onset of any nonlinearities. It can be checked against the first method described to determine its accuracy in a particular case.

The dose metric calculations needed are determined by the method to be used for the noncancer or cancer analysis. If the NOAEL/uncertainty factor (UF) method is being used in an assessment, a dose metric needs to be calculated only for the NOAEL or LOAEL exposure for a particular study and endpoint. On the other hand, if dose-response modeling is going to be performed, such as in the BMD approach, dose metrics generally would be calculated for all

exposure groups. The dose metrics are then used in the dose-response model in place of the usual exposure concentrations or administered doses. It is important to remember that when this is done, the result of the dose-response modeling will also be in terms of a value of the dose metric rather than an exposure concentration or administered dose. Dose-response modeling is more properly conducted on the dose metrics because it is expected that the observed effects of a chemical will be more simply and directly related to a measure of target tissue exposure than to a measure of administered dose.

To convert an animal dose metric (e.g., at the BMD) to an equivalent exposure concentration or administered dose, the pharmacokinetic model must be run repeatedly, varying the exposure concentration or administered dose, until the dose metric value is obtained. In the case of calculating the acceptable human exposure corresponding to a given toxicity study, the physiological, biochemical, and exposure parameters in the model are set to appropriate human values and the model is iterated until the dose metric obtained for the human exposure of concern (often continuous or daily lifetime exposure) is equal to the dose metric obtained for the toxicity study. One effective way to do this is to run the model at regular dose intervals (e.g., log or half-log) over a wide dose range. These results can be used to generate a regression line describing the relationship between the internal dose metric and the exposure dose or concentration. This regression line can be used to accurately estimate the exposure giving a particular internal dose metric, as can be confirmed by running the model for that exposure. Plotting the relationship between exposure and the internal dose metric is also valuable because it demonstrates where nonlinearities occur.

The human dose metrics used for deriving dose-response values can be calculated in an analogous way to the dose metric for the toxicity study; i.e., if the dose metric in the toxicity study was expressed in terms of an average daily value, the dose metric used for calculating the associated human exposure should also represent an average daily value. However, it should be remembered that the exposure scenarios may be different, e.g., continuous human inhalation in contrast to a 6-hr/day exposure in the animal toxicity study. When a steady-state dose metric is used in both an experimental animal and the human, the calculation of a steady-state dose metric in the human generally requires running the model for a much longer period of time than in the animal. For short-term exposures, where the model has been run for the total duration of the toxicity study and the average dose metric value has been calculated, the dose metric used to calculate associated human exposure is usually obtained for an exposure over the same time

period. An exception to this rule is the case where it is anticipated that the short-term exposure of concern for the human may represent a short-term excursion against a background of chronic exposure. In this case, a more conservative approach may be preferred, in which a steady-state dose metric calculation is used for the human.

The following section describes applications of PBPK models in risk assessment. These applications relate to high-dose to low-dose extrapolation, interspecies extrapolation, estimating intraspecies variability, route-to-route extrapolation, and duration extrapolation as required for RfD derivation, RfC derivation, and cancer risk assessment.

4.3. REVIEW OF EXTRAPOLATIONS POSSIBLE WITH PBPK MODELS

Risk assessment applications typically require that extrapolations be made from the critical studies (i.e., animal toxicology or human epidemiology studies) to the human exposure situation. These extrapolations of the critical studies are the focus of pharmacokinetic modeling. To a significant extent, the models utilize in vitro or in vivo data to enable the model to address these extrapolations (e.g., characterize pharmacokinetics at high and low doses, parameterize models for test species and humans). To a more limited degree, extrapolations are made in the course of model development, most typically due to limitations on available data for humans, such that it is assumed parameters scale in some manner from animals to humans. As pharmacokinetic modeling strives to become increasingly predictive in nature, it is likely that predictive tools (e.g., methods to predict partition coefficients) will play larger roles in model development.

4.3.1. Interspecies Extrapolation

The application of PBPK models for interspecies extrapolation of tissue dosimetry is performed in several steps. First, a model for the appropriate species in potential critical toxicity studies is developed. Increasingly, a priori predictions of the PBPK model are compared with experimental observations to evaluate the adequacy of the structure and the parameter estimates of the rodent model. This sometimes involves refining some or all of the parameters by allowing modeling software to estimate the best value for these parameters. The next step involves using species-specific or allometrically scaled physiological parameters in the model and replacement of the chemical-specific parameters (e.g., metabolic rates, protein binding constants) with appropriate estimates for the species of interest (e.g., humans). Thus, in this approach, the

qualitative determinants of pharmacokinetics are considered to be invariant among the various mammalian species. Qualitative differences between species, if any, can also be factored into the existing structure of PBPK models (e.g., if different metabolic pathways existed among species) but, obviously, data describing these species differences are required.

For conducting interspecies extrapolation of pharmacokinetic behavior of a chemical, quantitative estimates of model parameter values (i.e., partition coefficients, physiological parameters, and metabolic rate constants) in the second species are required. The tissue:air partition coefficients of chemicals appear to be relatively constant across species, whereas blood:air partition coefficients show some species-dependent variability. Therefore, the tissue:blood partition coefficients for human PBPK models have been calculated by dividing the rodent tissue:air partition coefficients by the human blood:air partition values (Krishnan and Andersen, 2001). The tissue:air and blood:air partition coefficients for volatile organic chemicals may also be predicted using appropriate data on the content of lipids and water in human tissues and blood (Poulin and Krishnan, 1996a, b).

Whereas the adult physiological parameters vary coherently across species, the kinetic constants for metabolizing enzymes do not necessarily follow any type of readily predictable pattern, making the interspecies extrapolation of xenobiotic metabolism difficult. Therefore, the metabolic rate constants for xenobiotics are best obtained in the species of interest. In vivo approaches for determining metabolic rate constants are not always feasible for application in humans. The alternative is to obtain such data under in vitro conditions (e.g., Lipscomb et al., 1998, 2003). A parallelogram approach may also be used to predict values for the human PBPK model on the basis of metabolic rate constants obtained in vivo in rodents as well as in vitro using rodent and human tissue fractions (Lipscomb et al., 1998; Reitz et al., 1988b). Alternatively, for chemicals exhibiting high affinity (low K_m) for metabolizing enzymes, V_{max} has been scaled to the 0.75 power of body weight, keeping the K_m species invariant. This approach may be useful as a crude approximation, but it may be used only when other direct measurements of metabolic parameters are not available or feasible.

An example of rat-human extrapolation of the kinetics of toluene using a PBPK model is presented in Figure 2-2 (Chapter 2). Here the structure of the PBPK model developed in rats was kept unchanged, but the species-specific parameters were determined either by scaling or experimentally, as described above (Tardif et al., 1997). The model was then able to predict accurately the kinetics of toluene in humans. Whenever the human data for a particular chemical

are not available for evaluation purposes, a corollary approach permitting the use of human data on similar chemicals may be attempted (Jarabek et al., 1994).

There are some instances where PBPK models can be used for interspecies extrapolation of toxicity studies without the need of an animal PBPK model. For example, an RfC for methanol has been proposed (Starr and Festa, 2003) using a mice developmental toxicity study (Rogers et al., 1993) where blood methanol levels were also reported. By using the blood methanol level at the POD from the mice study, a previously published human methanol PBPK model (Bouchard et al., 2001) was used to predict the inhalation concentration associated with the same internal blood methanol level in humans. This example highlights the advantage afforded by toxicity studies that also include pharmacokinetic measurements.

4.3.2. Estimating Intraspecies Variability

Intraspecies variability for the dose metric can be assessed using PBPK models to estimate the magnitude of interindividual variability (pharmacokinetic component) for RfC and RfD derivations. For this purpose, population distributions of parameters, particularly those relating to physiology and metabolizing enzymes (i.e. genetic polymorphisms), are specified in a Monte Carlo approach, such that the PBPK model output corresponds to distributions of dose metric in a population. Using the Monte Carlo approach, repeated computations based on inputs selected at random from statistical distributions for each input parameter (e.g., physiological parameters, enzyme content/activity with or without the consideration of polymorphism) are conducted to provide a statistical distribution of the output, i.e., tissue dose. Using the information on the dose metric corresponding to a high percentile (e.g., 95th) and the 50th percentile, the magnitude of interindividual variability can be computed for risk assessment purposes (Figure 4-3).

Even though past efforts largely have characterized the impact of the distributions of parameters in the adult population, variability analyses also need to address different life stages (e.g., pregnancy, children, aged). Generally, age-specific changes in physiology, tissue composition, and metabolic activity (reviewed in O'Flaherty, 1994) can be incorporated into the same model structure used for adults (Corley et al., 2003). Published examples of modeling different ages describe predictions for a range of chemicals with different properties (Clewell et al., 2004, 2002b; Ginsberg et al., 2004; Sarangapani et al., 2003). However, some life stages, notably pregnancy and lactation, require different model structures (i.e., describing the mother

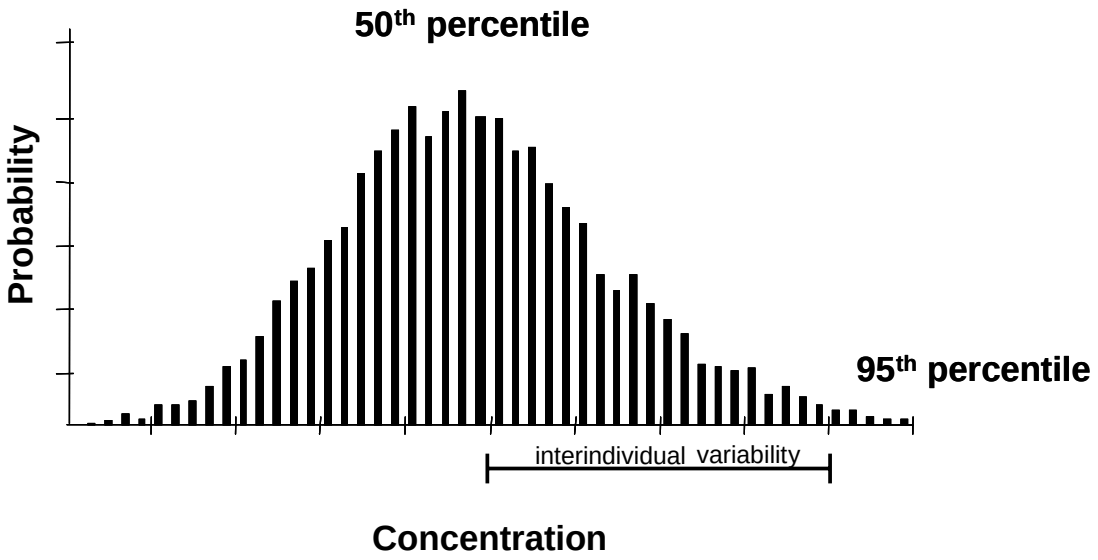


Figure 4-3. Estimation of an interindividual variability. In this example, interindividual variability describes the variation between the 50th (median) and 95th percentile values of a dose metric simulated with a probabilistic PBPK model.

and the offspring) (Corley et al., 2003; Gentry et al., 2003, 2002). Characterization of population variability across ages and life stages as well as adult variability is an ongoing area of development. PBPK models represent a powerful tool for quantitatively characterizing population pharmacokinetic variability for application to risk assessment. For more information, the reader is referred to *Use of PBPK Models to Quantify the Impact of Human Age and Interindividual Differences in Physiology and Biochemistry Pertinent to Risk* (U.S. EPA, 2006).

4.3.3. Route-to-Route Extrapolation

There are two different approaches to route extrapolation involving PBPK models. The first one is to use an animal model to extrapolate a POD for one route to a POD by another route on the basis of equivalent dose metric. The second approach would involve the estimation of the human POD for one route from the available animal POD for another route on the basis of equivalent dose metric.

The extrapolation of the kinetic behavior of a chemical from one exposure route to another is performed by including appropriate equations to represent each exposure pathway.

For simulating the intravenous administration of a chemical, a single input representing the dose administered to the animal can be included in the equation for mixed venous concentration. Oral gavage of a chemical dissolved in a carrier solvent may be modeled by introducing a first-order or a zero-order uptake rate constant, and dermal absorption has been modeled by including a diffusion-limited compartment to represent skin as a portal of entry (Krishnan and Andersen, 2001). After the equations describing the route-specific entry of chemicals into systemic circulation are included in the model, it is possible to conduct extrapolations of pharmacokinetics and dose metrics. This approach is illustrated in Figure 4-4 for oral-to-inhalation extrapolation of the kinetics of chloroform in rats. For simulating the inhalation pharmacokinetics, the oral dose was set to zero, whereas for simulating chloroform kinetics following oral dosing the inhaled concentration was set to zero (Figure 4-4). Accordingly, 4-hr inhalation exposure to 83.4 ppm chloroform is equal to an oral dose of 1 mg/kg, as determined with PBPK models on the basis of equivalent dose metric (i.e., parent chemical AUC in blood) (Figure 4-4). Note that the peak concentrations differ by about 10-fold; thus, if peak concentration was thought to be the appropriate dose metric, higher inhalation exposures would be required to produce the same peak as a 1-mg/kg oral dose.

4.3.4. Duration Adjustment

On the basis of equivalent dose metric, the duration-adjusted exposure values can be obtained with PBPK models (Simmons et al., 2005; Bruckner, 2004; Brodeur et al., 1990; Andersen et al., 1987). For example, if the appropriate dose metric were the AUC of a chemical, it would initially be determined for the exposure duration of the critical study using the PBPK model and then the atmospheric concentration for a continuous exposure (during a day, window of exposure, or lifetime) yielding the same AUC is determined by iterative simulation. Figure 4-5 depicts an example of 4-hr to 24-hr extrapolation of the pharmacokinetics of toluene in rats, based on equivalent 24-hr AUC (2.4 mg/L/hr). The rats exposed to 50 ppm for 4 hr and 9.7 ppm for 24 hr of toluene would receive the same dose metric. Again, it should be noted that extrapolations across long durations may not be warranted, as life stage changes and pharmacodynamic adaptations (e.g., sensitization and desensitization) may be operational (Clewell et al., 2002a).

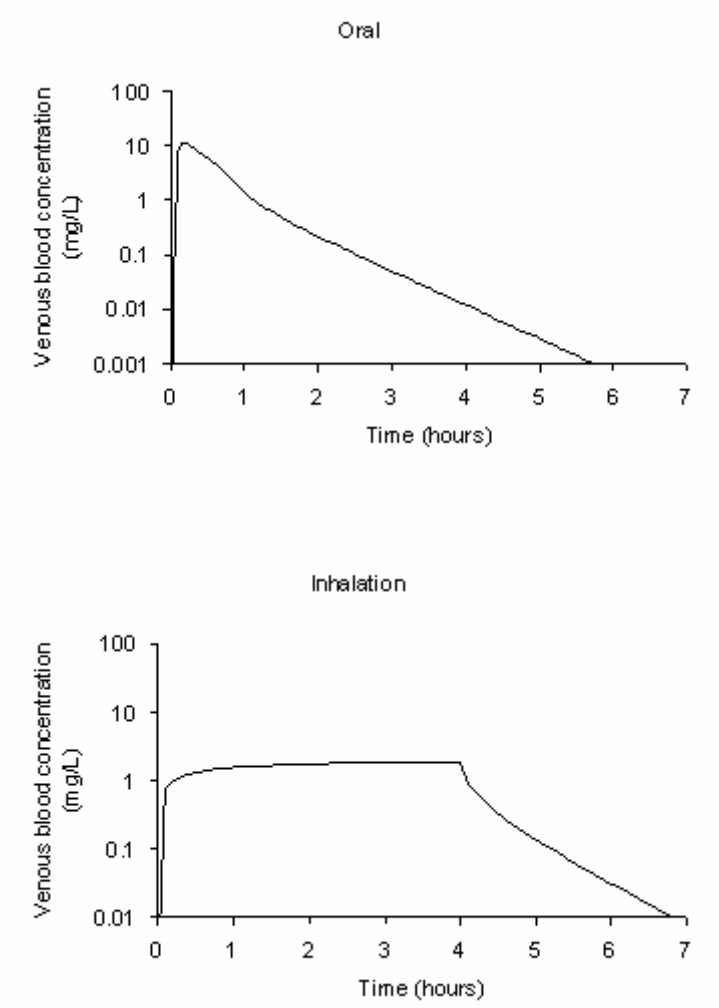


Figure 4-4. Oral-to-inhalation extrapolation of the pharmacokinetics of chloroform on the basis of same area under the curve in blood (7.06 mg/L/hr). The oral dose was 1 mg/kg and the inhaled concentration was 83.4 ppm (4 hr).

Source: Adapted from Corley et al. (1990).

4.3.5. High-Dose to Low-Dose Extrapolation

PBPK models facilitate high-dose to low-dose extrapolation of tissue dosimetry by accounting for the dose-dependency of relevant processes (e.g., saturable metabolism, enzyme induction, enzyme inactivation, protein binding, and depletion of glutathione reserves) (Clewell and Andersen, 1987). The description of metabolism in PBPK models has frequently included a capacity-limited metabolic process that becomes saturated at high doses. Nonlinearity arising from mechanisms other than saturable metabolism, such as enzyme induction, enzyme

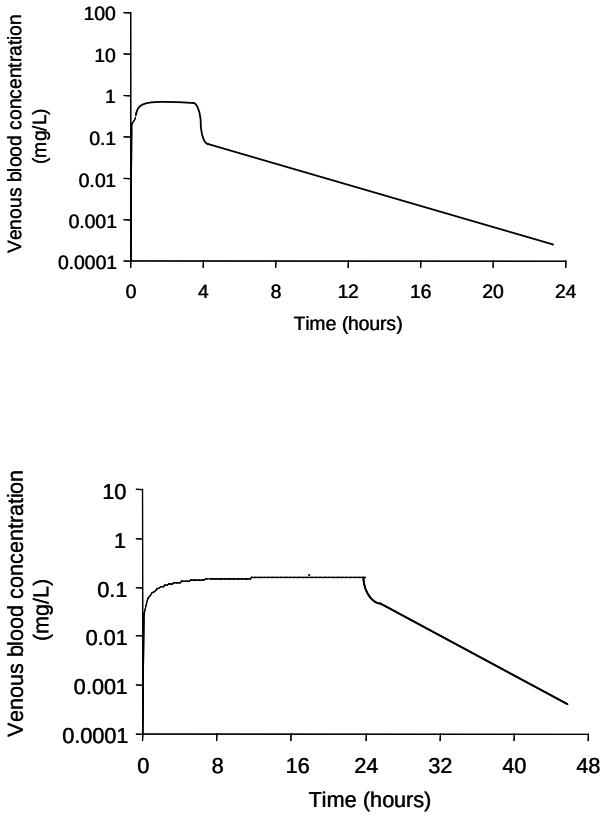


Figure 4-5. Duration adjustment (4 hr to 24 hr) of toluene exposures in rats, based on equivalent AUC (2.4 mg/L/hr). The rats were exposed to 50 ppm toluene for 4 hr and 9.7 ppm for 24 hr.

Source: Adapted from Tardif et al. (1997).

inactivation, depletion of glutathione reserves, and binding to macromolecules, have also been described with PBPK models (e.g., Krishnan et al., 1992; Clewell and Andersen, 1987). A PBPK model intended for use in high-dose to low-dose extrapolation needs equations and parameters describing dose-dependent phenomena if they occur in the range of interest for the assessment. Because the determinants of nonlinear behavior may not be identical across species and age groups (e.g., maximal velocity for metabolism, glutathione concentrations), these parameters are required for each species/age group. During the conduct of high-dose to low-

dose extrapolation, no change in the parameters of the PBPK model is required except for the administered dose or exposure concentration.

An example of high-dose to low-dose extrapolation is presented in Figure 4-6. In this figure, both the blood AUC and the amount metabolized over a period of time (12 hr) are plotted as a function of exposure concentrations of toluene. For conducting high-dose to low-dose simulation in this particular example, only the numerical value of the exposure concentration (which is an input parameter for the PBPK model) was changed during every model run. All other model parameters remained the same. The model simulations shown in Figure 4-6 indicate the nonlinear nature of blood AUC as well as the amount of toluene metabolized per unit time in the exposure concentration range simulated. In such cases, the high-dose to low-dose extrapolation of tissue dosimetry should not be conducted assuming linearity but, rather, should be performed using tools such as the PBPK models.

4.4. ROLE OF PBPK MODELS IN REFERENCE CONCENTRATION AND REFERENCE DOSE DERIVATION

4.4.1. Reference Concentration

The RfC corresponds to an estimate (with uncertainty spanning perhaps an order of magnitude) of continuous inhalation exposure (mg/m^3) for a human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects during a lifetime (U.S. EPA, 1994). Notationally, RfC is defined as:

$$RfC = POD_{[HEC]}/UF$$

where:

$POD_{[HEC]}$ = POD (NOAEL, LOAEL, or BMC) dosimetrically adjusted to an HEC

UF = uncertainty factors to account for the extrapolations associated with the POD (i.e., interspecies differences in sensitivity, human intraspecies variability, subchronic-to-chronic extrapolation, LOAEL-to-NOAEL extrapolation, and incompleteness of database)

The starting point for an RfC derivation is the identification of the POD for the critical effect in a key study. Subsequent steps involve (a) adjustment for the difference in duration between experimental exposure (e.g., 6 hr) and expected human exposure (24 hr), (b) calculation

of the HEC, and (c) application of uncertainty factors (UFs). The benefit of using PBPK models in the RfC process is discussed below. Specifically, the role of PBPK models in determining the POD, duration adjustment factor, and HEC is presented in Sections 4.4.2 through 4.4.6. It

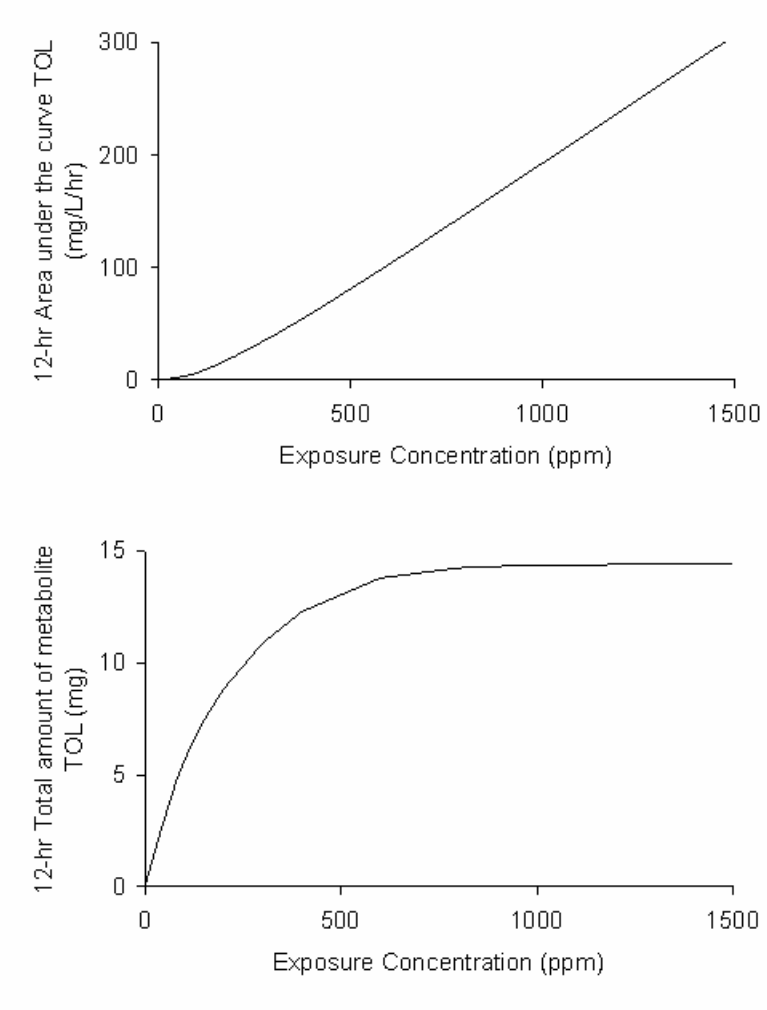


Figure 4-6. High-dose to low-dose extrapolation of dose metrics using PBPK model for toluene. Inhalation exposures were for 4 hr, and areas under the curve and amount metabolized were calculated for 12 hr. Note that there is a slight curve in the top graph around 125 ppm.

Source: Adapted from Tardif et al. (1997).

should be noted that although the various extrapolations were presented in a hierarchical fashion earlier in this document (e.g., interspecies, intraspecies, route, duration, and high-dose to low-

dose), the order of extrapolations is changed in this section to more closely parallel the RfC and RfD derivation processes.

4.4.2. Reference Concentration: Point of Departure

It is important to realize that currently the POD for RfC derivation cannot be identified or established with only pharmacokinetic data or PBPK models in the absence of dose-response data. Integrated pharmacokinetic-pharmacodynamic models (e.g., Timchalk et al., 2002; Gearhart et al., 1994, 1990) may be capable of predicting response and thus estimating a POD in the future, but this is a research effort that is not yet ready for risk assessment applications. At present, PBPK modeling can be useful for conducting route-to-route extrapolation, duration adjustments, inter- and intraspecies extrapolations on the basis of equivalent delivered dose from PODs identified in toxicity, epidemiology, or clinical studies.

4.4.3. Reference Concentration: Route-to-Route Extrapolation

Typically, the POD used in the RfC process would be the inhalation route-specific NOAEL, LOAEL, or BMC. These PODs essentially correspond to exposure concentrations in an experimental or field study (NOAEL, LOAEL) or to the lower confidence limit (95th percentile) of the exposure concentration (BMCL) associated with a specified response level (generally in the range of 1 to 10% above background; e.g., $BMCL_{10\%}$) derived from statistical analysis of experimental dose-response data (U.S. EPA, 2000a, 1994).

When information on the POD is available only for a noninhalation route of exposure (e.g., oral route), route-to-route extrapolation can be conducted (Pauluhn, 2003). Historically, the NOAEL (mg/kg/day) associated with an oral exposure route was converted to milligrams per day and then to the equivalent inhaled concentration on the basis of human breathing rate and body weight. Data on the route-specific fraction absorbed, when available, are used to determine the equivalent inhalation concentration on the basis of equivalent absorbed doses (U.S. EPA, 1999a). Such simplistic approaches, however, assume that the rates of ADME and tissue dosimetry of chemicals are the same for a given total dose, regardless of the exposure route and intake rate. These approaches essentially neglect the route-specific differences in pharmacokinetics, such as first-pass clearance. First-pass clearance can arise when chemicals undergo extensive metabolism in tissues at portals of entry; this may include the intestines and liver for orally absorbed compounds or the lungs for inhaled compounds (Benet et al., 1996).

Therefore, route-to-route extrapolation using a more complete pharmacokinetic modeling approach, such as PBPK modeling, is preferable, as described in Section 4.3.3.

4.4.4. Reference Concentration: Duration Adjustment

An RfC addresses continuous exposure of human populations, so the POD used in its derivation should correspond to 24-hr/day exposures (U.S. EPA, 1994). PODs are frequently obtained from animal exposures or occupational exposures that occur for 6 to 8 hr/day, 5 days/wk, so an adjustment to a continuous 24-hr exposure, resulting in a lower concentration for continuous exposures, is conducted on the basis of hours per day and days per week (i.e., $6/24 \times 5/7$) (U.S. EPA, 2002). This simple adjustment assumes that "Haber's Rule" applies, i.e., that for a given chemical $C \times t = k$, where C and t are the concentration (mass per unit volume) and time needed (at that concentration) to produce some toxic effect, and k is a constant associated with that toxic effect. The rule leads to the conclusion, for example, that doubling the concentration will halve the time needed to produce a comparable effect level. In pharmacokinetics, the integration of $C \times t$ over the exposure-response time frame of interest is also referred to as the AUC. If the AUC is not the dose metric most associated with the adverse effect (e.g., sometimes peak concentration is more critical) or various $C \times t = k$ regimens do not result in a comparable effect level, then "Haber's Rule" is not applicable (U.S. EPA, 2002). When data indicate that a given toxicity is more dependent on concentration than on duration (time), this adjustment would not be used. If the appropriate measure of internal dose is uncertain, the Agency uses adjustment to a continuous inhalation exposure based on the $C \times t$ relationship as a matter of health-protective policy (U.S. EPA, 2002). For additional insights into "Haber's Rule" (as one in a family of power functions) and its use in risk assessment, the reader is referred to Miller et al. (2000).

PBPK models can be used to estimate the value of a proposed internal dose metric that would result from various administered doses (U.S. EPA, 2002; Jarabek, 1994). PBPK models do not address pharmacodynamic events and assume that these events do not alter the kinetics for within-day exposures (<24 hr). Consistent with the Agency's policy (U.S. EPA, 2002), the dose metric of a chemical for the exposure scenario of the critical study is initially determined using the PBPK model (e.g., 6 hr/day, 5 days/wk); then the atmospheric concentration for a

continuous exposure (24 hr/day) during a lifetime or a particular window of exposure that yields the same dose metric is determined by iterative simulation.

4.4.5. Reference Concentration: Dosimetric Adjustment Factor (Interspecies Extrapolation)

In the RfC process, a DAF is applied to the duration-adjusted POD to account for pharmacokinetic differences between test species and humans to derive an HEC (U.S. EPA, 1994). The DAF depends on the nature of the inhaled toxicant and the MOA as well as the endpoint (local effects vs. systemic effects). Dosimetry data, if available, in the test animals and humans (including deposition data, region-specific dosimetry, blood concentration of systemic toxicants) are used to estimate the DAF. In the absence of such data, knowledge of critical parameters or mathematical models in the test species and humans can be useful in estimating the DAF.

For highly reactive or water-soluble gases that do not significantly accumulate in blood (e.g., hydrogen fluoride, chlorine, formaldehyde, volatile organic esters), the DAF is derived for estimates of the delivery of chemical to different regions of the respiratory tract, based on regional mass transfer coefficients and differences in surface area and ventilation rates (U.S. EPA, 1994). For poorly water-soluble gases that cause remote effects (e.g., xylene, toluene, styrene), PBPK models are identified as the preferred approach. Absent a PBPK model, the DAF is determined on the basis of the ratio of blood:air partition coefficients in animals and humans (U.S. EPA, 1994). For gases that are water soluble with some blood accumulation (e.g., acetone, ethyl acetate, ozone, sulfur dioxide, propanol, isoamyl alcohol) and have the potential for both respiratory and remote effects, some combination of the above approaches may be used.

An alternative to the use of DAFs, discussed in the RfC guidance (U.S. EPA, 1994) is to employ more elaborate or chemical-specific models to make interspecies extrapolations. A variety of computational tools are available to determine the uptake and deposition of gases and particulates in nasal pathways and the respiratory tract (U.S. EPA, 2004; Bogdanffy and Sarangapani, 2003; Hanna and Lou, 2001; Tran et al., 1999; Bush et al., 1998; Asgharian et al., 1995; Jarabek, 1994; Kimbell et al., 1993). PBPK models are frequently used for systemically distributed gases and vapors, but in conjunction with other models (e.g., CFD), they can be used for locally acting gases with contact site effects. A limitation of DAFs is that they do not account for metabolism of the more reactive gases, so PBPK modeling approaches would clearly

be preferable for these compounds if adequate data are available. Further applications of PBPK models to the more reactive gases and agents are expected to continue to be developed in the near future.

Intraspecies extrapolations and the application of UFs in RfC derivation are addressed in Section 4.4.12.

4.4.6. Example of PBPK Model Use in Reference Concentration Derivation

The RfC derivations for m-xylene and vinyl chloride exemplify the application of PBPK models. In the case of m-xylene, using the adjusted NOAEL of 39 mg/m^3 as input to the rat PBPK model, the steady-state blood concentration was established (0.144 mg/L) (Tardif et al., 1997). The human model was then run to determine the exposure concentration yielding that same dose metric (HEC = 41 mg/m^3) (U.S. EPA, 2003). In an alternative approach, the dose metric associated with the unadjusted NOAEL (217 mg/m^3, 6 hr/day, 5 days/wk, 13 wks) in the rat was determined using the PBPK model (time-weighted average blood concentration = 0.198 mg/L). Then, the human PBPK model was used to determine the 24-hr exposure concentration that would produce this target dose metric (39 mg/m^3). Dividing this value by the appropriate UFs (3 for interspecies pharmacodynamic differences, 10 for interindividual variability, 3 for subchronic to chronic extrapolation, and 3 for database deficiency), the RfC was determined (0.1 mg/m^3).

In the case of vinyl chloride, the RfC was derived from the NOAEL for the oral route (U.S. EPA, 2000b). The PBPK model was initially used to derive the dose metric associated with the rat NOAEL (0.13 mg/kg/day). Because systemic toxicity resulted in the same endpoint regardless of exposure route, a human PBPK model was exercised to determine the continuous inhalation exposure concentration associated with the same dose metric (2.5 mg/m^3) (Clewell et al., 1995). Using a total UF of 30 (3 for toxicodynamic component of interspecies UF and 10 for intraspecies variability), the RfC was established (0.1 mg/m^3).

Box 4-1. Role of PBPK models in the RfC process

- Route-to-route extrapolation of the point of departure
- Duration adjustment calculation
- Dosimetric adjustment factor (i.e., interspecies)
- Pharmacokinetic component of human variability

If the available human PBPK model is probabilistic in nature, accounting for the population distribution of parameters (biochemical, physiological, and physicochemical), the magnitude of the interindividual variability can be estimated (Delic et al.,

2000). In that case, the intraspecies uncertainty factor might be set to 3 (to account only for pharmacodynamic differences). The potential role of the PBPK model in the RfC process is summarized in Box 4-1.

4.4.7. Reference Dose

An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (Dourson et al., 1992; Barnes and Dourson 1988). It is expressed in units of milligrams per kilogram per day. An RfD is calculated as follows:

$$RfD = POD/UF$$

where:

POD = NOAEL, LOAEL, or BMD

UF = uncertainty factors related to extrapolations associated with the POD (i.e., interspecies extrapolation, human variability, subchronic-to-chronic extrapolation, LOAEL-to-NOAEL extrapolation) or incompleteness of the database.

An RfD derivation begins with the identification of the POD for the critical effect. Subsequently, the UFs are applied as appropriate. PBPK models are potentially useful in deriving the RfD by estimating the POD and extrapolation factors, as described in Sections 4.4.8 through 4.4.11.

4.4.8. Reference Dose: Point of Departure

As with RfC derivation, PBPK models are not able to establish a POD in the absence of experimental dose-response data. Development of a BBDR model linked with a PBPK model could potentially improve the quantification of the dose-response and POD for RfD derivation. There has been some success in developing BBDR models for simple adverse effects (e.g., cholinesterase inhibition, cytotoxicity, hematoxicity) (Ashani and Pistinner, 2004; Cox, 1996; Gearhart et al., 1994, 1990; Reitz et al., 1990a), but these models are not routinely used to estimate PODs for RfD derivation, partly due to limitations on data needed to calibrate and test the models. New technologies (e.g., toxicogenomics) may offer the potential for generating

needed data, and more integrated PBPK and BBDR models may be an attainable goal in the near future.

4.4.9. Reference Dose: Route-to-Route Extrapolation and Duration Adjustment

The RfD derivation generally uses an oral NOAEL, LOAEL, or BMD as the POD. The oral route-specific NOAEL and LOAEL correspond to experimentally tested doses, whereas the BMD is obtained from statistical modeling of dose-response data (U.S. EPA, 2000a).

When an oral POD is unavailable, PBPK models can be useful in deriving such values on the basis of results obtained for other dosing routes (e.g., inhalation, intravenous, dermal), as previously described. Extrapolation of inhalation data using simple assumptions about ventilation rate, chemical concentration, and body weight will be inaccurate due to pharmacokinetic factors such as first-pass clearance, discussed above for route-to-route extrapolation in RfC derivation. In addition, comparisons of oral PODs with dosimetry based route extrapolation of inhalation results can be valuable because the vehicle (e.g., corn oil) in oral gavage studies sometimes alters the toxicity response.

As with RfCs, chronic RfDs are intended for continuous exposure of human populations. For PODs derived from gavage studies, typically administered 5 days/wk, it has been standard practice to adjust for continuous exposure using a 5/7 adjustment. Alternatively, PBPK models can be used to model human 7-day/wk exposures and thus estimate a dose metric corresponding to a POD determined in experimental animal bioassays.

4.4.10. Reference Dose: Interspecies Extrapolation

As with RfC derivation, PBPK models can be employed to account for pharmacokinetic differences between test species and humans and covert a POD to a human equivalent dose (U.S. EPA, 2002). Estimation of human pharmacokinetic variability and the application of UFs in RfD derivation are discussed in Section 4.4.12.

4.4.11. Example of PBPK Model Use in Reference Dose Derivation

The RfD derivation for ethylene glycol monobutyl ether exemplifies the current approach of PBPK model application (U.S. EPA, 1999b). In this case, the LOAEL identified in an animal study (59 mg/kg/day) was provided as input to the PBPK model to determine the Cmax of the metabolite butoxy acetic acid in blood (BAA_{max}) (Corley et al., 1997). The dose metric

(BAA_{max}) associated with the LOAEL was established in the test species (103 μM). The human PBPK model was then run to determine the exposure dose that would give the target dose metric (103 μM) (Corley et al., 1997). The resulting human equivalent dose of 7.6 mg/kg/day was divided by the appropriate UFs (30; 10 for interindividual differences and 3 for LOAEL-to-NOAEL extrapolation) to establish the RfD (0.3 mg/kg/day). In this particular case, the interspecies pharmacodynamic factor was set to 1 because in vitro studies suggested that humans are less sensitive than rats to the hematologic effects of ethylene glycol monobutyl ether (U.S. EPA, 1999b).

When the BMD is available, a similar approach is used to establish the RfD. In the case of ethylene glycol monobutyl ether, initially the dose metric associated with the BMD was established (BAA_{max} = 64 μM) and then the human PBPK model was used to back-calculate the equivalent dose (5.1 mg/kg/day). Using the appropriate UF (10 for interindividual variability), the RfD was derived (0.5 mg/kg/day) (U.S. EPA, 1999a). If the human PBPK model accounted for the population distribution of parameters, the pharmacokinetic component of the interindividual variability could be addressed as illustrated in the dose-response analysis with methyl mercury (Clewell et al., 1999). PBPK models, by facilitating the simulation of tissue dose of the toxic moiety of chemicals, address specific areas of uncertainty associated with derivation of the RfD, as shown in Box 4-2.

Box 4-2. Role of PBPK models in the RfD process

- Route-to-route extrapolation
- Duration adjustment
- Pharmacokinetic component of interspecies extrapolations
- Pharmacokinetic component of human variability

4.4.12. Uncertainty Factors: Role of PBPK Models

The UFs and variability factors used in RfC and RfD derivation account for extrapolations from test animals to humans (interspecies, UF_A), across duration of exposure (subchronic to chronic), from LOAEL to NOAEL, for variability within the human population to protect the most sensitive population (intraspecies variability, UF_H), and for poor quality or missing data in the database (database deficiency) (U.S. EPA, 1994; Jarabek, 1994). The total of all UFs generally should not exceed 3,000 (U.S. EPA, 2002). If the NOAEL for a chemical with an adequate database has been identified in a chronic study, only the UF_A and UF_H are used in the assessment. The conventional default value for UF_A of 10 is used in RfC and RfD derivation as an approximation of cross-species scaling resulting in equivalent effects. Similarly, the

default value for UF_H of 10 is presumed adequate to account for variability in the human kinetic and dynamic processes following exposure and to protect potentially sensitive human subpopulations.

The values for UF_A and UF_H are based on empirical information for pharmacokinetics and pharmacodynamics (e.g., isoenzyme levels, enzyme activity levels, tissue volumes, breathing rates, cell proliferation rates) (Dorne et al., 2002, 2001a, b; Walton et al., 2001) and science policy and historical use. Extrapolations across species or estimates of interindividual variability (e.g., differences arising from genetic polymorphisms), however, are best done on the basis of chemical specific determinants of disposition and effects. Initially, evaluation of specific determinants of interspecies differences or human variability is useful, but simple pooling of these specific determinants without accounting for covariance or nonlinear interactions can lead to unrealistic estimates for either UF_A or UF_H (Lipscomb, 2004). The net impact of various determinants on the UF_A and UF_H is more properly evaluated within the integrated and physiologically based context of a PBPK or BBDR model.

When data are available to go beyond default uncertainty values, these UFs can be subdivided into their toxicokinetic and toxicodynamic components (IPCS, 2005; U.S. EPA, 2005b). The World Health Organization's International Programme on Chemical Safety (IPCS) has produced guidance on the development of chemical-specific adjustment factors (CSAFs) (IPCS, 2005). Although the principles of using chemical-specific data in developing values for UFs has long been endorsed by EPA (e.g., U.S. EPA, 1994), and many of the guiding principles in the IPCS document are also components of EPA's risk assessment approach, the Agency does not use CSAFs per se, due in part to differences in calculation methods. For instance, the Agency often separates the pharmacokinetic and pharmacodynamic components of interspecies variability equally (i.e., $10^{0.5}$ or 3.16, generally rounded to 3 each), whereas the IPCS advocates $10^{0.6}$ (4.0) and $10^{0.4}$ (2.5), respectively (IPCS, 2005).

When sufficient chemical-specific data are available for PBPK modeling, such models are useful for characterizing the magnitude of the pharmacokinetic component of the UF_A as well as the UF_H used in the RfC and RfD processes. When using PBPK models to adjust for pharmacokinetic differences between species, a factor of 3 (one-half order of magnitude) is generally retained to account for remaining uncertainties (U.S. EPA, 2003, 1994; Clewell et al., 2002a; Jarabek, 1995a). However, chemical-specific information on the pharmacodynamic aspect of inter- and intraspecies differences may inform a further reduction or increase of these

UFs from default values. It should be recognized that PBPK and BBDR models are not currently suitable for characterizing the magnitude of LOAEL-NOAEL, subchronic-chronic, or database UFs, although research in these areas is ongoing (Thomas et al., 1996a).

4.5. ROLE OF PBPK MODELS IN CANCER RISK ASSESSMENT

The dose-response assessment portion of cancer risk assessment may vary, depending on MOA considerations. A CSF can be based on a linear extrapolation from the POD (i.e., high-dose to low-dose extrapolation), or a nonlinear analysis may be applied (U.S. EPA, 2005b). Either approach may also require interspecies or route-to-route extrapolations for the POD. The role of PBPK models in conducting these extrapolations is discussed in Sections 4.5.1 through 4.5.5.

4.5.1. Interspecies Extrapolation

For gases and particulates, the default procedure for interspecies extrapolation involves the derivation of an HEC, as described in Section 2.5.4 (Jarabek, 1995a, b; U.S. EPA, 1994). For oral exposures, when a PBPK model is not available, the EPA endorsed scaling of doses for carcinogens between species (e.g., rat to humans) according to body mass raised to the three-fourths power ($BW^{0.75}$) (U.S. EPA, 2005b, 2002, 1992b). This procedure presumes that equal doses in these units (i.e., in $mg/kg^{0.75}/day$), when administered daily over a lifetime, will result in equal lifetime cancer risks across mammalian species. The three-fourths power scaling relationship (sometimes called "Kleiber's law" from his original proposition in a 1932 article) is generally attributed to differences in metabolic rate. The leading biological rationale for a less-than-full-power relationship for general metabolic processes (i.e., $< BW^1$) is that exchange surfaces and distribution networks constrain the concentration and flux of metabolic reactants (Enquist et al., 1998; West et al., 1997). There remains considerable dissent as to the generality of the $BW^{0.75}$ scaling factor, the underlying biological rationale, and the value of the exponent (i.e., many proponents advocate a $BW^{0.67}$ scaling based solely on surface area differences), particularly for toxicological effects of xenobiotic chemicals in contrast to endogenous anabolic and catabolic processes (Agutter and Wheatley, 2004). Nonetheless, $BW^{0.75}$ scaling remains the current EPA default approach (U.S. EPA, 1992b).

The nature and slope of the dose-response relationship for carcinogens may not be identical in test species and humans due to pharmacokinetic and pharmacodynamic differences

(Monro, 1994). If appropriate data are available in both the test species and humans (e.g., tissue or blood concentrations), then interspecies extrapolations of an equivalent carcinogenic or safe dose can be conducted. In the absence of a complete data set, PBPK models provide a means to characterize the relationship between the applied dose and the internal dose of a carcinogen in the species of interest for subsequent extrapolation to humans (Andersen et al., 1987).

4.5.2. Intraspecies Variability

Intraspecies variability in pharmacokinetics or pharmacodynamics has not usually been considered in cancer risk assessment. CSFs have been used without further adjustment to account for susceptible populations. The recent supplemental guidance, however, suggests that an additional adjustment factor to the cancer slope or unit risk value be considered to account for enhanced susceptibility in early life (i.e., to neonates and young children) from exposure to carcinogens exhibiting a mutagenic MOA (U.S. EPA, 2005a). Furthermore, when assessing the less-than-lifetime exposures occurring in childhood, the guidelines stipulate consideration of adult-children differences in key exposure factors (e.g., skin surface area, drinking water ingestion rates) (U.S. EPA, 2005c).

PBPK models can be useful in evaluating pharmacokinetic differences among adults and children and their impact on the internal disposition of chemical carcinogens (Ginsberg et al., 2004; Clewell et al., 2004; Price et al., 2003b; Gentry et al., 2003; Clewell et al., 2002b). However, the quantitation of differences in tissue dose between adults and children would not be account for pharmacodynamic differences related to early-life exposures of neonates and children.

4.5.3. Route-to-Route Extrapolation

As with RfC and RfD derivation, PBPK models can facilitate the conduct of route-to-route extrapolation by accounting for the route-specific rate and magnitude of absorption, first-pass effect, and metabolism (Clewell and Andersen, 1994). The slope factor or the POD associated with one exposure route can be translated into applied dose for another exposure route by simulating the tissue dose of toxic moiety associated with the exposures by each route (U.S. EPA, 2000b; Gerrity et al., 1990).

4.5.4. High-Dose to Low-Dose Extrapolation

The oral CSF or the IUR can be determined by modeling the relationship between the cancer response and the administered dose or exposure concentration (U.S. EPA, 2005a). According to the revised cancer guidelines, either a nonlinear (i.e., RfC or RfD) or linear (i.e., unit risk estimate) extrapolation based on the POD can be conducted, as appropriate for the MOA of the carcinogen (U.S. EPA, 2005a). The use of internal dose or delivered dose in such analysis has been encouraged.

Because high doses of chemicals are often administered in rodent cancer bioassays, the number of tumors observed in such studies is not always directly proportional to the exposure dose. Thus, the dose-response relationships can appear complex, in part due to nonlinearity in the pharmacokinetic processes occurring at high exposure doses. In other words, the target tissue dose of the toxic moiety may be disproportional to the administered doses used in animal bioassays (Figure 2-1, Chapter 2). Therefore, dose-response analysis based on an appropriate dose metric may result in linearization of the relationship (Clewell et al., 2002a, 1995; Andersen et al., 1987). The slope factor derived using the dose metric-response curve has units of (dose metric)$^{-1}$. For nonlinear analyses, a POD can be converted using a PBPK model to the dose metric at which no significant incidence of cancer is expected on the basis of MOA of the chemical and dose-response data.

An integrated PBPK-BBDR model would improve the characterization of a chemical carcinogen dose-response relationships (e.g., a PBPK model coupled to a clonal expansion and progression model); however, most such coupled models are still in the development stage (U.S. EPA, 2005b). PBPK models improve estimation of the internal dose metric for a chemical carcinogen and play an important role in reducing the uncertainties associated with some of the extrapolations used in the cancer risk assessment process (Box 4-3).

Box 4-3. Role of PBPK models in cancer risk assessment

- Interspecies extrapolations of pharmacokinetically equivalent doses
- Route-to-route extrapolation
- High-dose to low-dose extrapolation
- Intraspecies variability to protect sensitive subpopulations

4.5.5. Example of PBPK Model Use in Cancer Risk Assessment

For assessing the cancer risk associated with human exposures, the exposure concentration is used as input to human PBPK models to estimate the dose metric, which is then multiplied with the dose metric-based slope factor. In the cancer risk assessments using PBPK

models, it is assumed that the tissue response associated with a given level of dose metric in the target tissue is the same in test animals and in humans (e.g., Andersen et al., 1987). It is a reasonable assumption that can be revised as a function of species-specific mechanistic information available for a given chemical.

The demonstration of the applicability of PBPK models in cancer risk assessment was first accomplished with dichloromethane, which caused liver and lung tumors in mice exposed to 2,000 or 4,000 ppm 6 hr/day, 5 days/wk for lifetime (Andersen et al., 1987). In this case, the mouse PBPK model was used to calculate the tissue dose of metabolites and parent chemical arising from exposure scenarios comparable to those of the cancer bioassay study, and their relationship to the observed tumor incidence was then examined. Because the parent chemical was nonreactive, Andersen et al. (1987) considered it an unlikely candidate responsible for the tumorigenicity. Hence, the relationship between the tissue exposure to its metabolites and tumor incidence was examined (Table 4-2). Whereas the dose metric based on oxidative pathway varied little between 2,000 and 4,000 ppm, the flux through the glutathione pathway increased with increasing dose of dichloromethane and corresponded well with the degree of dichloromethane-induced tumors at these exposure concentrations.

Table 4-2. Relationship between tumor prevalence and dichloromethane metabolites produced by microsomal and glutathione pathways for the bioassay conditions (methylene chloride-dose response in female mice)

Exposure (ppm)	Microsomal pathway dose[a]		Glutathione pathway dose[a]		Tumor number	
	Liver	(Lung)	Liver	(Lung)	Liver	(Lung)
0	—	—	—	—	6	(60)
2,000	3,575	(1,531)	851	(123)	33	(63)
4,000	3,701	(1,583)	1,811	(256)	83	(85)

[a] Tissue dose is cumulative daily exposure (mg metabolized/volume tissue/day). Reprinted from *Toxicology & Applied Pharmacology*, vol. 87, Andersen et al., Physiologically based pharmacokinetics and the risk assessment process for methylene chloride, pp. 185–205, 1987, with permission from Elsevier.

The model prediction of the target tissue dose of the glutathione conjugate resulting from 6-hr inhalation exposures to 1–4,000 ppm dichloromethane is presented in Figure 4-7. The

estimation of target tissue dose of dichloromethane-glutathione conjugate by linear back-extrapolation gives rise to a 21-fold higher estimate than that obtained by the PBPK modeling approach. This discrepancy arises from the nonlinear behavior of dichloromethane metabolism at high-exposure concentrations. At exposure concentrations exceeding 300 ppm, the

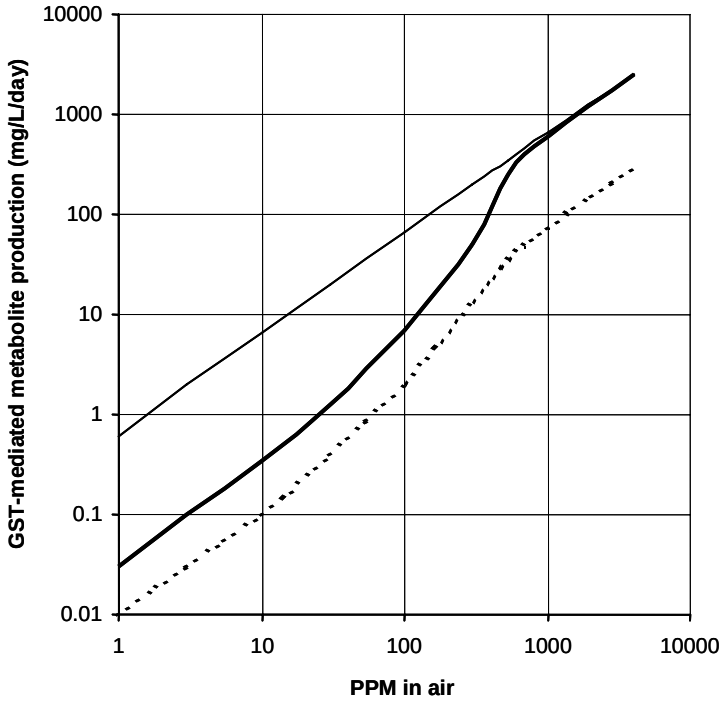


Figure 4-7. PBPK model predictions of glutathione (GST)-pathway metabolites in mouse liver. The three curves are for a linear extrapolation from the bioassay exposures of 2,000 and 4,000 ppm (upper curve), the expected tissue dose, based on model parameters for the mouse (middle line), and the expected dose expected in humans, based on human model parameters (bottom line). The curvature occurs because oxidation reactions that are favored at low inhaled concentrations become saturated as inhaled concentration increases above several hundred ppm. Reprinted from *Toxicology & Applied Pharmacology*, vol. 87, Andersen et al., Physiologically based pharmacokinetics and the risk assessment process for methylene chloride, pp. 185–205, 1987, with permission from Elsevier.

cytochrome P-450-mediated oxidation pathway is saturated, giving rise to a corresponding disproportionate increase in the flux through glutathione conjugation pathway. By accounting for the species-specific differences in metabolism rates and physiology in the PBPK model, the target tissue dose for humans was estimated to be some 2.7 times lower than that for the mouse.

The target tissue dose-based slope factor has subsequently been used for characterizing the cancer risk associated with human exposures (Haddad et al., 2001a; Reitz et al., 1989; Andersen et al., 1987). The case of dichloromethane exemplifies how PBPK models can be used to improve the dose-response relationship on the basis of appropriate dose metrics, thus leading to scientifically sound conduct of interspecies and high-dose to low-dose extrapolations essential for cancer risk assessments.

4.6. MIXTURE RISK ASSESSMENT

PBPK models facilitate risk assessment of chemical mixtures by estimating the change in dose metrics due to multichemical interactions (Haddad et al., 2001b). For conducting tissue dosimetry-based assessments for mixtures, adequately evaluated PBPK models for the mixture in the test species and in humans are required and the dose-response values for the individual chemicals (e.g., CSF, RfD, RfC) known. The approach for using PBPK models in risk assessment of mixtures of systemic toxicants or carcinogens exhibiting threshold mechanism of action, would consist of (Haddad et al., 2001b)

1. Characterizing the dose metrics associated with dose-response values for the mixture components,
2. Obtaining predictions of dose metrics of each mixture component in humans, based on information on exposure levels provided as input to the mixture PBPK model; and
3. Determining the sum total of the ratios of the results of steps (1) and (2) for each component during mixed exposures.

Similarly, for carcinogens with slope factor (Haddad et al., 2001b),

1. The dose metric-based slope factor can be established for each component using the animal PBPK model,
2. The dose metric associated with human exposure concentrations can be established using mixture PBPK models, and
3. The results of steps (1) and (2) can be combined to determine the potentially altered cancer response during mixed exposures.

Risk assessments based on the use of PBPK models for single chemicals and mixtures, as detailed in previous sections, account for only the pharmacokinetic aspect or, more specifically,

target tissue exposure to toxic moiety. If these tissue exposure simulations are combined with pharmacodynamic models, then better characterization of dose-response relationships and prediction of PODs (NOAEL, BMD, BMC) may become possible.

4.7. LINKAGE TO PHARMACODYNAMIC MODELS

The identification of PODs by simulation may become possible with the use of BBDR models. These models would require the linkage of quantitative descriptions of pharmacokinetics and pharmacodynamics via mechanism of action. Accordingly, the output of PBPK models is linked to the pharmacodynamic model using an equation that reflects the researcher's hypothesis of how the toxic chemical participates in the initiation of cellular changes leading to measurable toxic responses. For example, certain DNA adducts cause mutations, cytotoxic metabolites kill individual cells, and expression of growth factors can act as a direct proliferation stimulus. In each of these cases, the temporal change in the dose metric simulated by the PBPK model is linked with mathematical descriptions of the process of adduct formation, cytotoxicity, or proliferation in the BBDR models to simulate the quantitative influence of these processes on tumor outcome. Figure 4-8 presents an example of the relationship between dose metric (simulated by the PBPK model) and fraction of liver cells killed (simulated by pharmacodynamic model) for chloroform. In this case, the pharmacodynamic model consisted of differential equations to simulate time-dependent changes in the number of hepatocytes in the liver as a function of basal rates of cell division and death, chloroform-induced cytolethality, and regenerative replications (Page et al., 1997; Conolly and Butterworth, 1995).

Table 4-3 presents a list of pharmacodynamic models for cancer and noncancer endpoints. A characteristic of several of these pharmacodynamic models is that they are able to simulate the normal physiological processes (e.g., cell proliferation rates, hormonal cycle) and additionally account for the ways in which chemicals perturbate such phenomena, leading to the onset and progression of injury. Pharmacodynamic models that can be linked with PBPK models are not available for a number of toxic effects and modes of action. This situation is a result, in part, of the complex nature of these models and the extensive data requirements for development and evaluation of these models for various exposure and physiological conditions.

With the availability of integrated pharmacokinetic-pharmacodynamic models, the scientific basis of the process of estimating PODs and characterizing the dose-response curve

will be significantly enhanced. Additionally, such a modeling framework will facilitate a quantitative analysis of the impact of pharmacodynamic determinants on the toxicity outcome, such that the magnitude of the pharmacodynamic component of the interspecies and intraspecies factors can be characterized more confidently. Even though some PBPK models have been used in RfD, RfC, and unit risk estimate derivation for a number of substances (Table 4-1), the need for applying such models (where possible) should be continuously explored.

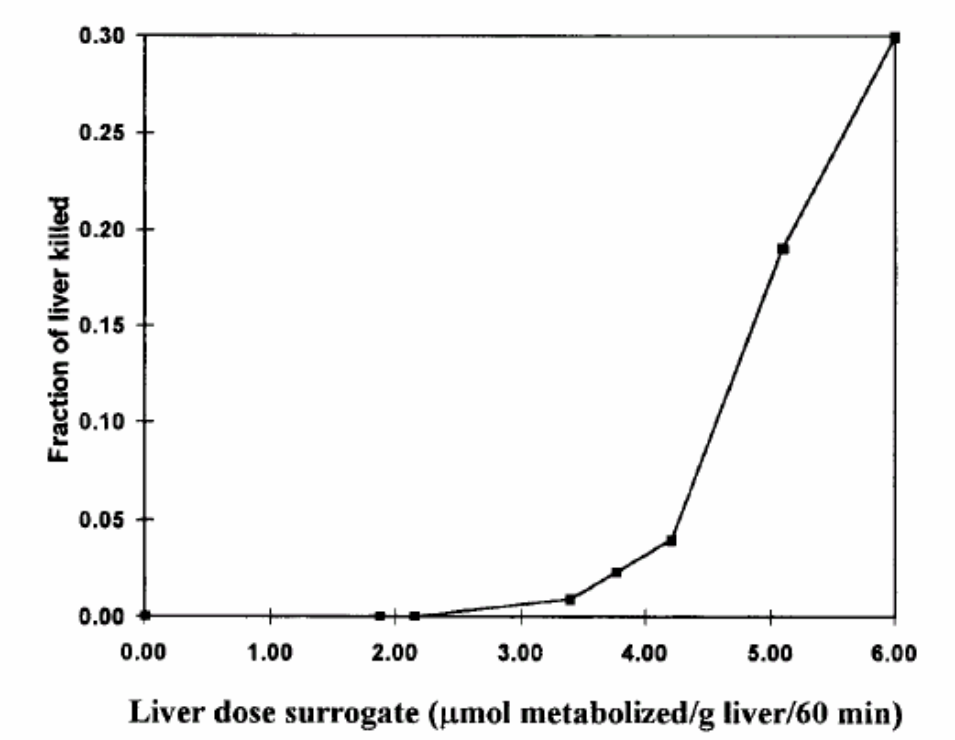


Figure 4-8. Relationship between the dose metric (μmol metabolized/g liver/hr) simulated by PBPK model and the cell killing inferred from pharmacodynamic model for chloroform. Reprinted from *Fundamentals of Applied Toxicology*, vol. 37, Page et al., Implementation of EPA revised cancer assessment guidelines: incorporation of mechanistic and pharmacokinetic data, pp. 16–36, 1997, with permission from Oxford University Press.

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Table 4-3. Examples of biologically based models of endpoints and processes of toxicological relevance

Toxicity endpoint or process	Features	Chemical studied	References
Cancer	Simulation of relative roles of initiation, promotion, cytolethality, and proliferation	2-acetylamino fluorine Chloroform Dimethylnitrosamine Formaldehyde Polychlorinated biphenyls Pentachlorobenzene Saccharin	Conolly et al. (2003); Tan et al. (2003); Thomas et al. (2000); Conolly and Andersen (1997); Conolly and Kimbell (1994); Chen (1993); Luebeck et al. (1991); Cohen and Ellwein (1990); Moolgavkar and Luebeck (1990); Moolgavkar and Knudson (1981); Moolgavkar and Venzon (1979); Armitage and Doll (1957)
Cholinesterase inhibition	Simulation of dose-dependent inhibition of plasma cholinesterase, red blood cell acetyl cholinesterase and brain acetyl cholinesterase, and nontarget B-esterase	Organophophates	Timchalk et al. (2002); Gearhart et al. (1994, 1990)
Developmental toxicity	Simulation of altered cell kinetics as the biological basis of developmental toxicity	Methyl mercury	Faustman et al. (1999); Leroux et al. (1996)
Estrus cycle	Simulation of interactions of estradiol and lutenizing hormone	Endocrine-modulating substances	Andersen et al. (1997)
Gene expression	Simulation of induction of CYP1A1/2 protein expression in multiple tissues	Tetrachlorodibenzodioxin	Santostefano et al. (1998)
Granulopoiesis	Simulation of loss of proliferating cells and loss of functional cells	Cyclophosphamide	Steinbach et al. (1980)
Nephrotoxicity	Simulation of induction of renal 2µ globulin in male rat kidney as a function of proteolytic degradation and hepatic production	2,2,4-Trimethyl-2-phenol	Kohn and Melnick (1999)
Teratogenic effect	Sensitivity distribution of embryo as a function of age and stage of development	Hydroxyurea	Luecke et al. (1997)

GLOSSARY

Absorbed dose: The amount crossing a specific absorption barrier (e.g., the exchange boundaries of the skin, lung, and digestive tract) through uptake processes.

Applied dose: The amount presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism).

Area under the curve (AUC): The concentration of a chemical in tissues or blood integrated over time. It is a measure of tissue exposure to chemicals over a period of time.

Bayesian statistics: An approach that considers a model's parameters as random variables with a probability distribution for describing each parameter. The distribution based only on prior information and assumptions is called the *prior distribution*. Analysis of new data yields a *posterior distribution* that reconciles the prior information and assumptions with the new data.

Benchmark dose (BMD) or benchmark concentration (BMC): A dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response) compared to background.

Biologically based dose-response (BBDR) model: A predictive model that describes biological processes at the cellular and molecular level linking the target organ dose to the adverse effect.

Cancer slope factor (CSF): An estimate of the increased cancer risk from a lifetime exposure to an agent. This estimate, usually expressed in units of proportion (of a population) affected, is generally reserved for use in the low-dose region of the dose-response relationship. It is often an upper bound, approximating a 95% confidence limit.

Clearance: Volume containing the amount of drug eliminated per unit time by a specified organ; it has the dimension of a flow per unit time.

Critical effect: The first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases.

Delivered dose: The amount of a substance available for biologically significant interactions in the target organ.

Diffusion limited uptake: Compounds (typically high molecular weight and those with significant protein binding) where membrane diffusion is often the rate-limiting process.

Dose metric: The target tissue dose that is closely related to ensuing adverse responses. Dose metrics used for risk assessment applications should reflect the biologically active form of chemical, its level, and duration of internal exposure, as well as intensity.

Dose-response assessment: The process of determining the relationship between the magnitude of administered, applied, or internal doses and biological responses. Response can be expressed as measured or observed incidence or change in level of response, percent response in groups of

subjects (or populations), or the probability of occurrence or change in level of response within a population.

Exposure assessment: The process of identifying and evaluating the human population exposed to a toxic agent by describing its composition and size as well as the type, magnitude, frequency, route, and duration of exposure.

First-order process: A linear metabolic process where a constant fraction of chemical is metabolized per unit time.

First-pass effects: Metabolism that occurs before a compound can enter the general circulation. For example, an orally administered compound may undergo metabolism in the intestines and/or liver prior to systemic distribution.

Flow-limited diffusion: The chemical diffuses readily between blood and tissue compartments and exchange is limited primarily by blood flow.

Half-life: Interval of time required for one-half of a given substance present in an organ to leave it through processes other than physical decay. It is a constant only for mono-exponential functions.

Human equivalent concentration (HEC): The human concentration (for inhalation exposure) of an agent that is believed to induce the same magnitude of toxic effect as the exposure concentration in experimental animal species. This adjustment may incorporate pharmacokinetic information on the particular agent, if available, or use a default procedure.

Integration interval: The time interval at which the calculations of the change in concentration or amount of chemical in various compartments of the model are performed.

Internal dose: A more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The amount of the chemical available for interaction by any particular organ or cell is termed the delivered or biologically effective dose for that organ or cell.

Markov-chain Monte-Carlo simulation: An approach that has frequently been used within a Bayesian statistical framework to (a) sample each model's parameters from their prior distributions, (b) fit the model with the sampled parameters to several additional experimental data sets, and (c) compare the model's predictions with the experimental results to obtain posterior distributions for the model's parameters that improve the model's fit. These steps are repeated thousands of times until each parameter's posterior distribution converges to a more robust distribution that reflects a wider database.

Pharmacokinetic models: Mathematical descriptions simulating the relationship between external exposure levels and the biologically effective dose at a target tissue over time. Pharmacokinetic models take into account absorption, distribution, metabolism, and elimination of the administered chemical and its metabolites**.**

Pharmacodynamic models: Mathematical descriptions simulating the relationship between a biologically effective dose and the occurrence of a tissue response over time.

Physiologically based pharmacokinetic (PBPK) model: A model that estimates the dose to target tissue by taking into account the rate of absorption into the body, distribution and storage in tissues, metabolism, and excretion on the basis of interplay among critical physiological, physicochemical, and biochemical determinants.

Point of departure (POD): The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD, BMC), or a no-observed-adverse-effect level or lowest-observed-adverse-effect level for an observed incidence or change in level or response.

Potential dose: The amount ingested, inhaled, or applied to the skin.

Reference concentration (RfC): An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. [Durations include acute, short-term, subchronic, and chronic].

Reference dose (RfD): An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. [Durations include acute, short-term, subchronic, and chronic].

Steady state: A variable is said to have attained steady state when its value stays constant in a given interval of time, i.e., when its derivative is zero.

Target organ: The biological organ(s) most adversely affected by exposure to a chemical or physical agent.

Terminal half-life: The terminal half-life is the interval of time for the concentration of the drug in a compartment to decrease 50% in its final phase.

Uncertainty: Uncertainty occurs because of lack of knowledge. Uncertainty can often be reduced with greater knowledge of the system or by collecting more and better experimental or simulation data.

Uncertainty factors (UFs)/variability factors: Generally, 10-fold default factors used in operationally deriving the reference dose and reference concentration from experimental data. The factors are intended to account for (a) variation in sensitivity among the members of the human population (i.e., interindividual variability), (b) uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty), (c) uncertainty in extrapolating from data obtained in a

study with less-than-lifetime exposure to lifetime exposure (i.e., extrapolating from subchronic to chronic exposure), (d) uncertainty in extrapolating from a lowest-observed-adverse-effect level rather than from a no-observed-adverse-effect level, and (e) uncertainty associated with extrapolation when the database is incomplete.

Variability: Variability refers to true heterogeneity or diversity. Differences among individuals in a population are referred to as interindividual variability; differences for one individual over time are referred to as intraindividual variability.

Volume of distribution: The volume of distribution is the ratio between the administered dose and plasma or blood concentration of a chemical.

Zero-order process: A saturated metabolic process where a constant amount of chemical is eliminated per unit time.

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