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Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk 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)
BMD	benchmark dose
BMC	benchmark concentration
Cmax	maximal concentration
CSF	cancer slope factor
DAF	dosimetric adjustment factor
HEC	human equivalent concentration
HI	hazard index
IUF	interspecies uncertainty factor
IUR	inhalation unit risk
IVF	intraspecies variability factor
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
MBDE	mass balance differential equation
MOA	mode of action
MVK	Moolgavkar-Venzon-Knudson
OSF	oral slope factor
PK	pharmacokinetic
PBPK	physiologically based pharmacokinetic
POD	point of departure
QSAR	quantitative structure-activity relationship
RfD	reference dose
RfC	reference concentration

PREFACE

This draft report, *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. These 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 EPA scientists and risk assessors who may be less familiar with the field. In addition, this report can be informative to PBPK modelers within and outside the Agency, as it provides an assessment of the types of data and models that the EPA requires for consideration of a model for use in risk assessment. This draft report will be externally peer-reviewed by an independent panel of experts prior to making the report final.

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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?

Because PBPK modeling efforts to date have largely focused on chemicals that cause systemic effects (i.e., water-insoluble gases and some nonvolatile organics), this document draws upon the experience and literature on these efforts.

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 U.S. Environmental Protection Agency risk assessment framework. Useful resources are provided as appendices, including (1) a list of publications relating to PBPK modeling and its use in health risk assessment, (2) a compilation of parameters of several published PBPK models, and (3) a list of published algorithms for estimating parameters for PBPK models of environmental chemicals.

PBPK models consist of a series of equations for compartments, fluid flows, and chemical reactions that represent real biological tissues and physiological processes in the body and 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 applied dose in a quantitative dose-response assessment, with the intent of reducing the uncertainty inherent in

using the applied dose to derive risk values. This reduction in uncertainty and improved scientific basis for the risk 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., to 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 applied dose.

Examples of PBPK model applications in risk assessments include interspecies extrapolation of the dose-response (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 should, 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. As a resource, appendices to this document provide a compilation of parameters of several published PBPK models, a list of available animal-alternative algorithms for parameter estimation, and key references.

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) the model has been evaluated and peer-reviewed for the adequacy of its structure and parameters, and (3) the model 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 use to derive a given point of departure (e.g., NOAEL, LOAEL, BMD, BMC) 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 version of the PBPK model is also developed to simulate kinetics in humans for estimating the applied dose to a human needed to achieve the relevant internal dose predicted from the animal toxicity or, less frequently, 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 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. The document focuses primarily 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), as well as some nonvolatile organics. This document primarily draws on the experience and literature on these efforts. The approaches are not intended, for example, to apply directly to agents, gaseous or particulate, where the target organ is the respiratory tract; the reference concentration (RfC) methodology (U.S. EPA, 1994) should be consulted for these circumstances. However, the elements of discussion found in this document are also conceptually applicable, in a broader sense, to other kinds of dosimetry models and a wider range of substances.

In developing this document, it was assumed that risk assessors are familiar with the basic concepts of PBPK modeling and that model developers are familiar with the basic concepts of risk assessment; therefore, the document contains only brief descriptions of PBPK modeling and risk assessment methods. Appropriate references to secondary review articles and reports from which readers can obtain additional information 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 by the additional insight it provides; 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 be familiar with potential applications in health risk assessment.

1.3. ORGANIZATION OF THE DOCUMENT

Three key chapters form the main 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. This chapter 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. The issues relating to the evaluation of PBPK models that are discussed in this chapter will facilitate the assessor's decision on whether or not an available model is adequate and scientifically defensible for use in reducing uncertainties in a given risk assessment. In this regard, 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 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 extrapolation, interspecies extrapolation, intraspecies extrapolation, route-to-route and scenario extrapolation, mixture risk assessment, and linkage with pharmacodynamic models. This chapter also highlights how PBPK models are used in cancer and noncancer assessments.

The appendices provide a list of publications relating to PBPK modeling and its use in health risk assessment, a compilation of parameters of several published PBPK models, and a list of published algorithms for estimating parameters for PBPK models of environmental chemicals.

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.

A range of modeling approaches is used to characterize exposures and resulting delivered doses. This variety of approaches reflects differences in chemical and physical characteristics (e.g., stable or reactive gases, particulate matter, lipophilic organics, water-soluble compounds) and their ability to cause contact site or systemic toxic effects (Andersen and Jarabek, 2001; Overton, 2001; U.S. EPA, 1994; 2004). Many drugs and toxic chemicals that cause systemic effects are absorbed following oral exposures, so compartmental and noncompartmental pharmacokinetic modeling has focused on these characteristics (O'Flaherty 1981; Renwick 2001). Compartmental modeling, generally described as a set of mathematical equations that describe empirical pharmacokinetic data concerning a chemical, evolved to include mathematical descriptions of biological processes (e.g., partition coefficients, tissue volumes), which is the focus of this document.

Because the respiratory tract is a frequent site of both exposure and toxicity, it has been a particular focus for a range of modeling approaches. These approaches include those developed for gases of various reactivity and solubility, as well as for particulate matter (see U.S. EPA, 1994). More refined approaches are available and useful for reactive gases and particulate matter, including two-dimensional and three-dimensional modeling such as computational fluid dynamics models (Kimbell et al., 1993; Martonen et al., 2001; Overton, 2001, U.S. EPA, 2004). Predicting 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).

Another significant factor influencing modeling approaches is the role of metabolism. For example, a range of volatile compounds can cause nasal or other respiratory toxicities that require metabolism of the chemical in respiratory tract tissue. These kinds of chemicals have been modeled using elaborate PBPK models of the nose (with or without representation of the rest of the body) that have been linked with computational fluid dynamic models to describe the deposition of chemical in different regions of the nose (Frederick et al., 2002; Bogdanffy et al., 2001). Similarly, compounds that are relatively water soluble require specific approaches that address aspects such as fractional absorption or "wash-in, wash-out" effects (Johanson, 1991; Medinsky et al., 1993; Perkins et al., 1995).

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

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 (Andersen et al., 1987; Clewell and Andersen, 1997; Aylward et al., 1996; Benignus et al., 1998; Melnick and Kohn, 2000).

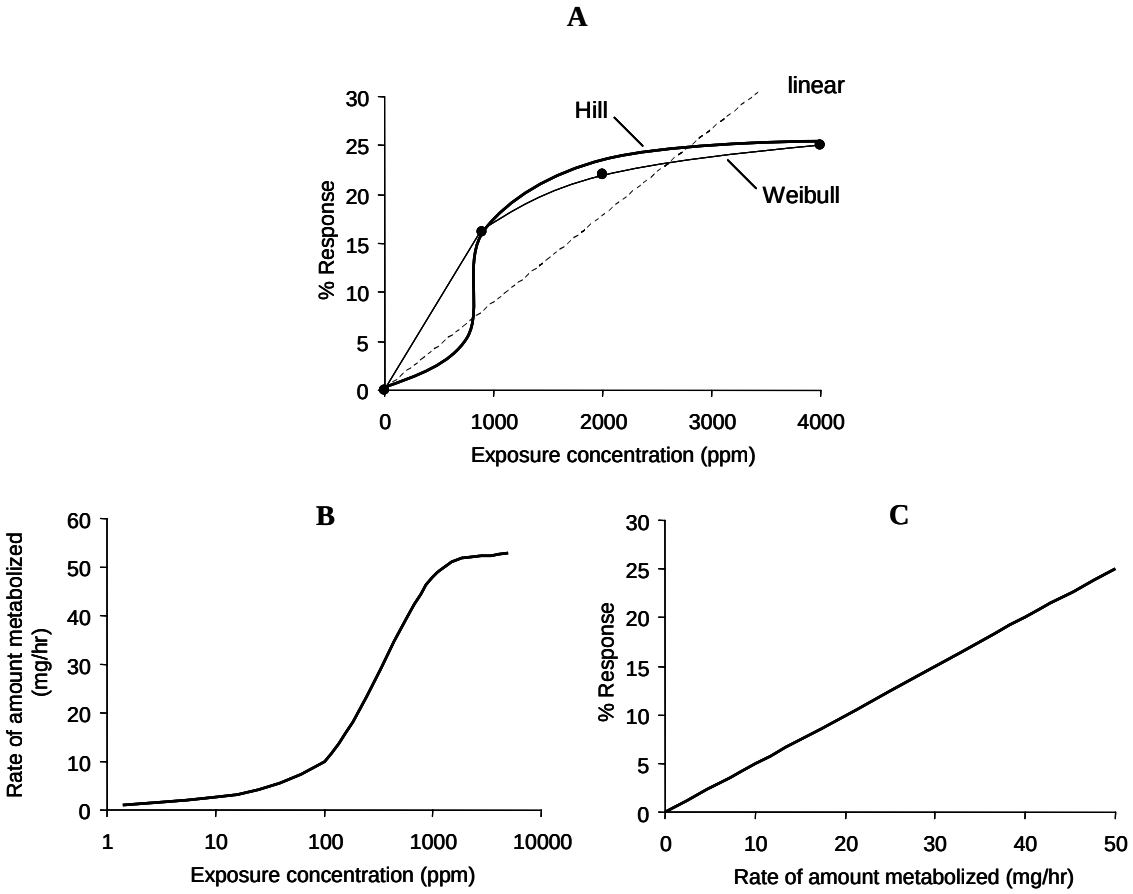

Figure 2-1. Relationship between the exposure concentration and adverse response for a hypothetical chemical. Panel A depicts the case of a chemical for which the correlation between dose and response is weak or complex, along with equally plausible curve fits (linear, Hill, and Weibull). This dose-response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

The use of blood and tissue concentrations for relating dose and response in exposed organisms has long been recognized in pharmacology (e.g., Wagner, 1981). The target tissue dose closely related to ensuing adverse responses is often referred to as the "dose metric" (Andersen and Dennison, 2001). Dose metrics used for risk assessment applications should 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 specific window of susceptibility), and intensity (peak, average, or integral), as well as 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 over lifetime (i.e., area under the concentration versus time curve [AUC]) in target tissue has been considered to be the appropriate dose metric (Collins, 1987; Voisin et al., 1990; Clewell et al., 2002).

When the carcinogen 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 window of susceptibility for a particular gestational event (e.g., Welsch et al., 1995; Luecke et al., 1997).

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

Parent chemical
- Peak concentration
- Average concentration
- Amount or quantity
- AUC

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

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 its MOA (Clewell et al., 2002). Box 2-1 lists dose metrics that can be used to derive dose-response relationships for risk assessment.

2.3. PHARMACOKINETIC DATA NEEDS IN RISK ASSESSMENTS

The 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, based on the relationship between administered dose and observed responses in laboratory or field studies. For noncancer and nonlinear cancer assessments, adjustments to the POD are then made to account for uncertainties in the estimate of a POD for humans and to protect the most sensitive human subpopulation exposed by specific routes (e.g., inhalation, oral). This process frequently requires conducting interspecies, intraspecies, high-dose to low-dose, duration, and exposure route extrapolations of responses. Based on information about the MOA of the compound, an

appropriate approach for the extrapolation can be identified. Such an approach implies that when the value of the 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). Alternatively, if one were to assume that the pharmacokinetics in humans were identical to those in rats, an equivalent 24 hr exposure would be calculated to be 12.5 ppm (i.e., 6 hr × 50 ppm is equivalent to 24 hr × 12.5 ppm).

For reactive gases that cause contact site toxicity, the concentration of chemical or its delivery rate to the target may be the relevant dose metric, whereas for anesthetic effects of volatile organics, the current concentration in the blood has been an effective dose metric. In these cases, the acute effects are more —or entirely— dependent upon concentration than on time, so the extrapolations would be dependent upon that dose metric, potentially without adjustments for duration of exposure.

The most robust pharmacokinetic data set needed for risk assessment would consist of the time-course data on the dose metric associated with exposure scenarios and doses used in the critical studies chosen for the assessment (e.g., animal bioassays or human 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 should be obtained for the window of susceptibility, route and scenario of exposure associated with the critical study as well as for the window of susceptibility, appropriate route, and exposure scenarios in humans. If such a pharmacokinetic data set is available for each scenario of interest, then there is no real need for any pharmacokinetic models, even though their availability can facilitate simulation of other potential scenarios of interest and critical determinants of tissue dose.

In almost all cases, dose metric measurements associated with human exposures to environmental chemicals will not be available. Even the available animal pharmacokinetic data may not correspond to the active toxic moiety, relevant route, or appropriate dose levels. In the absence of experimental data on the biologically active form of the chemical in target tissues, data on blood concentration of the parent chemical, urinary metabolite levels, or fraction

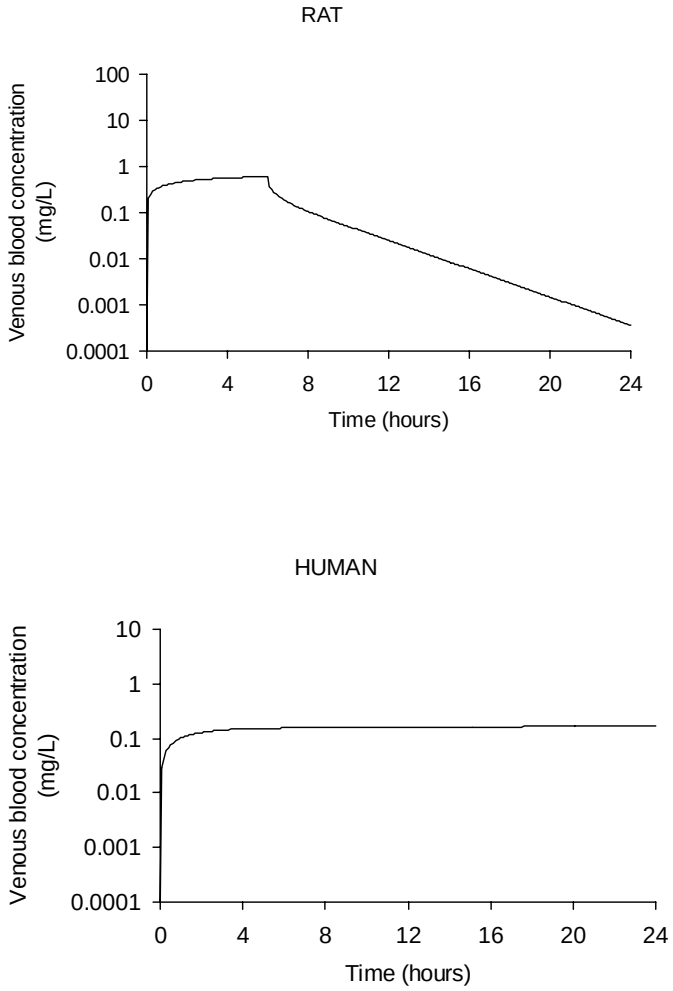

Figure 2-2. Rat-human extrapolation of exposure concentrations of toluene on the basis of equivalent dose metrics (AUC of toluene in blood; 3.8 mg/L-hr). Rat exposures are for 6 hr to 50 ppm; human exposures are for 24 hr to 17.7 ppm. Both exposures yield the same AUC, as determined using species-specific 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.

absorbed may be used as a surrogate of dose metrics. These 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

Regulatory agencies have a mandate to develop guidelines for toxicity testing and formulate risk values based on the current understanding of the dose-response relationship. Reference values correspond to an estimate of an exposure level for a given duration that is likely to be without an appreciable risk of adverse effects over the lifetime in humans, including sensitive subpopulations. The reference values developed at EPA include reference concentration (RfC) for chronic inhalation exposures and reference dose (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, such as cancer slope factor (CSF) and inhalation unit risk (IUR) estimates. The underlying assumption in these processes is that the exposure concentration (or applied dose) results in a toxic chemical moiety in a target organ that will be less than or equal to a level 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 of chemicals in the target tissue(s) in humans. Even in animal studies, it is more practical to get measures of blood, plasma, and urinary concentrations of toxic chemicals and their metabolites than to get data on the time course on the actual tissue dose of a toxic moiety relevant for use in risk assessment. Pharmacokinetic models are therefore used to simulate the tissue concentration of toxic substances. There are both noncompartmental and compartmental pharmacokinetic models (Renwick, 2001); however, we will focus on compartmental models.

Among the compartmental pharmacokinetic models, those that allow for prediction of target organ concentration are most appropriate and useful for conducting extrapolations essential for risk assessment. These models are commonly referred to as PBPK models (Himmelstein and Lutz, 1979; Rowland, 1985; Leung, 1991; Andersen, 1995; Krishnan and Andersen, 2001). By simulating the kinetics and dose metric of chemicals, they attempt to reduce the uncertainty related to the interspecies, intraspecies, high-dose to low-dose, route-to-route, and exposure scenario extrapolations essential in the context of RfC, RfD, and cancer unit risk estimate processes. The following sections discuss how the PBPK models are used in the context of health risk assessment.

2.5. ROLE OF PBPK MODELS IN RfC DERIVATION

2.5.1. Reference Concentration

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

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

where:

$POD_{[HEC]}$ = POD (no-observed-adverse-effect level [NOAEL], lowest-observed-adverse-effect level [LOAEL], or benchmark concentration [BMC]) dosimetrically adjusted to a human equivalent concentration (HEC)

UF = uncertainty factors to account for the extrapolations associated with the POD (i.e., interspecies differences in sensitivity, intraspecies extrapolation, 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 (1) adjustment for the difference in duration between experimental exposure (e.g., 6 hr) and expected human exposure (24 hr); (2) calculation of the HEC, based on dosimetric adjustments; and (3) application of uncertainty factors. 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, HEC, and uncertainty factors is presented in Sections 2.5.2 through 2.5.5.

2.5.2. Point of Departure

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

Route-to-route extrapolation can be conducted on the basis of equivalent potential doses when information on the POD was available only for a noninhalation route of exposure (e.g., oral

route) (Pauluhn, 2003). For example, the NOAEL (mg/kg/day) associated with a particular exposure route (e.g., oral) was converted to milligrams per day and then to equivalent inhaled concentration, based on breathing rate and body weight of the test species. This simplistic approach assumes that the rates of absorption, distribution, metabolism, excretion, and tissue dosimetry of chemicals are the same for a given total dose, regardless of the exposure route and intake rate. Such an approach essentially neglects the route-specific differences in pharmacokinetics—more specifically, absorption, clearance, and first-pass effects. When data on the route-specific fraction absorbed are available, they are used to determine the equivalent inhalation concentration on the basis of equivalent absorbed doses (U.S. EPA, 1999a), but preference would be given to more complete pharmacokinetic modeling.

The POD for RfC derivation cannot be identified or established with only pharmacokinetic data or PBPK models, independent of the dose-response data. On the other hand, better characterization of dose-response relationships has been demonstrated by the use of integrated pharmacokinetic-pharmacodynamic models (e.g., Gearhart et al., 1990, 1994; Timchalk et al., 2002). Such models might be capable of predicting response and thus determining a POD if sufficient data are available. More commonly, however, PBPK modeling will be useful for conducting route-to-route extrapolation on the basis of equivalent delivered dose from PODs identified from NOAEL, LOAEL, or BMC (e.g., oral to inhalation).

2.5.3. Duration Adjustment

RfCs are intended for continuous exposure of human populations, such that the POD used in its derivation should 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/d, 5 d/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). This simple adjustment, generally referred to as Haber's Rule, implies that the AUC (i.e., concentration × time [C × t]) and not peak concentration is the dose metric associated with the toxicity endpoints (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 neither dose metric is demonstrable experimentally to be the appropriate measure of internal dose, the Agency uses adjustment to a continuous inhalation

exposure based on the C × t relationship as a matter of health-protective policy (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 (Jarabek, 1994; U.S. EPA, 2002). This approach is based on the expectation that the pharmacodynamic aspect does not change between the various durations of within-day exposures (<24 hr). Consistent with the Agency's policy (U.S. EPA, 2002), the AUC of a chemical for the exposure scenario of the critical study should be determined initially using the PBPK model (e.g., 6 hr/d, 5 d/wk); then the atmospheric concentration for continuous exposures (24 hr/d) during lifetime or particular window of susceptibility yielding the same AUC or Cmax can be determined by iterative simulation.

2.5.4. Dosimetric Adjustment Factor

In the RfC process, a dosimetric adjustment factor (DAF), intended to account for pharmacokinetic differences between test species and humans, is applied to the duration-adjusted POD (NOAEL, LOAEL, or BMC) to derive HECs (U.S. EPA, 1994). The DAF depends on the nature of the inhaled toxicant and MOA as well as the endpoint (local effects vs. systemic effects). The 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.

For highly reactive or water-soluble gases that do not significantly accumulate in blood (e.g., hydrogen fluoride, chlorine, formaldehyde, volatile organic acids, and esters), the DAF is derived using simulated delivery of chemical to different regions of the respiratory tract, based on regional mass transfer coefficients, as well as differences in surface area and ventilation rates (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 potential for both respiratory and remote effects, the DAF could be calculated on the basis of uptake, defined by overall mass transfer coefficient, as well as flow-limited perfusion distribution models. The DAF for poorly water-soluble gases that cause remote effects (e.g., xylene, toluene, styrene) is determined on the basis of blood:air partition coefficients and flow-limited perfusion models (U.S. EPA, 1994).

A variety of computational tools are available for determining the uptake and deposition of gases and particulates in nasal pathways and the respiratory tract (Kimbell et al., 1993; Jarabek, 1994; Asgharian et al., 1995; Tran et al., 1997; Bush et al., 1998; Hanna et al., 2001, Bogdanffy and Sarangapani, 2003; U.S. EPA, 2004). 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., computational fluid dynamics), to more locally acting gases. Their use (discussed above) in estimating dose metrics over time allows for a more refined duration adjustment. Also, the other RfC approaches 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. There is little doubt that further applications of PBPK models to the more reactive gases and agents will be developed and realized in the near future.

2.5.5. Uncertainty Factors: Role of PBPK Models

The uncertainty/variability factors used in RfC derivation include one or more of the following: an interspecies uncertainty factor (IUF) of 3 to account for possible pharmacodynamic differences; an intraspecies variability factor (IVF) of 10 for variability in kinetics and dynamics among humans; subchronic-to-chronic extrapolation factor of 10; a LOAEL-to-NOAEL factor of 10; and a database deficiency factor of 1 to 10 (U.S. EPA, 1994; Jarabek, 1994). The total of all uncertainty factors generally should not exceed 3,000 (U.S. EPA, 2002). With the exception of the IVF, the magnitude of the factors listed above cannot be estimated using pharmacokinetic data or PBPK models alone, although they can be determined either by conducting appropriate experiments or by using integrated pharmacokinetic-pharmacodynamic models. Experiments to estimate the IVF for each toxic chemical in each population of interest is neither plausible nor ethical. It has been possible, however, to estimate the IVF by analyzing available data on pharmaceutical products collected in human volunteers and patients over the years (Hattis et al., 1999; Silverman et al., 1999).

The IVF of 10 conventionally used in RfC derivation implies that for the same level of response or nonresponse, the potential doses among individuals may differ by as much as—but not more than—an order of magnitude. The factor of 10 is a composite of the interindividual differences in pharmacokinetics and pharmacodynamics (Renwick and Lazarus, 1998). The magnitude of the pharmacokinetic component of the IVF can be estimated with the use of PBPK models and applied in conjunction with the remaining interindividual pharmacodynamic

variability factor of 3, and this is entirely consistent with what is done under the RfC process to account for dosimetry and pharmacodynamic differences between species (Jarabek, 1994; U.S. EPA, 2002). The PBPK models are also potentially useful for estimating the magnitude of the pharmacokinetic component of the uncertainty factors for LOAEL-to-NOAEL and subchronic-to-chronic extrapolations, but the feasibility of such estimations has not yet been explored systematically (Thomas et al., 1996b).

2.5.6. Summary

The potential role of the PBPK model in the RfC process is summarized in Box 2-2.

Box 2-2. Role of PBPK models in the RfC process

- Route-to-route extrapolation of the POD
- Duration adjustment calculation
- Dosimetric adjustment factor
- Intraspecies variability factor (PK component)

2.6. ROLE OF PBPK MODELS IN RfD DERIVATION

2.6.1. Reference Dose

An RfD is 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 (Barnes and Dourson 1988; Dourson et al., 1992). 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, intraspecies extrapolation, 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 uncertainty factors are applied as appropriate. Regarding the RfD process, PBPK models are potentially useful in determining the POD as well as the extrapolation factors, as described in Sections 2.6.2 and 2.6.3.

2.6.2. Point of Departure

Where possible, the RfD derivation 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 the LOAEL or NOAEL has not been identified in an oral dosing study but has been obtained for another route of exposure, the route-to-route dose conversion in the past assumed that absorption by both routes is 100% and that equal potential doses administered via inhalation and oral route are equitoxic (Pauluhn, 2003). In other words, this overly simplistic approach assumed that the rates of absorption, distribution, metabolism, excretion, and tissue dose of toxic moiety of chemicals are identical, regardless of the exposure route, particularly if the same dose (mg/kg/d) is administered by both routes. The data on route-specific fraction absorbed, when available, have been used to improve the scientific basis of the route-to-route extrapolation process (e.g., U.S. EPA, 1999a).

When the oral NOAEL, LOAEL, or BMD is unavailable for a given route of exposure, PBPK models can be particularly useful in deriving such values on the basis of results obtained for other dosing routes (e.g., inhalation, intravenous, dermal). PBPK models facilitate the conduct of route-to-route extrapolation (e.g., inhalation to oral) to establish the oral route-specific POD (NOAEL, LOAEL, BMD), based on equivalent internal dose. The use of PBPK models represents a significant improvement in the scientific basis of the conventional route-to-route extrapolation approach, because it takes into account the route-specific rates and magnitude of absorption, clearance, and first-pass effects.

PBPK models, however, are not able to establish an oral POD in the absence of some route-specific experimental dose-response and kinetic data. Development of a biologically based dose-response (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) (Gearhart et al., 1990, 1994; Reitz et al., 1990a; Cox, 1996; Ashani and Pistinner, 2004), but these models are not routinely used to estimate PODs for RfD derivation because of the lack of data needed to calibrate and test the models. New technologies (e.g., toxicogenomics) offer the potential for generating the needed data, and more integrated PBPK and BBDR models may be an attainable goal in the near future.

2.6.3. Uncertainty Factors

The uncertainty factors used in RfD derivation include extrapolations across species (interspecies) and across duration of exposure (subchronic to chronic), degree of response (e.g., LOAEL-to-NOAEL), variability within a population (intraspecies variability), and to account for poor or absent data in the database (database deficiency). If the NOAEL for a chemical with an adequate database has been identified in a chronic study, only the IUF and IVF are used in the assessment. The IUF of 10 conventionally used in RfD derivation addresses the possibility that the same level of response or nonresponse is associated with a HED that is 10 times lower than the dose administered to animals. Similarly, the IVF of 10 is presumed to account for both the differences in tissue dosimetry and tissue response within a human population.

If in vivo pharmacokinetic and pharmacodynamic data are available for humans and the test species as well as for the various subgroups of the population, chemical-specific adjustment factors can be derived to replace the default uncertainty factors. The IUF and IVF may also be estimated from an understanding of the key pharmacokinetic and pharmacodynamic determinants of a chemical's disposition in the body (e.g., isoenzyme levels, enzyme activity levels, tissue volumes, breathing rates, cell proliferation rates) (e.g., Dorne et al., 2001a, b, 2002; Walton et al., 2001). Extrapolating among species or estimating interindividual variability must be performed carefully because the magnitude of variability associated with individual determinants is neither cumulative nor additive. A one-by-one evaluation of the determinants of interspecies differences or interindividual variability is useful for understanding the mechanistic basis and their relative importance, but such an isolated approach can easily lead to unrealistic estimates of the overall magnitude of an IVF or IUF. The net impact of various determinants on the IVF and IUF is more properly evaluated by integrating the available information with a PBPK or BBDR model.

PBPK models are useful for characterizing the magnitude of the pharmacokinetic component of the IUF as well as the IVF used in the RfD process. When using PBPK models to adjust for pharmacokinetic differences, a factor of 3 is generally used to account for the potential interspecies differences and interindividual variability in pharmacodynamics, as with the RfC process (Jarabek et al., 1995; Clewell et al., 2002; U.S. EPA, 2003). However, chemical-specific information on the pharmacodynamic aspect of inter- and intraspecies differences may inform on further reduction of these uncertainty factors. It should be recognized that in their current state

of advancement, PBPK and BBDR models are not suitable for characterizing the magnitude of LOAEL-NOAEL and subchronic-chronic factors.

Although a thoroughly characterized BBDR model might address the LOAEL-NOAEL issue, it is unlikely that such models will be capable of extrapolating from subchronic to chronic effects because of the multiplicity of feedback systems and plasticity within organisms, nor will such models be capable of accounting for the potential for different adverse effects over the lifespan.

2.6.4. Summary

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 2-3.

Box 2-3. Role of PBPK models in the RfD process

- Route-to-route extrapolation
- Interspecies uncertainty factor (PK component)
- Intraspecies variability factor (PK component)

2.7. ROLE OF PBPK MODELS IN CANCER RISK ASSESSMENT

The dose-response assessment portion of cancer risk assessment may vary, depending on MOA considerations. It can involve the determination of a slope factor based on linear extrapolation from the POD (i.e., high-dose to low-dose extrapolation) or it may involve the use of the POD for nonlinear analysis (U.S. EPA, 2005a). Either approach may also require extrapolation of animal data to humans. In addition, some of the assessments may require route-to-route extrapolations. The role of PBPK models in conducting these extrapolations is discussed in the following sections (2.7.1 through 2.7.4).

2.7.1. High-Dose to Low-Dose Extrapolation

The oral slope factor or IUR has frequently been 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, however, either a nonlinear (i.e., RfC or RfD) or linear (i.e., unit risk estimate) extrapolation based on the POD can be conducted, as justified by the MOA of the carcinogen (U.S. EPA, 2005a). Further, 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 tumors observed in such studies are not always directly proportional to the exposure dose. Rather, the cancer dose-response relationships appear complex, in part due to nonlinearity in the pharmacokinetic processes occurring at high doses. In other words, the target tissue dose of the toxic moiety is often disproportional to the administered doses used in animal bioassays (Figure 2-1). Therefore, dose-response analysis based on the appropriate dose metric often results in the linearization of the relationship (Andersen et al., 1987; Clewell et al., 1995, 2002). The slope factor derived using the dose metric versus the response curve will have units of $(\text{dose metric})^{-1}$; similarly, NOAELs are converted using PBPK models 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.

Linkage of PBPK models with BBDR models (e.g., clonal expansion and progression models as well as two-stage models) would represent the ideal framework for characterizing the health risk associated with human exposure to chemical carcinogens. However, such models still continue to be developed and improved upon, and currently there is not a standard model that is used or recommended (U.S. EPA, 2005a).

2.7.2. Interspecies Extrapolation

The default procedure for conducting interspecies extrapolation (e.g., rat to humans) of the oral dose of carcinogens involves scaling in proportion to body weight to the 0.75 power (U.S. EPA, 2005a). This scaling approach is based on the observation that several physiological, anatomical, and biochemical processes in mammalian species are closely related to body surface area (Voisin et al., 1990; U.S. EPA, 2002). In other words, this default approach implies that the delivered doses (following oral exposure) are related to applied dose by 0.75 power of body weight, independent of the species. For gases and particulates, however, the default procedure for interspecies extrapolation would involve the derivation of a HEC, as described in Section 2.4.4 (U.S. EPA, 1994; Jarabek, 1995a, b).

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). Interspecies extrapolations of an equivalent dose of carcinogens can be conducted if appropriate data (tissue or blood concentrations of potential toxic moiety or promutagenic adducts) are available in both the test species and humans. In the absence of such data, PBPK models can be used to characterize the relationship between applied dose and

delivered dose of carcinogens in the species of interest for subsequent extrapolation to humans (Andersen et al., 1987).

2.7.3. Route-to-Route Extrapolation

Route-to-route extrapolation is necessary if the cancer dose-response data were obtained in test species exposed by a route different from the anticipated human exposure and the effect is considered pertinent to the human exposure route. Route-to-route extrapolation is considered appropriate for a chemical that induces tumors at a site different from the portal of entry and if the chemical is absorbed to give an internal dose. Simplistic route-to-route extrapolations are performed by assuming that the relationship between applied dose and tissue dose of toxic moiety (dose metric) is the same, regardless of the exposure route. The uncertainty associated with this approach is that the first-pass effect as well as the rates and extent of absorption and metabolism may vary from one route to another (Pauluhn, 2003). The data on route-specific fraction absorbed are now used to improve the scientific basis of the route-to-route extrapolation process (e.g., U.S. EPA, 1999a).

PBPK models, by accounting for the route-specific rate and magnitude of absorption, first-pass effect, and metabolism, facilitate the conduct of route-to-route extrapolation (Clewell and Andersen, 1994). The slope factor or the NOAEL associated with an exposure route can be translated into applied dose by another route on the basis of equal delivered doses. Basically, by simulating the tissue dose of toxic moiety associated with the applied doses given by different routes, PBPK models facilitate conducting route-to-route extrapolation of the NOAEL or slope factor of chemical carcinogens (Gerrity et al., 1990; U.S. EPA, 2000b).

2.7.4. Intraspecies Variability

Intraspecies variability in pharmacokinetics or pharmacodynamics has not usually been considered in the context of cancer risk assessment. In characterizing risk, the CSF has been used without adjustment for susceptible populations. The newer supplemental guidance suggests the use of an adjustment factor to the cancer slope or unit risk value to account for enhanced susceptibility related to early-life exposures of neonates and young children, particularly for carcinogens exhibiting a mutagenic MOA (U.S. EPA, 2005b). Further, when assessing the less-than-lifetime exposures occurring in childhood, the guidelines stipulate adjustments for adult-

children differences in exposure factors (e.g., skin surface area, drinking water ingestion rate) (U.S. EPA, 2005b).

PBPK models are potentially of use in evaluating the pharmacokinetic basis of the adult-children differences in tissue dose of carcinogens (Price et al., 2003b; Gentry et al., 2003; Ginsberg et al., 2004). However, the quantitation of the tissue dose differences between adults and children would not be sufficient to account for pharmacodynamic differences related to early-life exposures of neonates and children. PBPK models can still be of use in determining the adjustment factor for early-life exposures of neonates and young children on the basis of equivalent internal doses.

2.7.5. Summary

PBPK models, by facilitating the simulation of tissue dose of toxic moiety of chemical carcinogens, play an important role in reducing the uncertainties associated with some of the extrapolations used in the cancer risk assessment process (Box 2-4).

Box 2-4. Role of PBPK models in cancer risk assessment

- High-dose to low-dose extrapolation
- Route-to-route extrapolation
- Interspecies extrapolations of pharmacokinetically equivalent doses

2.8. USE OF PHARMACOKINETIC DATA AND MODELS IN EXPOSURE ASSESSMENT

2.8.1. Conventional Approaches

The conventional approach to exposure assessment involves the calculation of potential dose for each route of exposure, with knowledge of 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). For calculating absorbed dose, pharmacokinetic data such as time-course data on concentration or total quantity in alveolar air, urine, or blood would be required (Paustenbach, 2000). The conversion of biomarker data, absorbed dose, or potential dose into delivered dose is not straightforward and requires the use of appropriate modeling techniques. The need for the use of pharmacokinetic models is further emphasized by the fact that the tissue dose is not always directly proportional to the exposure concentration or potential dose.

2.8.2. Role of PBPK Models

PBPK models allow the conversion of potential dose or exposure concentration to tissue dose, which can then be used for risk characterization purposes. As the dose-response relationships are being increasingly established on the basis of tissue dose, it is also essential that the potential doses calculated during exposure assessments be translated into tissue doses to facilitate risk characterization. PBPK models are additionally useful in investigating and establishing the relationship between biomonitoring or biomarker data and potential dose as well as the relationship between biomarker levels and tissue dose of chemicals by iterative simulation (e.g., Fennell et al., 1992; Krishnan et al., 1992; Csanady et al., 1996; Timchalk et al., 2001).

This ability of PBPK models has been explored for establishing biological exposure indices (e.g., breath, blood, or breath concentrations) associated with worker exposure to threshold limit values of solvents (Perbellini et al., 1990; Leung, 1992; Kumagai and Matsunaga, 1995; Thomas et al., 1996b; Droz et al., 1999). PBPK models also offer a framework for reconstructing human exposure over a period of time (Vinegar et al., 1990; Roy and Georgopoulos, 1998; Canuel et al., 2000). Further, such dose reconstructions and tissue dose resulting from multiroute (oral, inhalation, dermal) and multimedia (air, water, food, soil) exposures can be computed with the use of PBPK models (e.g., Georgopoulos et al., 1994; Roy et al., 1996; Rao and Ginsberg, 1997; Corley et al., 2000; Levesque et al., 2002). The net tissue dose associated with a particular exposure situation (multiroute and multisource) can then be obtained with PBPK models and used for risk characterization purposes.

2.8.3. Summary

The potential roles of PBPK models in exposure assessment are summarized in Box 2-5.

Box 2-5. Role of PBPK models in exposure assessment

- Conversion of potential dose into tissue dose
- Tissue dose estimation for aggregate exposures
- Dose reconstruction based on exposure biomarkers

2.9. PHARMACOKINETIC DATA NEEDS IN RISK ASSESSMENT: SUMMARY

Adverse tissue responses are more directly and closely related to tissue dose of the toxic moiety than is the concentration of the parent chemical in the environment. Therefore, there is no question that the scientific basis of, and confidence in, risk assessments are enhanced by the use of tissue dose information. Because the tissue dose is not directly and simply related to exposure concentration or potential dose at all times, there is some uncertainty regarding the use

of potential dose in risk assessment. If the relationship between potential dose and tissue dose of the toxic moiety of chemicals is linear and proportional at all dose levels used in animals and humans, then there is no need for PBPK models. However, this is not usually the case. Therefore, the PBPK models are used for addressing specific areas of uncertainty in dose-response assessment and exposure assessment on the basis of the simulation of tissue dose of potential toxic moiety.

In the context of dose-response assessment, PBPK models are needed for conducting

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

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

1. Converting potential 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.

The PBPK models intended for use in dose-response and exposure assessments should be evaluated for their adequacy. The next chapter presents the process and criteria for evaluating PBPK models intended for use in risk assessment.

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

PBPK models intended for risk assessment applications should be evaluated for their adequacy. There are no published documents that specify either model validation standards or criteria of adequate PBPK models. However, several publications have dealt with good modeling practices as well as approaches for evaluating and documenting biological models intended for risk or safety assessments (Yates, 1978; Andersen et al., 1995; Clark et al., 2004). Evaluation of PBPK models intended for risk assessment applications should be undertaken along the lines of model purpose, model structure, mathematical representation, parameter estimation (calibration), and computer implementation aspects, as detailed in the following sections.

3.1. MODEL PURPOSE

The purpose for which a PBPK model is developed basically determines its structure and level of detail. Not all PBPK models are developed for the purpose of supporting risk assessments. Some are actually used as a tool for hypothesis testing for particular modes of action and for guiding improved experimental design. The structure of a PBPK model used strictly for responding to the needs of a curiosity-driven researcher may not serve the intent and purposes of a risk assessor. PBPK models, therefore, are of varying complexities and capabilities, according to the intended purpose. PBPK models, in general, are developed for one of the following three purposes:

1. Integrate diverse sets of pharmacokinetic data on a particular chemical,
2. Investigate the pharmacokinetic basis of toxicity of a chemical that appears complex at the administered dose level, and
3. Predict tissue dosimetry for situations other than what has been or could be tested experimentally (see Section 3.6).

Models that permit the integration of diverse sets of data are useful in uncovering mechanistic determinants of pharmacokinetics of specific chemicals (e.g., Clewell and Andersen, 1997; Haddad et al., 1998). However, PBPK models resulting from such efforts are not routinely evaluated for their predictive value but still are useful for the purpose of uncovering unusual

determinants of pharmacokinetics (e.g., suicide inhibition, diffusion-limited uptake). On the other hand, PBPK models developed to improve the understanding of toxicological behavior of chemicals that appears complex at the administered dose level (e.g., species, sex, or route differences in response) may be either predictive or integrative. Typically, the PBPK models of use to the risk assessor are those that have predictive value at the quantitative level with respect to the production or pharmacokinetics of potential toxic moieties (parent chemical or metabolite).

A PBPK model capable of predicting the pharmacokinetics and tissue dose of the potential toxic moiety of a chemical is the preferred one for risk assessment applications.

3.2. MODEL STRUCTURE

The structure of a PBPK model in large part depends upon the purpose for which the model is developed and the philosophy of the modeler. There is virtually no limit to the number and size of compartments in a model intended to describe molecular/cellular events. 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. PBPK models differ from single-compartment models by representing many more physiological and biochemical processes that are relevant to the toxicokinetics of the chemical in question. This complexity is represented by differentiating or lumping tissues into specific compartments each with a unique set of physiological (i.e., blood flows) and biochemical parameters (i.e., partition coefficients). A PBPK model intended for risk assessment applications should, preferably, include the target organ as one of the compartments. Minimally, it should facilitate the calculation of 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) should be included. It is often acceptable to mathematically describe absorption, distribution, metabolism and excretion (ADME) of chemicals in PBPK models without physically representing 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. In some cases, a compartment is subdivided into several sub-compartments based on mechanistic and biological considerations. For example, the liver can be divided into separate compartments depending on the localization of enzymatic activity. Figure 3-1 represents examples of PBPK model structures that have been commonly used to simulate the kinetics of volatile and nonvolatile substances. Note that all these models facilitate the simulation of the concentration of chemicals or their metabolites in the target organ or a surrogate tissue (usually blood).

It is important to realize that compartments in PBPK models are usually assumed to be homogenously and completely mixed reactors. This means that the concentration of the chemical anywhere in the tissue is the same and equal to the concentration of the chemical as it leaves the tissue in venous blood. This assumption is necessary to simplify the differential equations representing the mass balance of the chemical in the tissue. The numerical solution of the final set of equations representing the PBPK model is further simplified because of the complete-mixing assumption.

The structure of a PBPK model intended for risk assessment applications should 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.

3.3. MATHEMATICAL REPRESENTATION

In PBPK modeling, each tissue compartment is generally described with a mass balance differential equation (MBDE) that consists of a series of clearance terms with units of volume per time, i.e., liters per hour or milliliters per minute. The clearance terms, in most cases, relate to tissue uptake, tissue-to-blood transfer, metabolism, or excretion of chemicals. The uptake of a chemical in systemic circulation by a tissue is described according to Fick's law of simple diffusion, which states that the flux of a chemical is proportional to its concentration gradient. Descriptions of passive and 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 high-molecular-weight compounds, however, membrane diffusion is often the rate-limiting process, and in such cases chemical uptake is described with differential equations for the tissue blood and cellular matrix subcompartments (Rowland, 1985; Leung, 1991; Andersen, 1995; Krishnan and Andersen, 2001) (Tables 3-1 and 3-2).

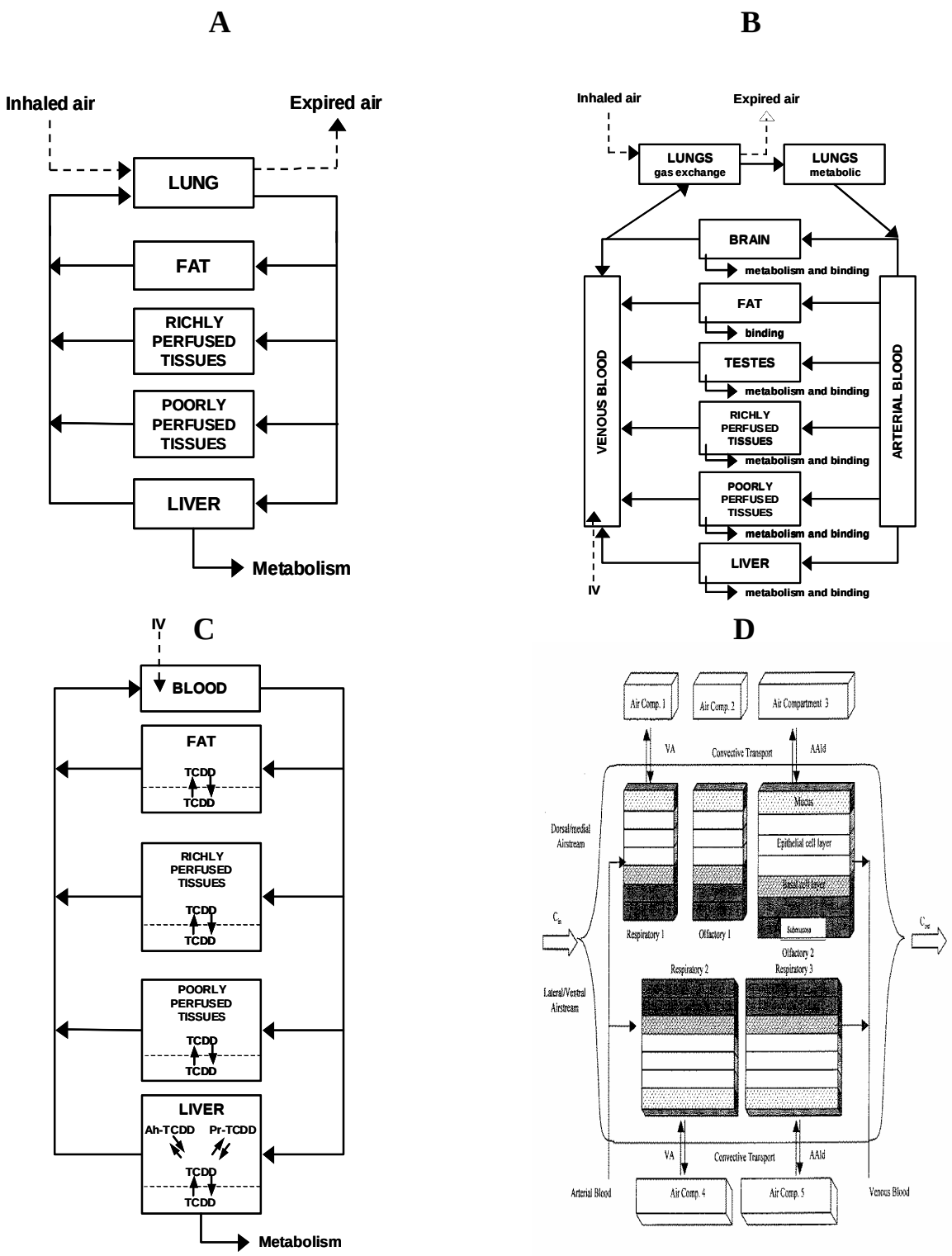

Figure 3-1. Conceptual representations of PBPK models for (A) toluene, (B) ethylene oxide, (C) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and (D) vinyl acetate. The input and output arrows associated with individual compartments represent arterial and venous blood flows. Note that model D contains 5 compartments for the nasal cavity alone (the site of toxicity). From Bogdanffy et al. (1999), Krishnan et al. (1992), and Andersen et al. (1993).

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]	$$Ca_n = \frac{Q_c \times Cv_{n-1} + Q_p \times Cinh_n}{Q_c + \frac{Q_p}{P_b}}$$
Liver	$$\frac{dA_{l\,n}}{dt} = Q_l \times \left(Ca_n - Cv_{l\,n-1}\right) - \frac{V_{\max} \times Cv_{l\,n-1}}{Km + Cv_{l\,n-1}}$$ $$A_{l_n} = \frac{dA_{l_n}}{dt} \times dt + A_{l_n}$$ $$C_{l_n} = \frac{A_{l_n}}{V_l}$$ $$Cv_{l_n} = \frac{C_{l_n}}{P_l}$$
Fat	$$\frac{dA_{f\,n}}{dt} = Qf \times \left(Ca_n - Cv_{f\,n-1}\right)$$ $$A_{f\,n} = \frac{dA_{f\,n}}{dt} \times dt + A_{f\,n}$$ $$C_{f\,n} = \frac{A_{f\,n}}{V_f}$$ $$Cv_{f\,n} = \frac{C_{f\,n}}{P_f}$$
Richly perfused tissues	$$\frac{dA_{r\,n}}{dt} = Qr \times \left(Ca_n - Cv_{r\,n-1}\right)$$ $$A_{r\,n} = \frac{dA_{r\,n}}{dt} \times dt + A_{r\,n}$$ $$C_{r\,n} = \frac{A_{r\,n}}{V_r}$$ $$Cv_{r\,n} = \frac{C_{r\,n}}{P_r}$$
Poorly perfused tissues	$$\frac{dA_{s\,n}}{dt} = Qs \times \left(Ca_n - Cv_{s\,n-1}\right)$$ $$A_{s\,n} = \frac{dA_{s\,n}}{dt} \times dt + A_{s\,n}$$ $$C_{s\,n} = \frac{A_{s\,n}}{V_s}$$ $$Cv_{s\,n} = \frac{C_{s\,n}}{P_{s\,n}}$$

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	$Cv_n = \frac{Ql \times Cv_{l_n} + Qf \times Cv_{f_n} + Qr \times Cv_{r_n} + Qs \times Cv_{s_n}}{Qc}$
Alveolar air	$Calv_n = \frac{Ca_n}{P_b}$

[a] Equations are from Ramsey and Andersen (1984).
[b] The 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
n = current time
n-1 = previous simulation time
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	$V_{t1}\frac{dC_1}{dt} = Q_t \times (C_{in} - C_{out}) - [PA] \times (C_1 + C_2)$
Cellular matrix	$V_{t2}\frac{dC_2}{dt} = [PA] \times (C_1 - C_2)$

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)

The rate of the amount of chemical consumed by macromolecular binding process is described in PBPK models as a second-order reaction or by using equations based on reversible equilibrium relationship. The rate of metabolism in PBPK models has been described as a first-order, second-order, or saturable process. Conjugation reactions, on the other hand, are traditionally described as a second-order process with respect to the concentrations of the cofactor and the chemical. Alternatively, descriptions based on ping-pong mechanism have also been used successfully. 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 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 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 actual details and complexity of the physiological and biochemical processes are not reflected by 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.

The MBDEs in PBPK models should have units of mass per time (e.g., mg/hr) or concentration per time (mg/L-hr^{-1}), and the type of equations chosen to describe tissue uptake, binding, and metabolism should be justified.

3.4. PARAMETER ESTIMATION

Knowledge of chemical-specific and species-specific parameters would be required to solve the equations constituting PBPK models. For a model to be used for estimating interindividual differences in tissue dosimetry, knowledge of the distributions of input parameters is essential. However, for all other purposes, the knowledge of the average value or the range of plausible values of model parameters is sufficient. Typically, PBPK models require the numerical values of physiological parameters, such as alveolar ventilation rate, cardiac output, tissue blood flow rates and tissue volumes, and clearance parameters related to renal, hepatic, and biliary excretion pathways, as well as certain partition coefficients (blood:air, skin:water, skin:air, and tissue:blood). Additional parameters (e.g., tissue DNA levels, hematocrit, number and concentration of binding proteins) may be required in some cases.

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. Even though peer-reviewed compilations of ranges and reference values of physiological parameters for adult animals and humans are available (Arms and Travis, 1988; Leggett and Williams, 1991; Fiserova-Bergerova, 1995; Brown et al., 1997; Davies and Morris, 1997) (Tables 3-3 through 3-6), this is not the case with respect to physiological parameters for specific subgroups of populations (e.g., developing and lactating animals, pregnant women, children). There are some reports on physiological parameters for specific subgroups but no compilations as yet of definitive ranges or reference values (Luecke et al., 1994; Schoeffner et al., 1999; Haddad et al., 2001a; Hattis et al., 2003; Price et al., 2003a, b; Pelekis et al., 2003).

In PBPK models for organic chemicals, the sum total of the volumes of compartments corresponding to soft tissues should be smaller than the body weight, usually about 91% of the body weight (100% [body weight] – 9% [weight of skeletal/structural components as percent body weight]). Even though the tissue volumes (L) are needed for PBPK modeling, tissue weights (kg) are usually used with the assumption of unit density (L = Kg). This assumption, which may seem questionable, is inconsequential for practical reasons, particularly with respect to the application of PBPK models in risk assessment.

The tissue flow rates in the model should add up to cardiac output. Fundamentally, maintaining the mass balance in PBPK models requires that the sum of the flows to the compartments be equal to the cardiac output. The ratio of cardiac output to alveolar ventilation rate is around 1 in a resting individual (e.g., Andersen et al., 1987). The specification of cardiac output independent of the value of ventilation rate is unacceptable, particularly if the their ratio (ventilation:perfusion ratio) 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 an individual animal or human being simulated. For predicting kinetics in another animal or human with a different body weight, the physiological parameters are calculated anew. To simplify this process, 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 body surface rather than body weight, the PBPK models

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

Physiological parameters	Mouse	Rat	Humans
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

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

specify these parameters as a power function of body weight, with the exponent varying from 0.67 to 0.75 (e.g., Andersen et al., 1987; Tardif et al., 1997).

An acceptable PBPK model should contain tissue volumes, flow rates, and ventilation:perfusion ratios that are within physiological limits. Particularly, 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

The calibration of PBPK models for partition coefficients has sometimes been done using in vivo data. In such cases, pharmacokinetic data collected following a single bolus dose or repeated doses leading to steady state are analyzed to determine the tissue:blood partition coefficients (Chen and Gross, 1979; Lam et al., 1982; Gabrielsson and Bondesson, 1987; Gallo et al., 1987). The steady-state data provide the most straight forward data for model calibration; however, they are valid only for tissues in which there are no significant binding or metabolic processes. In case of significant level of metabolism or binding, the calculation of tissue:blood partition coefficients should account for the amount of chemical consumed by such processes (Chen and Gross, 1979). The estimation of partition coefficients for PBPK models from in vivo data is acceptable as long as the same data set is not used for external evaluation later on.

The tissue:air, skin:water, skin:air, and blood:air partition coefficients required for PBPK modeling of volatile organic chemicals are conveniently determined in vitro using vial equilibration method (Sato and Nakajima, 1979; Gargas et al., 1989; Johanson and Dynesius, 1988; Fiserova-Bergerova and Diaz, 1986; Kaneko et al., 1994; Perbellini et al., 1995; Beliveau and Krishnan, 2000). Tissue:blood partition coefficients for nonvolatile chemicals can be determined in vitro using radioactive chemicals in ultrafiltration, equilibrium dialysis, or vial equilibration procedure (Lin et al., 1982; Igari et al., 1983; Sultatos et al., 1990; Jepson et al., 1994; Murphy et al., 1995). The partition coefficients estimated by these in vitro methods are acceptable, provided equilibrium is attained during the experimental conditions. The experimenter/modeler should have conducted a time-course analysis to choose an appropriate time point (at which equilibrium is attained) for determining partition coefficients in vitro.

Algorithms based on the consideration of solubility and binding of chemicals in biological matrices have 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, and octanol:water or oil:water partition coefficients of the chemical as well as the binding association constants, if applicable (e.g., Poulin and Krishnan, 1995, 1996a,b; Poulin and Thiel, 2000). 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 based on 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 (Payne and Kenney, 2002; Beliveau and Krishnan, 2003) (Appendix 1). Their use is acceptable, as long as the qualitative and quantitative aspects of structural features and physicochemical characteristics of the new chemical are within those used in calibrating the algorithm.

Partition coefficients required for PBPK models should 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 affinity constant) and tissue diffusion constants (for describing diffusion-limited uptake) required for PBPK modeling can confidently be determined using in vivo studies. For estimating 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, the use of exhaled breath and gas uptake studies has frequently been adopted with success (Filser and Bolt, 1979, 1981; Andersen et al., 1980; Gargas et al., 1986). 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). The use of in vivo data for parameter estimation is acceptable, as long as the same data set is not used for model evaluation purposes as well. The use of Bayesian approach is likely to enhance the precision of parameter estimations from in vivo data (e.g., Vicini et al., 1999).

Receptor binding and DNA-binding properties of chemicals have been successfully described with PBPK models on the basis of in vitro-derived data (Terasaki et al., 1984; Farris et al., 1988). The appropriateness of the usefulness 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 one gram of liver tissue and the average liver weight (in grams) of the species and age group of interest . In vitro data for humans is 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. Even though there are several examples of successful application based on appropriate in vitro-in vivo scaling methods (Reitz et al., 1989, 1996a; Gearhart et al., 1990; Hwang et al., 1996; De Jongh and Blaauboer, 1996, 1997; Iwatsubo et al., 1996; Kedderis and Held, 1996; Mortensen et al., 1997; Mortensen and Nilsen, 1998; Cole et al., 2001; Hissink et al., 2002; Lipscomb et al., 1998, 2003), the extrapolation of in vitro data to intact animal is not clear in all cases (e.g., Haddad et al., 1997, 1998). 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 (Andersen et al., 1991; Reitz et al., 1996b; Ploeman et al., 1997; Thrall et al., 2000; Kedderis and Lipscomb, 2001).

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 of the PBPK models require the use of numerical simulation methods because they contain differential equations and descriptions of nonlinear processes. Therefore, the PBPK model equations are written along with the integration algorithms and solved using programming languages, simulation software, or spreadsheets. Simulation languages and commercially available packages (Table 3-7) routinely make use of integration algorithms to obtain numerical solution to differential equations (Menzel et al., 1987; Burmaster and Murray, 1997; Easterling et al., 2000, Reddy et al., 2003) such that there is no need either for the modeler or the risk assessor to evaluate this aspect. 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

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 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.	Ramsey and Andersen (1984); Thomas et al. (1996); 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)
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 integration algorithm and interval should be specified by the user.	Johanson and Naslund (1988); Haddad et al. (1996)
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 (1997)
Berkely Madonna	Robert Macey and George Oster, University of California at Berkeley, CA	This program is a general-purpose differential equation solver. Developed on the Berkeley campus under the sponsorship of National Science Foundation and the National Institutes of Health. It is 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	This software facilitates the conduct of Bayesian analysis with PBPK models but has no graphical interphase.	Jonsson and Johanson (2003)

well as the integration interval used should be specified (e.g., Blancato and Saleh, 1994; Haddad et al., 1996).

The modeler should also 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 the acceptable speed, disk space, and run time memory required for PBPK modeling and parameter optimization; therefore, this aspect needs 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. The "bugs" in PBPK models written as a program may result either from typing errors or from illogical mathematical statements. To eliminate these errors, it is essential to carefully verify the model codes 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 written in correct programming language without any typing mistakes. The onus is on the modeler to ensure that the equations are entered correctly using the programming/simulation software, and risk assessors cannot be expected to go through the PBPK model codes to perform a routine error diagnostic check. Such verification should be done by the developer initially and then by individuals not involved in model development, such as peer-reviewers and co-workers (Clark et al., 2004).

The computer implementation of PBPK model need not be evaluated by risk assessors if a highly reputable commercial or open source simulation software has been used. The onus is on the model developer to ensure that the computer implementation of the PBPK model is free from errors. 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. MODEL EVALUATION

The purpose of model evaluation is to assess the adequacy of the model and its parameters to consistently describe the available data sets representing the pharmacokinetics of a chemical. A model that has been adequately evaluated would not only capture the critical

determinants of the pharmacokinetics of a chemical but also characterize the elements of uncertainty associated with the parameters.

The first step to ensure validity of the PBPK model is to check on the mass balance of the chemical in the whole body. The model should not create or destroy matter. Basically, when all the tissue levels, blood levels, and any excretion or metabolic transformation levels are added they should sum up to the same exposure dose level used in the model. Although trivial, this check is important in understanding the sources in the model that would falsely increase or decrease the level of the chemical in tissues. Another initial evaluation step is to check the model behavior when exposure is set to zero. The zero check is necessary to make sure that the model can represent steady-state levels of the chemical and to make sure that this level does not change with time in the absence of exposure.

The PBPK model evaluation to date has not been done rigorously from a statistical perspective. The most common 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 rationale underlying this qualitative approach to model evaluation is that 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 numbers (e.g., blood concentration values) but also at the level of the profile (i.e., bumps and valleys in the pharmacokinetic curve). Because PBPK modeling is not a fitting exercise, model simulations are not expected to touch upon each experimental data point. Rather, the shape of the simulated curve should correspond to that of the experimental data, and the simulated data should preferably be within two standard deviations of the mean of experimental values, even though this is not a rule of thumb used by the modeling community.

Figure 3-2 shows several examples of visual evaluation of the adequacy of PBPK models. The models used in cases A and C would be considered adequate because they simulate the behavior of the experimental data even though they do not accurately simulate every single experimental data. On the other hand, models used in cases B and D would not be considered adequate, and further work would be required to refine the models, either because they do not simulate the profile appropriately (B) or the model simulations are outside two standard deviations of the experimental data (D).

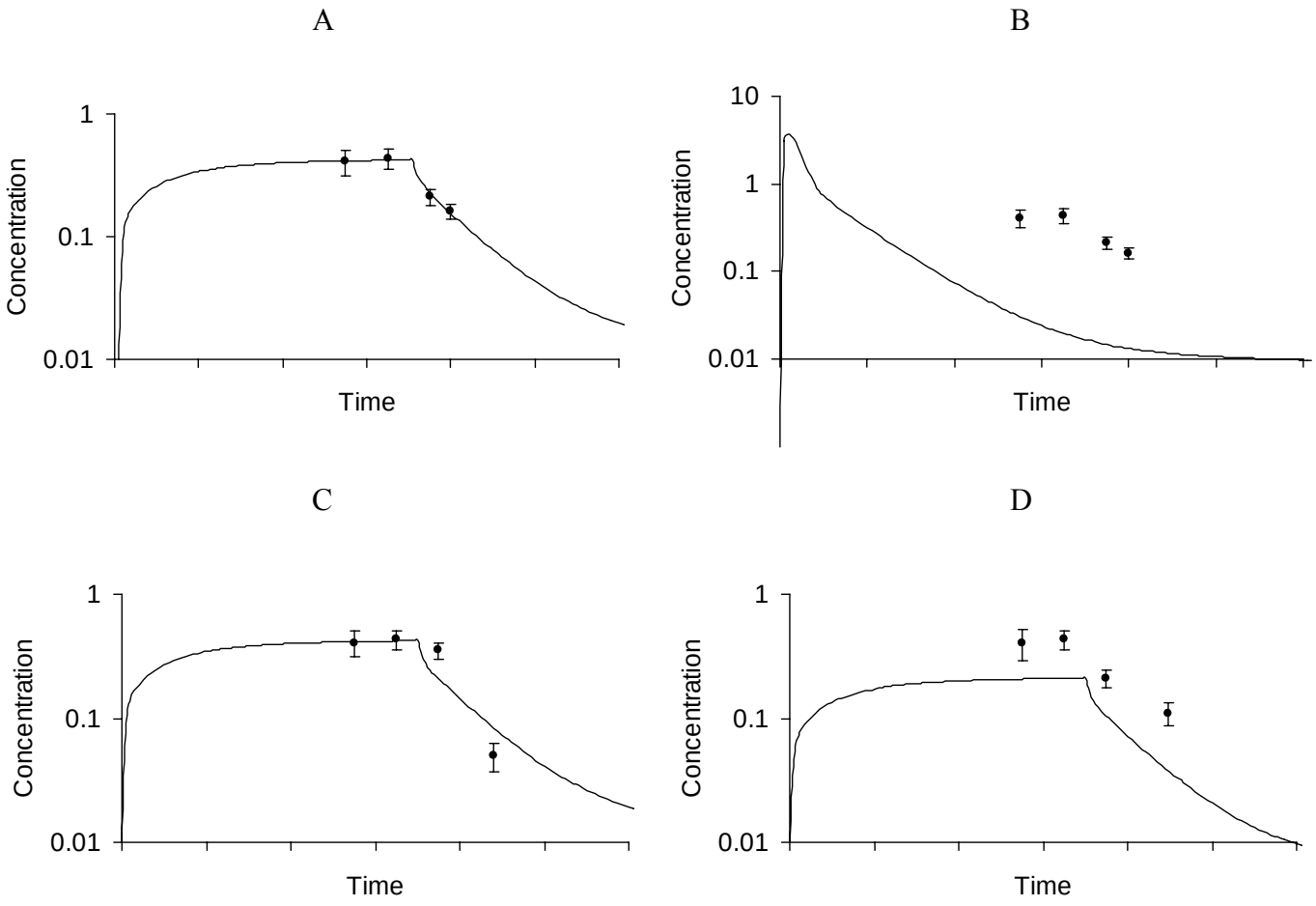

Figure 3-2. Comparison of PBPK model simulations (solid lines) with experimental data (symbols).

The above 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 behaviour. 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. Figure 3-2 (B) depicts a model whose structure is inadequate because the simulated profile is not consistent with the experimental data for the chemical. As a caveat, it is possible that the lack of fit in Figure 3-2 (B) is due to a problem with the model parameters, not the structure. If the model cannot fit the

pharmacokinetic profiles for any realistic parameter values, or only 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. 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 more than one model fits 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).

Prior to the discussion about evaluating model parameters, it is important to correct a common misunderstanding about what a "validated model" means. A model that has been calibrated against one dataset, and that adequately simulates a different data set, can be said to be "validated," but it is only validated to the extent to which those two dataset accurately represent the larger population, not in any global sense independent from the data used to develop the model. PBPK models are used to extrapolate to other exposure conditions or dosing regimens, but here again, only 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 is "validated" to predict outcomes for which it has not been adequately tested, many PBPK modelers today prefer to use the terms "calibrated" model (accompanied by a description of the data used to calibrate the model) and the "predictive capability" or utility of the model, again specifying the data that the model was adequately able to simulate. In this sense, the more quality data available to calibrate and test the model, the more confidence one can place in the model's predictive capability. PBPK modelers are in the process of developing better qualitative and quantitative descriptors for both models and the data used to develop models, to assist risk assessors in their evaluation and application of PBPK models.

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 datasets 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.

For external evaluations, none of the classical tests (t, Mann-Whitney, two-sample χ^2, two-sample Kolmogrov, etc.) that determine whether the underlying distributions of the two datasets are similar is applicable, since the output processes of almost all real world systems and simulations are non-stationary and autocorrelated. Furthermore, there is a question of 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. The more appropriate question is to ask 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 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 statistical test for the purpose of comparing PBPK model predictions with experimental data. For now, however, the visual inspection is the most frequently used approach, if not the best approach, to the external evaluation of the performance of PBPK models.

One approach for determining if 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 are set to zero. The increase in goodness-of-fit obtained by allowing those parameters to be non-zero can then be evaluated statistically using a χ^2 statistic, to determine if the additional degrees of freedom afforded by those parameters are justified (Collins et al., 1999).

There is increasing concern about the relevance and usefulness of external evaluation in PBPK modeling, particularly as it relates to humans. External evaluation requires that some of the available pharmacokinetic data not be used during model calibration phase, but kept for evaluating the performance of the model. Not everyone is in agreement with such an approach. Some people believe that all the data used for model evaluation should be used to improve the parameter estimates, so that no data are "wasted" towards that end. Such an iterative approach to

model evaluation and calibration maximizes the use of the available data. This is particularly an issue of relevance to human data since the actual parameters for each individual in a population might be 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 should not only take into account the existing information on parameters, but also be able to accommodate the new information based on fits to additional datasets. In this context, Bayesian analysis is being increasingly explored for use in PBPK modeling (Bois et al., 2000a, b; Jonsson and Johanson 2001, 2002). In the Bayesian approach, the prior information on parameters is updated with 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. Cross validation is another potentially useful approach in this regard (Keys et al., 2003). As used in structure-activity relationship modeling arena (Beliveau et al., 2003), cross validation involves using all the available data sets by repeated sub-sampling. 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 upon the purpose of the model and data availability.

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

3.7. SENSITIVITY, UNCERTAINTY, AND VARIABILITY ANALYSES

The variability, uncertainty, and sensitivity of parameters constituting the PBPK models may need to be evaluated, depending on the intended application(s) of the model.

3.7.1. Sensitivity Analysis

Sensitivity analysis deals with the quantitative evaluation of how parameters or input functions influence the outcome. Such an analysis conducted in the context of risk assessment would identify how each parameter influences the risk estimate and which specific one(s) drive(s) the risk estimate (U.S. EPA, 2001). By identifying the most sensitive parameters, sensitivity analysis facilitates focused use of resources for uncertainty and variability analyses.

Sensitivity analysis in the context of PBPK modeling involves determining the magnitude of change in pharmacokinetic simulations for a defined change in the input parameters. The results of sensitivity analysis are the 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 the 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 PBPK model parameter with respect to the dose metric (i.e., parent chemical AUC in target tissue). In this case, a sensitivity ratio of 2 associated with the breathing rate signifies that 1% change in the numerical value of this parameter will lead to a 2% change in the dose metric (Figure 3-3).

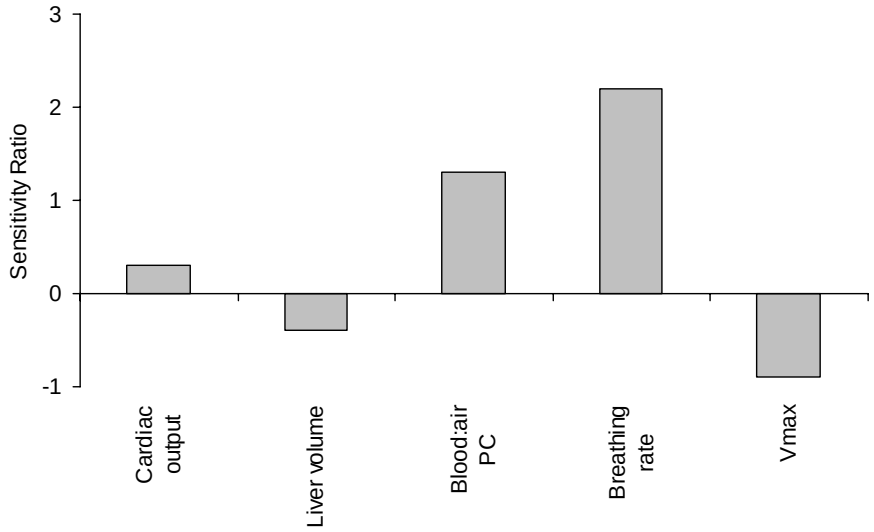

Figure 3-3. Sensitivity ratios associated with certain input parameters of a PBPK model.

Sensitivity analysis is informative for identifying the key parameters that are likely to affect the performance of the model and output such that efficient resource allocation for further research to enhance confidence in the parameter estimates can be done (e.g., Bois et al., 1991; Hetrick et al., 1991; Clewell et al., 1994). A sensitivity analysis is not obligatory for a PBPK

model to be acceptable for risk assessment applications, but it greatly strengthens the credibility of the model and guides resource allocation for risk assessment-oriented research.

One caveat when conducting individual parameter sensitivity analyses (such as described above) 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. 40% 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 V_{max}. 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, while very informative about the importance of individual parameters, over-estimates 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.

3.7.2. Variability Analysis

The focus of a variability analysis is to evaluate the range of values for a parameter expected to be present in individuals of a population and its impact on tissue dose simulations. PBPK models accounting for individual-specific parameters (e.g., enzyme levels, tissue volumes,

body weights, workload) may be constructed to simulate tissue dose variability in populations (e.g., Sato et al., 1991; Dankovic and Bailer, 1994). Alternatively, PBPK models for an average individual representing specific subgroups of populations (e.g., adult women, pregnant women, lactating women, children) may be constructed by accounting for subgroup-specific physiological, biochemical, and physicochemical parameters (e.g., Fisher et al., 1997; Krishnan and Andersen, 1998; Corley et al., 2003; Price et al., 2003b). These analyses, however, would not provide the probability or likelihood of a particular output for a population.

With information such as the tissue dose corresponding to the 95^{th} percentile and 50^{th} percentile, the magnitude of the interindividual variability factor can be computed. For this purpose, Monte Carlo simulation approaches based on prior distributions of input parameters (physiological parameters, enzyme content/activity with or without the consideration of polymorphism) have frequently been used (Thomas et al., 1996; El-Masri et al., 1999; Bogaards et al., 2001; Haber et al., 2002; Lipscomb and Kedderis, 2002; Timchalk et al., 2002; Lipscomb et al., 2003). This method involves repeated computations using inputs selected at random from statistical distributions for each parameter in order to provide a statistical distribution of the output, i.e., dose metric (Figure 3-4). The Monte Carlo approach to variability analysis has also helped in evaluating the net impact of the variability of critical biochemical and physiological parameters (e.g., Portier and Kaplan, 1989).

When conducting variability analysis, it is important that correlations in model parameters be included in the evaluation. 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 $f_{AC} = Q_A/Q$, 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.

More recent research has indicated the potential usefulness of Bayesian framework based on Markov Chain Monte Carlo simulation (Jonsson and Johanson, 2001, 2002). This method combines prior knowledge about parameters and data from new experimental studies to generate posterior parameter distributions, which in turn are used in Monte Carlo simulations for conducting variability and uncertainty analyses (Bois, 1999; Johanson et al., 1999; Bernillon and Bois, 2000).

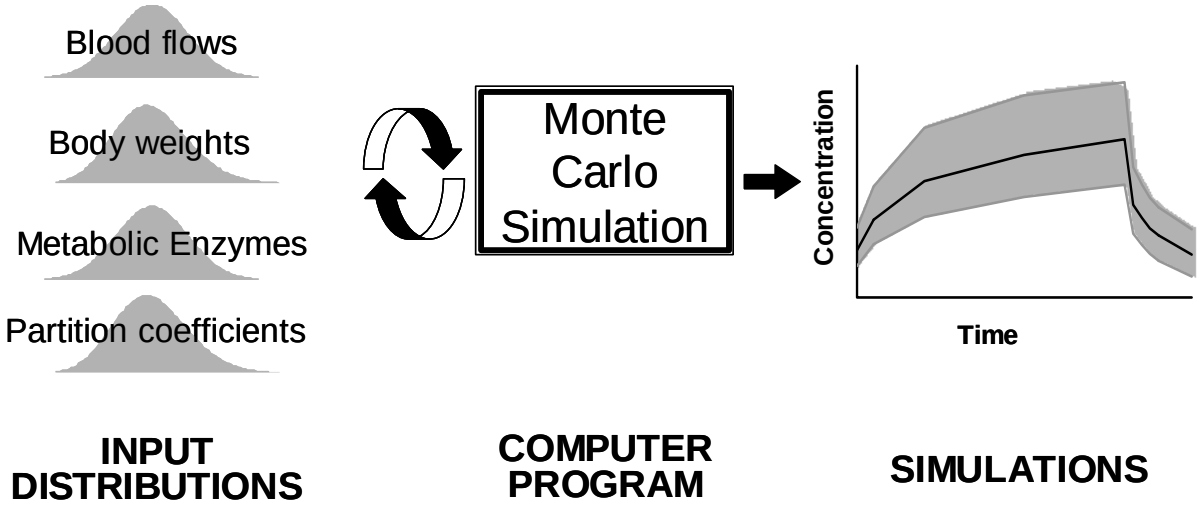

Figure 3-4. Illustration of the use of the Monte Carlo approach for simulating the distribution of internal concentration versus time on the basis of population distributions of PBPK model parameters.

Conducting a variability analysis with 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 factor (pharmacokinetic component).

3.7.3. Uncertainty Analysis

Uncertainty analysis in the context of PBPK modeling refers to the evaluation of the impact of the lack of precise knowledge about the numerical value of a parameter or model structure itself on dose metric simulations. The uncertainty regarding model structure and parameter values may contribute to uncertain predictions of dose metrics, particularly for low-dose exposure situations (Hattis et al., 1990). The 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 inadequacy of the model structure chosen for the study. In these cases, either a quantitative uncertainty analysis or model-directed mechanistic studies should help improve the predictive ability and robustness of the PBPK model (Clewell and Andersen, 1997; Haddad et al., 1998).

Quantitative uncertainty analyses of PBPK model have frequently been conducted using either a Monte Carlo approach or Bayesian framework based on Markov Chain Monte Carlo simulations with respect to specific endpoints (e.g., amount metabolized, tissue concentration of parent chemical at a specific time, cancer estimates) (e.g., Farrar et al., 1989; Krewski et al., 1995; Gelman et al., 1996; Elder, 1999). Alternatively, a stochastic response surface method or a fuzzy simulation approach may be used for uncertainty analysis with PBPK models (Isukapalli et al., 1998; Nesterov, 2001). The latter method is particularly useful when statistical distributions of parameters cannot be defined reliably 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 parameter(s) of the PBPK model should be conducted. Such analysis will provide an indication of the impact on tissue dose simulations of the imprecision or lack of accurate knowledge of a parameter value. However, if a PBPK model has been evaluated using various sets of data (e.g., species, dose levels, routes), then the benefits of uncertainty analysis are limited. Particularly, if the model with its set of parameters has been shown to predict adequately the kinetics of a chemical following various dose levels administered by more than one route in more than one species, conducting a quantitative uncertainty analysis cannot help improve any further the closeness of model predictions to experimental data. In other words, the improvement of the precision of parameter estimates, after a certain level, may not help improve the predictive ability of the model. Models that have been adequately evaluated may therefore be used for risk assessment applications without detailed uncertainty analysis. Where possible and relevant, uncertainty analysis should be performed to strengthen the credibility of the PBPK model and guide resource allocation to risk assessment-oriented research.

The conduct of sensitivity, uncertainty, and variability analyses should be based on acceptable statistical methods. EPA has published guiding principles for Monte Carlo analysis (U.S. EPA, 1997), but there is no such guidance for Bayesian and Markov Chain Monte Carlo methods. When using these methods, 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 should not 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 (Appendix 1). 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 may be obtained using in vitro systems. Other biochemical parameters, such as binding association constants and renal clearance, may be required, depending on the chemical. Additionally, route-specific absorption parameters such as the rate of oral absorption and the skin permeability constant are required for describing oral absorption and dermal absorption, respectively. Of these, the skin permeability coefficient can be obtained using available quantitative structure-activity relationships (QSARs) (Appendix 1). However, 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). The availability of the data set for external evaluation in humans, of course, 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, a parallelogram approach based on surrogate data has been used successfully. This approach uses two data sets (e.g. 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) in order 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 suffices 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 pharmacokinetic data and by structural and metabolic analogy. HCFC-123 was assumed to be able to reasonably simulate the in vivo pharmacokinetics in humans, even though human data were not available for this chemical for model evaluation purposes (Jarabek et al., 1994; Williams et al., 1996). 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 of lack of data on 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 (CH3, CH2, 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 metabolic constants required for constructing a first-generation PBPK model can be obtained. This QSAR approach is useful to initially develop PBPK models for other chemicals, as long as the number and nature of fragments do not exceed the ones in the calibration set used in the study (Beliveau et al., 2003).

Finally, in dealing with data-poor situations, the parameters, algorithms, and references provided in the appendices provide a good starting point. Useful data and equations for PBPK modeling may also be found in reliable electronic resources. For example, Nestorov (1998) outlines a web-based resource for PBPK modeling (http://www.capkr.man.ac.uk). The site provides instant access to resources such as data, methodology, and tools necessary to start a PBPK modeling effort. Another site pioneered by Dr. Loizou (England) will become available in the near future.

3.9. EVALUATION OF PBPK MODELS: SUMMARY

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

- The structure of a PBPK model intended for risk assessment applications should contain the target organ (or a surrogate tissue).

- The MBDEs in PBPK models should have units of quantity per time (e.g., mg/hr) or concentration per time ($mg/L\text{-}hr^{-1}$), and the type of equations chosen to describe ADME should be justified on the basis of hypothetical or known mechanisms of such processes.

- The tissue volumes, flow rates, and ventilation:perfusion ratios specified in the model should be within physiological limits. Particularly, the sum total of the tissue volumes should be within the body weight, and the sum total of tissue blood flow rates should equal cardiac output.

- The power function used for scaling of physiological flows on the basis of body weight should be within 0.67 and 0.75, unless species- or individual-specific data are available.

- The power function used for scaling physiological flows and maximal velocity of metabolism on the basis of body weight should be within 0.67 and 0.75.

- Maximal velocities of metabolism may also be scaled on the basis of body weight, 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 should be obtained from in vivo data, in vitro data, or theoretical algorithms in the application domain.

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

- The PBPK model should be implemented using commercially available software. If the modeler chooses to write his/her own program, then the appropriateness of the integration algorithm and integration interval should be justified.

- It is suggested that the PBPK model code be checked for accuracy of units, mass balance, blood flow balance, and behavior at zero dose. A model used in a risk assessment should be accompanied by sufficient documentation to support an independent evaluation and reconstruction of the model and simulation results. A more rigorous verification that may be considered by the risk assessor is to

independently re-code the model to assure that the documentation is thorough and that there are no "bugs" in the code.

- 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.

4. APPLICATION OF PBPK MODELS IN RISK ASSESSMENT

4.1. CHOOSING PBPK MODELS APPROPRIATE FOR USE IN RISK ASSESSMENT

Whether or not a PBPK model was initially intended for risk assessment purposes, it can be useful for risk assessment if it permits simulations of the tissue and blood concentrations of the toxic moiety (parent chemical or metabolite) associated with the animal toxicity or human epidemiological study serving as the basis for the derivation of health protective values (e.g., RfC, RfD, cancer slope factors). Specifically, the model should be able to simulate the dose metrics in the test species as well as in humans for the exposure route and exposure scenario of relevance. For use in risk assessment, a PBPK model:

- Should have been developed or calibrated for species and life stage of relevance to the risk assessment,
- Must have been peer-reviewed and evaluated for its structure and parameters,
- Should consist of parameters essential for simulating uptake via routes associated with human exposures as well as the critical study chosen for the assessment, and
- Should be able to provide predictions of the time-course of concentration of toxic moiety (parent chemical or metabolite) in the target organ or a surrogate compartment.

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 may not have been developed for the specific life stage and species used in the critical toxicological study forming the basis of a risk assessment. For example, PBPK models for methanol have been developed in rats, monkeys, and humans (Horton et al., 1992; Rogers et al., 1993; Bouchard et al., 2001), yet the critical study appears to be a developmental toxicity study in mice, although a newer two-generation study in rats may also be important (Clark et al., 2004). When the PBPK model has not been developed for the life stage or species used in the study forming the basis of the POD for an assessment, additional

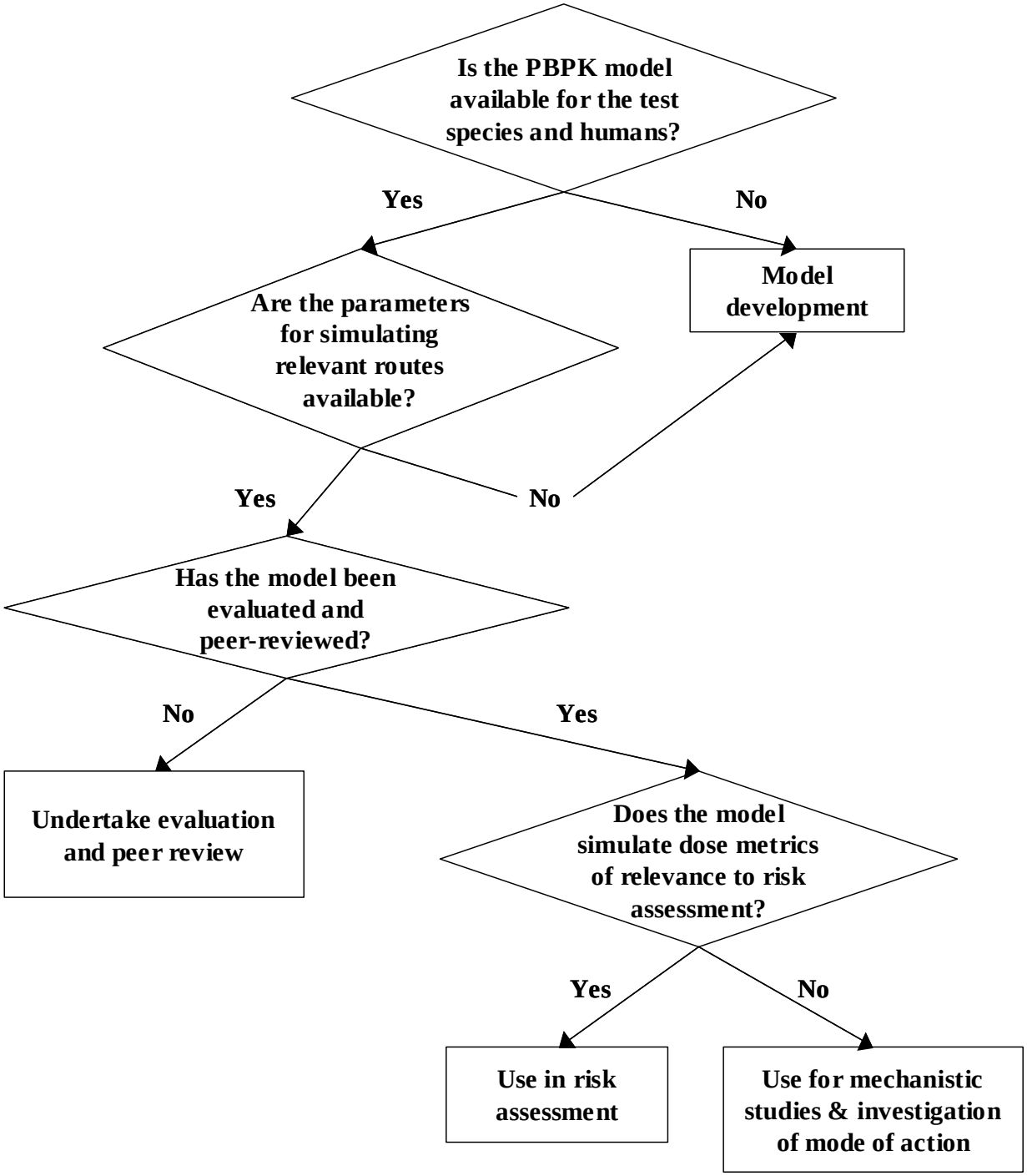

Figure 4-1. Decision tree for selecting PBPK models appropriate for use in risk assessment.

work may have to be undertaken to resolve the situation before the PBPK model can be used for that particular assessment.

The PBPK model for the relevant species and life stage should have been peer-reviewed to determine the adequacy of the structure, parameter estimation methods, and the evaluation efforts. If the PBPK model for a chemical is available in the species and life stage corresponding to the study forming the basis of an assessment but it has not been peer-reviewed, then efforts may be directed towards such a review. Box 4-1 enlists the fundamental questions and issues to be considered while conducting a peer-review evaluation of the adequacy of PBPK models intended for risk assessment applications. Finally, the peer-reviewed model(s) chosen for risk assessment applications should 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.

Peer-reviewed PBPK models that facilitate the prediction of dose

Box 4-1. Questions and issues to be considered while evaluating the adequacy of a PBPK model

- Is an appropriate target organ or a surrogate tissue identified as one of the model compartments?
- Are the known major sites of storage, transformation, and clearance included in the model structure?
- Is the form of equation used to describe chemical uptake justified on the basis of hypothesis of tissue uptake of the chemical?
- Have enzymatic processes been described appropriately? (If simulated exposure levels are well below saturation, use of first-order kinetics is acceptable.)
- Are the units throughout differential equations consistent (e.g., concentration should not be in mg/ml in one place and μM in another)?
- Are the input parameters related to the characteristics of the host, chemical, or environment?
- Is the sum total of compartment volumes within 100% of the body weight?
- Do the tissue blood flow rates add up to the cardiac output?
- Is the ventilation:perfusion ratio specified in the model within physiological limits?
- Is the volume of each tissue compartment within known physiological limits?
- Is the approach used to establish partition coefficients valid?
- Is the method used for estimating biochemical parameters adequate?
- Is the allometric scaling done appropriately?
- Is the integration algorithm used in the study known for solving differential equations and dealing with stiff and nonstiff conditions?
- Does the shape of the pharmacokinetic curve generated by the model match that obtained experimentally?

metrics of chemicals for relevant exposure route and life stage of species used in the critical study as well as humans are a prerequisite for their use in risk assessments.

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.

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, 1994, 2005a). The criteria and issues associated with the selection of PBPK models useful for risk assessment were considered in the previous section. 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 closely associated with the toxicity, varies from chemical to chemical, depending on the MOA and critical effect. 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 end point of concern. Analysis of available information on MOA of a chemical along with descriptive toxicity data from various studies, including those using inhibitors or inducers of hepatic metabolism, should be useful in identifying the potential toxic moiety (parent chemical or metabolite). Further, the available data on closely related chemicals may be used to infer the likely toxic moiety. If the induction of toxic effects by a chemical is not altered following treatment with a specific inhibitor of hepatic metabolism, then it would implicate the parent chemical as the toxic moiety. Alternatively, if the induction of the rate of metabolism enhances the extent of toxic effects produced, it would indicate the metabolite as the toxic moiety. Similarly, the toxicity data for various exposure routes and modes of administration may be compared to infer the potential toxic moiety (IPCS, 2001).

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

For chronic effects of chemicals, the integrated concentration of the toxic form of chemical in target tissue over time (i.e., AUC) is often considered a reasonable dose metric (Andersen et al., 1987; Collins, 1987; Voisin et al., 1990; Clewell et al., 2002). 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 hours) have been used as dose metrics (Andersen and Dennison, 2001). For developmental effects, the dose surrogate is defined in the context of window of susceptibility for a particular gestational event (e.g., Welsch et al., 1985). 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 of the toxic moiety, duration and extent of receptor occupancy, macromolecular adduct formation, or depletion of glutathione) (Clewell et al., 2002). Table 4-1 lists the dose metrics used successfully in a number of PBPK-based cancer and noncancer risk assessments.

When the appropriate dose metric cannot be identified readily, the evaluation of the relationship with the end point of concern should be undertaken with each of the dose metrics in order to identify the one that exhibits the closest or the best association (e.g., Andersen et al., 1987; Kirman et al., 2000). This becomes particularly important when there are little 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

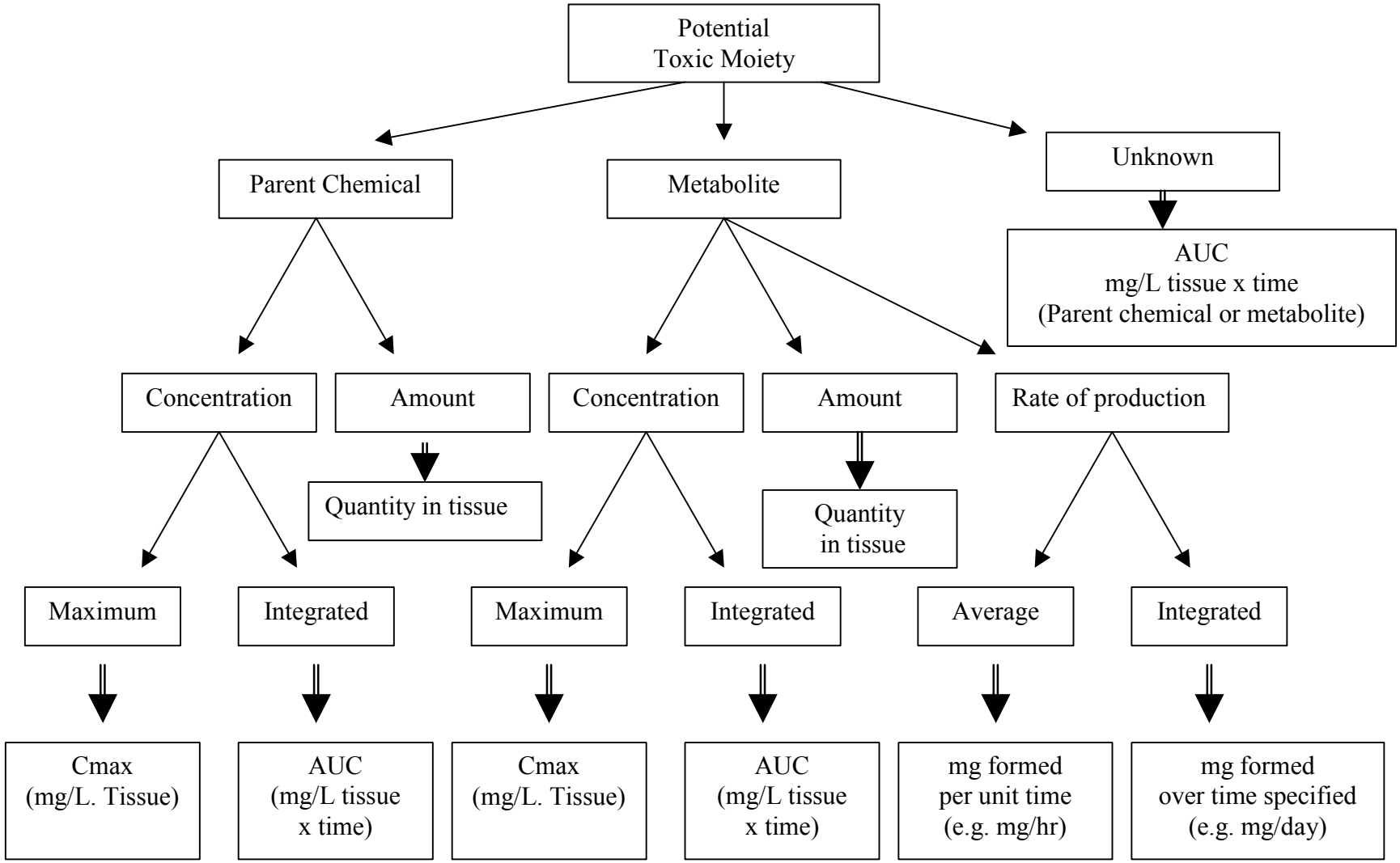

Figure 4-2. Examples of measure of tissue exposure to toxic moiety for risk assessment applications.

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	Levels of butoxyethanol/ butoxy acetic acid in forestomach	Poet et al. (2003)
Chloroform	Liver cancer Hepatic effects and kidney tumor	Amount of metabolites covalently bound to biological macromolecules per L liver per day; % cell kill/day Maximal rate of metabolism per unit kidney cortex volume	Reitz et al. (1990) Meek et al. (2002)
Chloropentafluorobenzene	Liver toxicity	AUC of parent chemical in liver	Clewell and Jarnot (1994)
Dichlorofluoroethane	Cardiac sensitization	Concentration of parent chemical at the end of exposure	Vinegar and Jepson (1996)
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)
Formaldehye	Cancer	DNA-protein crosslinks	Casanova et al. (1996); Schlosser et al. (2003)
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. (1992, 1993)
		Maximal concentration of parent chemical (g, 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)
Methyl methacrylate	Nasal lesions	Amount metabolized/time	Andersen et al. (1999, 2002)

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

Chemical	Endpoint	Dose metric	Reference
Methylene chloride	Cancer	Rate of glutathione metabolites produced/L liver/time	Andersen et al. (1987)
Pentafluoroethane	Cardiac sensitization	Concentration of parent chemical at the end of exposure	Vinegar and Jepson (1996)
Styrene	Lung tumors (mouse)	Steady state concentration of ring oxidation metabolite mediated by CYP2F	Cruzen et al. (2002)
TCDD	Biochemical responses	Body burden	Kim et al. (2002)
	Cancer risk	Time-weighted receptor occupancy Up/down regulation of receptor occupancy Number 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 & Clewell (2000)
	Cancer (liver lung and kidney)	Amount metabolized/kg/day; AUC for trichloroacetic acid or dichloroacetic acid in plasma; production of thioacetylating intermediate from dichlorovinylcysteine in kidney	Fisher and Allen (1993); Clewell et al. (2000)
Trifluoroiodomethane	Cardiac sensitization	Concentration of parent chemical at the end of exposure	Vinegar and Jepson (1996)
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. (1999, 2001)
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

other words, the level of the dose metric should be lower for exposure conditions with no effect and higher for toxic exposures, regardless of the route and exposure scenario (Clewell et al., 2002).

Where there is inadequate basis for giving priority to one dose metric over another, the most conservative one (the dose metric producing the highest risk or lowest acceptable exposure level) should be used in order to be health protective (Clewell et al., 2002). The use of appropriate dose metric helps 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.

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

4.3. EXAMPLES OF THE USE OF PBPK MODELS IN RISK ASSESSMENT

4.3.1. 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, 1997). 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 inactivation, depletion of glutathione reserves, and binding to macromolecules, have also been described with PBPK models (e.g., Clewell and Andersen, 1997; Krishnan et al., 1992). A PBPK model intended for use in high-dose to low-dose extrapolation should have the equations and parameters describing dose-dependent phenomena if dose-dependence occurs in the range of interest or 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 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 4-3. 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 4-3 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; rather it should be performed using tools such as the PBPK models.

4.3.2. Interspecies Extrapolation

The application of PBPK models for interspecies extrapolation of tissue dosimetry is performed in several steps. First, a rodent model is developed to describe the uptake and disposition of the chemical in question by integrating information on the physiological, biochemical, and physicochemical parameters. Then, *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. The next step involves using species-specific or allometrically scaled physiological parameters in the model and replacement of the chemical-specific parameters 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).

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

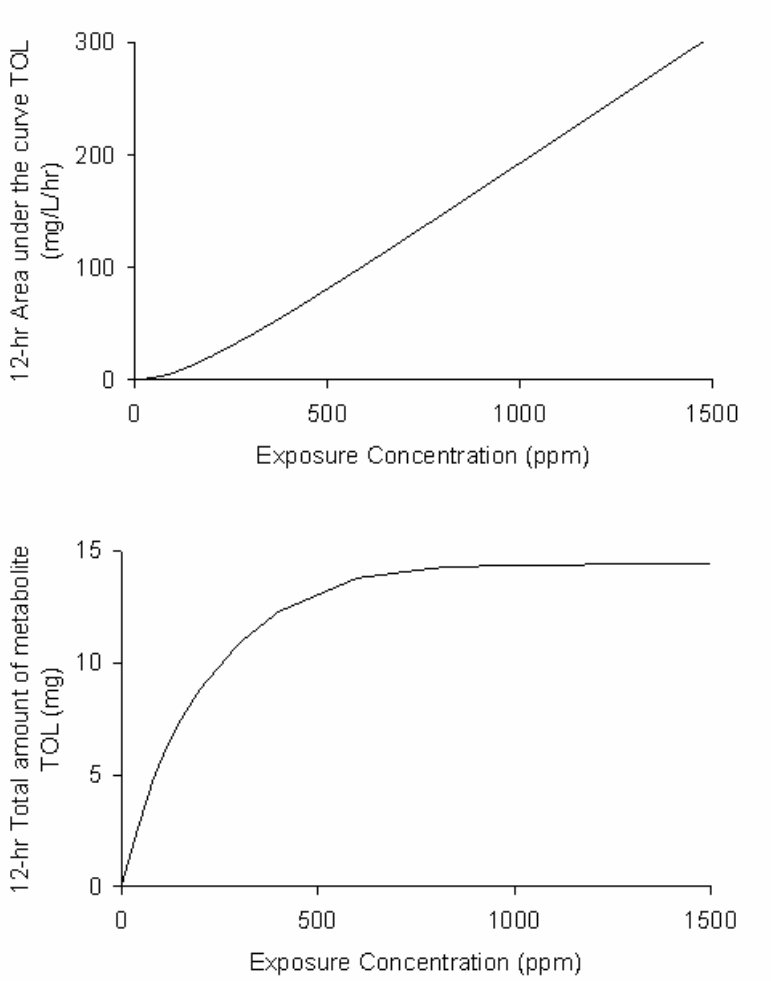

Figure 4-3. High-dose to low-dose extrapolation of dose metrics using PBPK model for toluene. Inhalation exposures were for 4 hr and AUCs and amount metabolized were calculated for 12 hr. Based on Tardif et al. (1997).

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 should be 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 (Reitz et al., 1988b; Lipscomb et al., 1998). Alternately, 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 should 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. 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 (Figure 2-2). 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).

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 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 adding 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 is 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 inhalation $\rightarrow$ oral extrapolation

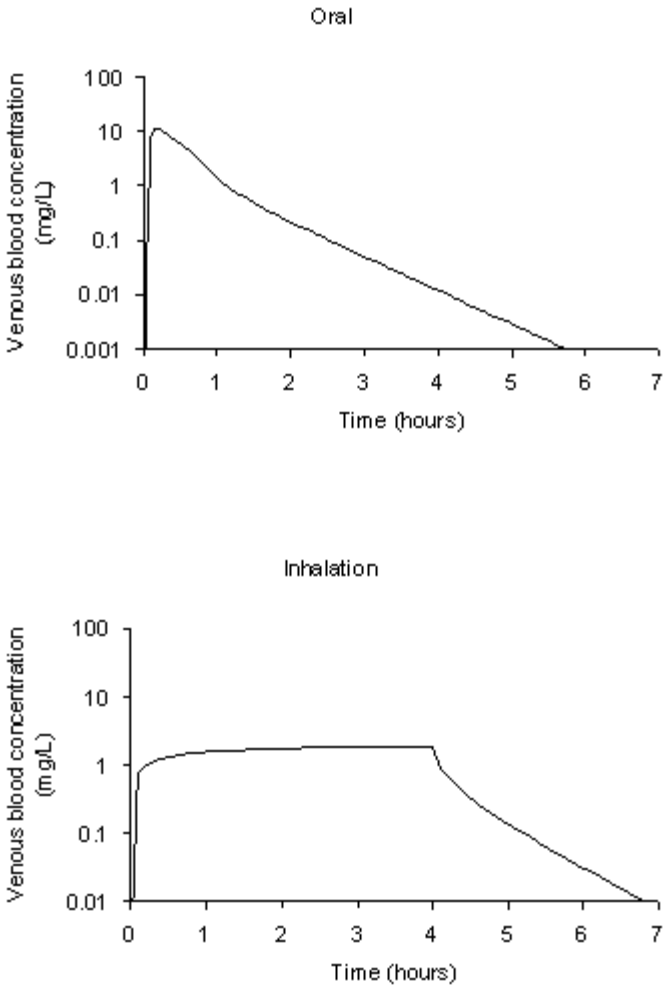

Figure 4-4. Oral-to-inhalation extrapolation of the pharmacokinetics of chloroform on the basis of same AUC in blood (7.06 mg/L-hr). The oral dose was 1 mg/kg and the inhaled concentration was 83.4 ppm (4 hr). Based on Corley et al. (1990).

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).

4.3.4. Duration Adjustment

On the basis of equivalent dose metric, the duration-adjusted exposure values can be obtained with PBPK models (Andersen et al., 1987; Brodeur et al., 1990). Accordingly, the AUC of a chemical for the exposure duration of the critical study is determined initially 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.

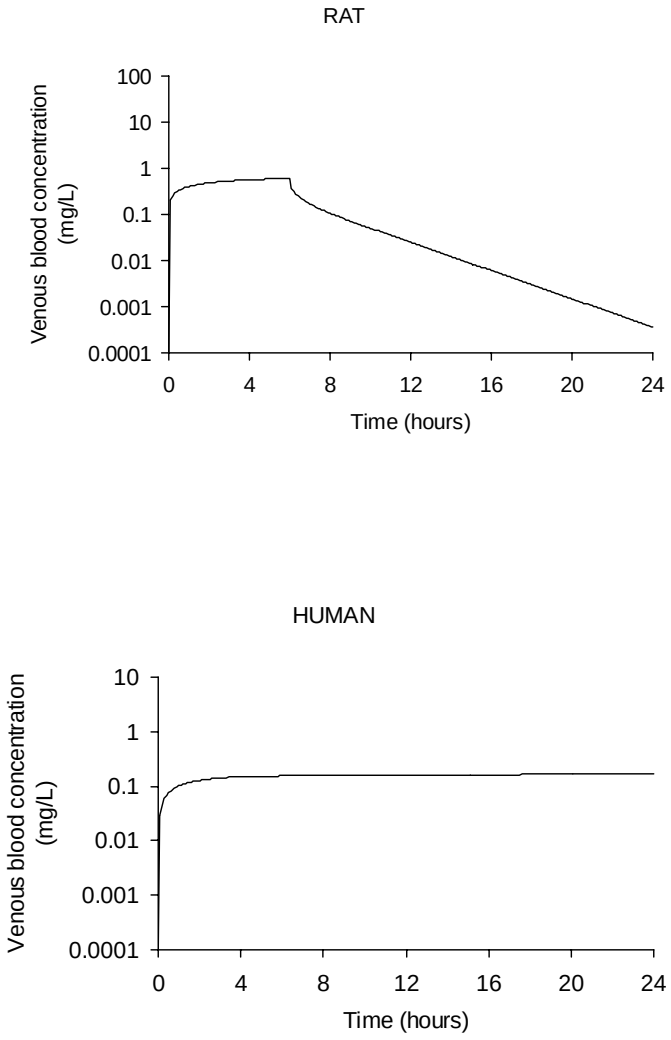

Figure 4-5. Duration adjustment (4 hr to 24 hr) of toluene exposures in rats, based on equivalent AUC (2.4 mg/L-hr). The rats were exposed to 50 ppm toluene for 4 hr and 9.7 ppm for 24 hr. Based on Tardif et al. (1997).

4.3.5. Intraspecies Extrapolation

Intraspecies extrapolation of the dose metric is conducted using PBPK models with the sole intent of estimating the magnitude of the interindividual variability factor (pharmacokinetic component) for RfC and RfD derivations. In this regard, the population distributions of parameters, particularly those relating to physiology and metabolizing enzymes, 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 (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 the 95th percentile and 50th percentile for unimodel, normal distribution (Naumann et al., 2001), the magnitude of the inter-individual variability factor can be computed for risk assessment purposes (Figure 4-6).

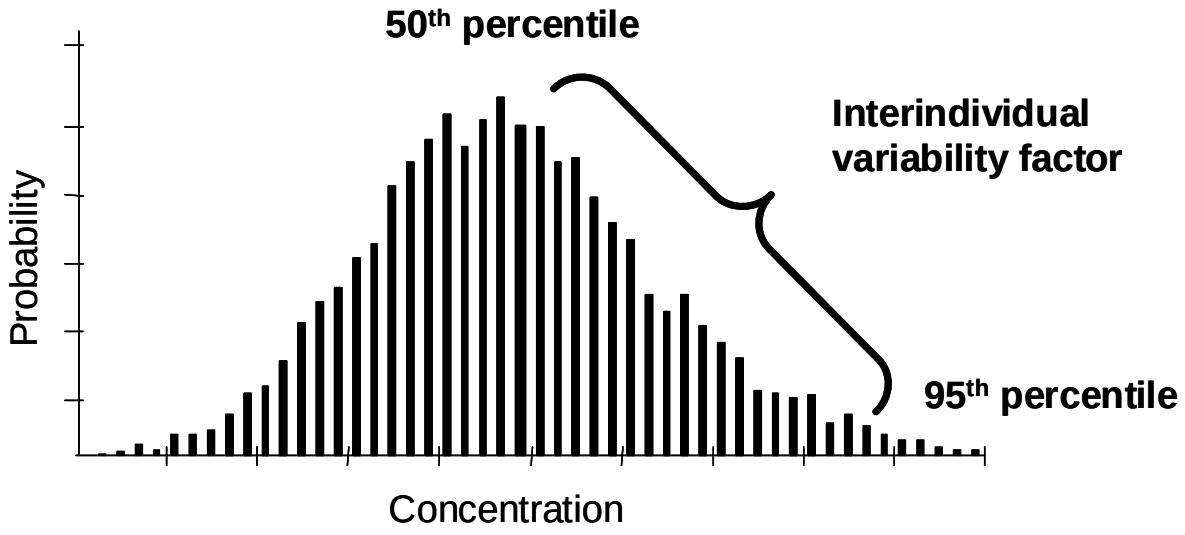

Figure 4-6. Estimation of the interindividual factor from the 50th (median) and 95th percentile values of a dose metric simulated with a probabilistic PBPK model.

Even though several past efforts have characterized the impact of the distributions of parameters in adult population, such variability analyses should also account for the life stage-specific changes in physiology, tissue composition, and metabolic activity (reviewed in O'Flaherty, 1994, Corley et al., 2003).

4.3.6. RfD Derivation

When a PBPK model is available for the oral route in test species and adult humans, RfD derivation has been accomplished as follows (e.g., U.S. EPA, 1999b):

1. The PBPK model with the parameters of the test species is exercised to determine the dose metric associated with the POD (NOAEL, LOAEL, or BMD),
2. The human PBPK model is exercised to determine the oral dose that is associated with the dose metric established in step (1) above, and
3. The resulting oral dose is divided by the appropriate uncertainty factors to establish the RfD.

A variant of the above approach involved dividing the dose metric associated with the POD by the uncertainty factors before exercising the human model to determine equivalent doses (e.g., Gentry et al., 2002). The outcome is essentially the same if the uncertainty factors are being applied to doses (internal or external) in the linear range of pharmacokinetic processes in the species of interest. Clewell et al., (2002) suggest that the uncertainty factors are more appropriately applied to the dose metric rather than to the external dose. The difference in outcome between the two approaches is likely to exist if the POD is in the partially or completely saturating range. Because the Agency has traditionally applied the uncertainty factors to the external dose and not to the internal dose, it may be useful to undertake a systematic evaluation of the outcome of applying the uncertainty factors to the external and internal doses for various chemicals and situations.

In the PBPK model-based approach to RfD derivation, the magnitude of uncertainty factors used is identical to that of the conventional approach except that the animal-to-human uncertainty factor is reduced to three to account for the pharmacodynamic aspect (e.g., Clewell et al., 1997; Gentry et al., 2002).

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-d) was provided as input to the PBPK model to determine the maximal concentration of the metabolite, butoxy acetic acid in blood (BAA_{max}) (Corley et al., 1997). The dose metric (BAA_{max}) associated with 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-d was divided by the appropriate uncertainty factors (30; 10 for interindividual differences and 3 for LOAEL-to-NOAEL extrapolation) to establish the RfD (0.3 mg/kg-d). 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-d). Using the appropriate uncertainty factor (10 for interindividual variability), the RfD was derived (0.5 mg/kg-d) (U.S. EPA, 1999b). If the human PBPK model was probabilistic in nature, accounting for the population distribution of parameters (biochemical, physiological, and physicochemical), the pharmacokinetic component of the interindividual variability factor could be addressed. Such a PBPK model-based assessment of interindividual variability factor in the derivation of the RfD has been shown with methyl mercury (Clewell et al., 1999).

4.3.7. RfC Derivation

When an inhalation PBPK model is available for the test species and humans, the HEC is derived as follows:

1. The PBPK model with the parameters of the test species is run to determine the dose metric associated with the POD (unadjusted or duration-adjusted NOAEL, LOAEL, BMC), and

2. The PBPK model with the parameters for an average individual is run to determine the continuous exposure concentration that is associated with the dose metric established in step (1) above.

Subsequently, appropriate uncertainty factors are used the same way as in the conventional approach (e.g., Clewell et al., 1997; Gentry et al., 2002; also see Section 4.3.6).

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/d, 5 d/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 uncertainty factors (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-d). Then, the human PBPK model was exercised to determine the continuous exposure concentration associated with the same dose metric (2.5 mg/m^3) (Clewell et al., 1995). Using a total uncertainty factor of 30 (3 for toxicodynamic component of IUF, 10 for interindividual variability factor), the RfC was established (0.1 mg/m^3).

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 factor can be estimated (Delic et al., 2000). In that case, the interindividual variability factor will be set to 3 (to account only for pharmacodynamic differences).

4.3.8. Cancer Risk Assessment (unit risk estimates, RfC, and RfD)

When data for the MOA in a cancer risk assessment suggest a threshold (curvilinear) dose-response relationship, the applications of PBPK models are similar to those in the reference dose process (i.e., RfD and RfC derivation). If a linear dose-response model is more applicable, or the MOA is unknown, then the following steps describe uses for a PBPK model (e.g., Andersen et al., 1987):

1. The relationship between administered dose or exposure concentration and dose metric is established for the test species using PBPK model,

2. The quantitative relationship between dose metric and the cancer incidence observed in the bioassay(s) is characterized to estimate the dose metric-based slope factor, and

3. The potential doses or exposure concentrations yielding the dose metric associated with various levels of risk (e.g., 1×10^{-6}) are back-calculated using the human PBPK model.

For assessing the cancer risk associated with human exposures, the exposure concentration is provided as input to human PBPK model to simulate 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/d, 5 d/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 is 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.

The model prediction of the target tissue dose of the glutathione conjugate resulting from 6-hour inhalation exposures to 1–4,000 ppm dichloromethane is presented in Figure 4-7 (Andersen et al., 1987). 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

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

Exposure (ppm)	Microsomal pathway dose[a]		Gluthathione pathway dose[a]		Tumor prevalence	
	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).

Source: Adapted from Andersen et al. (1987).

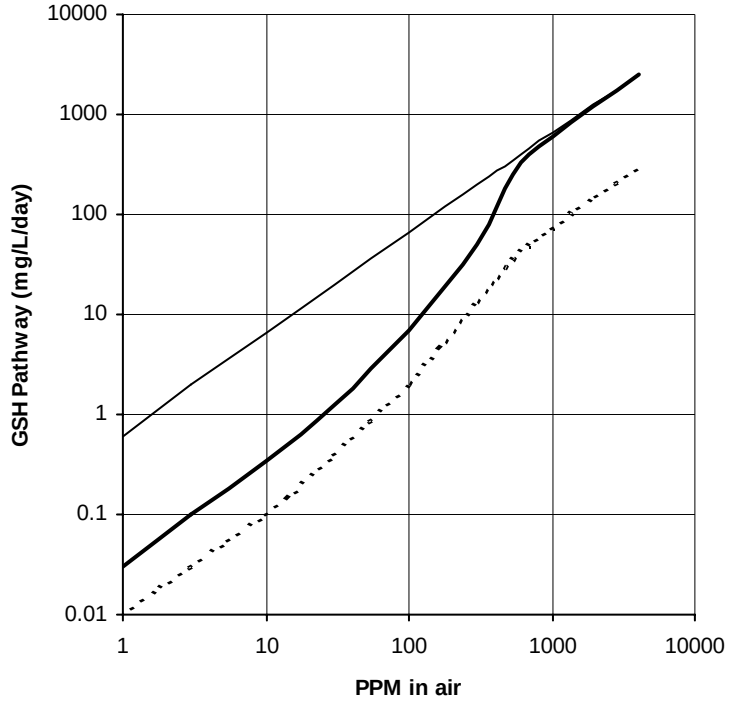

Figure 4-7. PBPK model predictions of glutathione-pathway metabolites in liver in mice. The three curves are for a linear extrapolation from the bioassay exposures of 2,000 and 4,000 ppm (upper curve), the expected tissue dose-based on model parameters for the mouse (middle line), and the expected dose expected in humans, based on human model parameters (bottom line). Similar curves were published in Andersen et al. (1987). The curvature occurs because oxidation reactions that are favored at low inhaled concentrations become saturated as inhaled concentration increases above several hundred ppm.

dichloromethane metabolism at high-exposure concentrations. At exposure concentrations exceeding 300 ppm, the 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 (Andersen et al., 1987; Reitz et al., 1989; Haddad et al., 2001). The case of dichloromethane exemplifies how PBPK models can be used for improving 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.

4.3.9. Mixture Risk Assessment

PBPK models permit the risk assessment of chemical mixtures by facilitating the simulation of change in dose metrics due to multichemical interactions (Haddad et al., 2001). For conducting tissue dosimetry-based assessments for mixtures, adequately evaluated PBPK models for the mixture in the test species and in humans are required. Further, the health-protective values for the individual chemicals (e.g., slope factor, RfD, RfC) should be 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., 2001):

1. Characterizing the dose metrics associated with the RfC or RfD of 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.

Notationally, for chemicals in mixture acting on the same target organ:

$$HI = \sum_{i=1}^{n} \frac{C_{tissue,\exp,i.}}{C_{tissue,ref,i.}}$$

where HI is the hazard index, $C_{tissue,exp,i}$ refers to the tissue concentration of the dose metric of component *i* predicted in the experimental animal at the POD using PBPK model that accounts for multiple interactions occurring in the mixture, and $C_{tissue,ref,i}$ is the dose metric associated with human exposure to the RfD or RfC of component *i*.

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

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

Notationally,

$$P(d) = \sum_i q^*_{i,tissue} \cdot d_{i,tissue}$$

where P(d) is the probability of excess cancer incidence in an exposed population, $q^*_{i,tissue}$ = dose metric-based cancer slope factor for each of the mixture components (i = 1,2, …, n), and $d_{i,tissue}$ refers to the dose metric for each mixture component simulated using PBPK models that account for multi-chemical interactions.

Risk assessments based on the use of PBPK models for single chemicals and mixtures, as detailed in Section 4.3, 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.

4.4. 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 researchers' 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 difference equations to simulate time-dependent changes in the number of hepatocytes in the liver as a function of basal

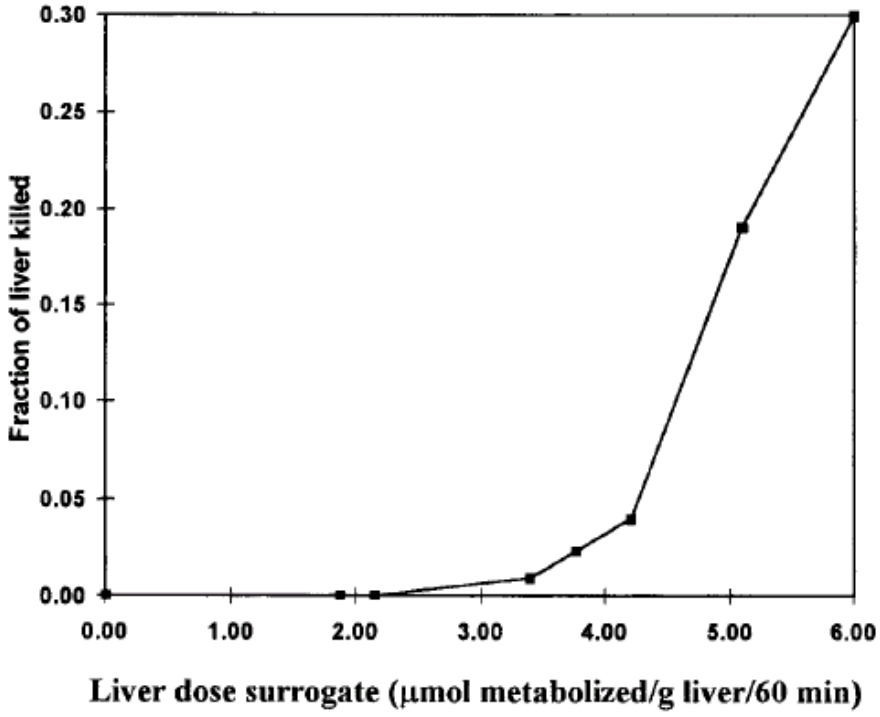

Figure 4-8. Relationship between the dose metric (μmol metabolized/g liver/hr) simulated by PBPK model and the cell killing inferred from pharmacodynamic model for chloroform.

Source: Page et al. (1997).

rates of cell division and death, chloroform-induced cytolethality, and regenerative replications (Conolly and Butterworth, 1995; Page et al., 1997).

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. The pharmacodynamic models, for linkage 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.

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

Toxicity end point or process	Features	Chemical studied	References
Cancer	Simulation of relative roles of initiation, promotion, cyclothality, and proliferation	2-acetylamino fluorine Chloroform Dimethylnitrosamine Formaldehyde PCBs Pentachlorobenzene Saccharin	Armitage and Doll (1957); Moolgavkar and Venzon (1979); Moolgavkar and Knudson (1981); Cohen and Ellwein (1990); Moolgavkar & Luebeck (1990); Luebeck et al. (1991); Chen (1993); Conolly and Andersen (1997); Conolly and Kimbell (1994); Thomas et al. (2000); Conolly et al. (2003); Tan et al. (2003)
Cholinesterase inhibition	Simulation of dose-dependent inhibition of plasma cholinesterase, red blood cell acetyl cholinesterase and brain acetyl cholinesterase, and nontarget B-esterase	Organophophates	Gearhart et al. (1990, 1994); Timchalk et al. (2002)
Developmental toxicity	Simulation of altered cell kinetics as the biological basis of developmental toxicity	Methyl mercury	Leroux et al. (1996); Faustman et al. (1999)
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	TCDD	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: 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 model: A predictive model that describes biological processes at the cellular and molecular level linking the target organ dose to the adverse effect.

Cancer scope factor: 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 interactions with biologically significant receptors in the target organ.

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.

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.

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 (i) sample each model's parameters from their prior distributions, (ii) fit the model with the sampled parameters to several additional experimental data sets, and (iii) 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. 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.

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: 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 NOAEL or LOAEL 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.

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.

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/variability factors: Generally 10-fold, default factors used in operationally deriving the RfD and RfC from experimental data. The factors are intended to account for (i) variation in sensitivity among the members of the human population (i.e., interindividual variability), (ii) uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty), (iii) 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), (iv) uncertainty in extrapolating from a LOAEL rather than from a NOAEL, and (v) 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.

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