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Physiologically Based Pharmacokinetic Model Use in Risk Assessment—Why Being Published Is Not Enough

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A panel of experts in physiologically based pharmacokinetic (PBPK) modeling and relevant quantitative methods was convened to describe and discuss model evaluation criteria, issues, and choices that arise in model application and computational tools for improving model quality for use in human health risk assessments (HHRAs). Although publication of a PBPK model in a peer-reviewed journal is a mark of good science, subsequent evaluation of published models and the supporting computer code is necessary for their consideration for use in HHRAs. Standardized model evaluation criteria and a thorough and efficient review process can reduce the number of review and revision iterations and hence the time needed to prepare a model for application. Efficient and consistent review also allows for rapid identification of needed model modifications to address HHRA-specific issues. This manuscript reports on the workshop where a process and criteria that were created for PBPK model review were discussed along with other issues related to model review and application in HHRA. Other issues include (1) model code availability, portability, and validity; (2) probabilistic (e.g., population-based) PBPK models and critical choices in parameter values to fully characterize population variability; and (3) approaches to integrating PBPK model outputs with other HHRA tools, including benchmark dose modeling. Two specific case study examples are provided to illustrate challenges that were encountered during the review and application process. By considering the frequent challenges encountered in the review and application of PBPK models during the model development phase, scientists may be better able to prepare their models for use in HHRAs.

Key Words: **PBPK; benchmark dose; risk assessment; Markov chain Monte Carlo; ontology.**

Pharmacokinetics (PKs) involves the study of the movement over time of parent chemical and its metabolite(s) in biological fluids, tissues, and excreta (Wagner, 1981); mathematical models, such as physiologically based pharmacokinetic (PBPK) models, are often constructed to interpret PK data. The disposition of the parent chemical and its metabolite(s) is dependent on rates of absorption, distribution, metabolism, and excretion (ADME). In turn, ADME rates are used in mathematical constructs to develop quantitative estimates for temporal concentrations of chemicals in target tissues where pharmacological or toxicological responses are observed.

Human health risk assessments (HHRAs) use the dose-response relationship to characterize and quantify potential health risks. Using target tissue, dose estimates, instead of external or applied doses, can improve the characterization of dose-response relationship and subsequent characterization of potential health risks. This improvement results from a direct relationship between internal dosimetry to biological response. When relevant and reliable estimates of internal dose of a compound or a key metabolite are available, the results of toxicology studies can often be better understood and evaluated in terms of the internal dose. Using an administered dose to characterize a dose-response relationship bypasses many critical ADME processes. Additionally, understanding ADME leads to a more complete use of biological and toxicological data to support route-to-route and animal-to-human extrapolation of dose-response information.

The selection of a dose metric is a key element to establishing an appropriate dose-response relationship. The dose metric is used to estimate the point of departure (POD) for the critical effect, and the POD is the dose at which the low-dose extrapolation begins in the HHRA (U.S. Environmental Protection Agency [EPA], 2011). Adjustments to the POD are made to account for uncertainties and to protect the most sensitive human population at risk of exposure to the chemical(s). This process frequently involves the conduct of interspecies, intraspecies, high to low dose, duration, and exposure route extrapolations from experimental data. In almost all cases, dose metric data associated with human exposures to environmental chemicals are not available. Additionally, available animal PK data may not correspond to the active toxic moiety relevant to

route or duration of exposure. In the absence of this type of data, PK models provide a quantitative format to evaluate extrapolation questions. PK models are classified as compartmental and noncompartmental (Renwick, 1994). Compartmental models that include physiological descriptions of biological tissues and processes describing ADME of chemicals (where key parameters, such as tissue volumes, are measured independent of chemical-specific PK data) are usually referred to as PBPK models. By integrating chemical-specific dosimetry data with physiological data and constraints, the uncertainty that exists regarding these extrapolations, and hence the resulting human risk estimates, can be reduced.

The increased use of PBPK modeling in HHRA, particularly in a regulatory context, has resulted in the need for expanded formal guidance on the use of PBPK models in these HHRAs. Several guidance documents have been released in an attempt to standardize the review and implementation of PBPK models in regulatory HHRAs (U.S. EPA, 2006; World Health Organization, 2010). The following areas of PBPK model evaluation with respect to use in HHRA have been delineated: model purpose, model structure, mathematical representation, parameter estimation, computer implementation, predictive capacity, statistical analyses, and model documentation (U.S. EPA, 2006). Because of their potential regulatory impact, it is critical that the PBPK models be systematically and fully evaluated and the model application in the HHRA be carefully considered and executed. Determination of model applicability for a given chemical and endpoint should consider what is known about the mode of action and relevant dose metrics. The decision as to whether or not to use a model is specific to the context for which the model application is being considered.

Recently, a panel of experts in PBPK modeling and relevant quantitative methods for application of models in HHRAs was convened (This manuscript is a report on the workshop held at the Society of Toxicology 2011 Annual Meeting [Washington, DC; 7–10 March 2011] on the topic of PBPK model use in risk assessment.) to discuss recurring challenges faced by PBPK model reviewers and risk assessors when attempting to utilize a PBPK model in an HHRA. Overall, the availability of model code was determined to be a key factor in determining whether a published PBPK model may be applied in an HHRA. When the expert reviewers acquire and review the code, several additional challenges may be encountered, including machine interpretability, model parameterization, and integration of the PBPK model with other HHRA methodologies, such as benchmark dose (BMD) modeling. The following sections describe these issues in more detail. Figure 1 highlights the key components of the continuum from model development to evaluation and application in an HHRA.

A Process for the Evaluation and Implementation of PBPK Models in HHRAs

PBPK models are developed to generate or test hypotheses and may be developed for a potential risk assessment application.

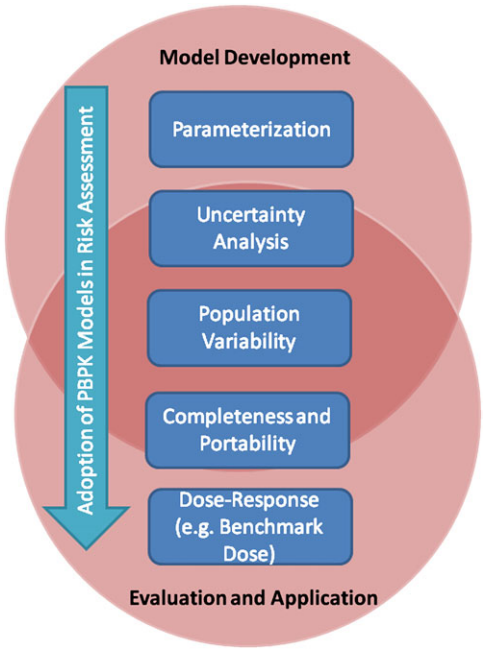

FIG. 1. This figure shows examples of key considerations during model development, evaluation, and application that are necessary before a PBPK model may be adopted for use in a HHRA.

However, these applications are frequently unknown at the time of model development. Thus, it is important for models that are developed and published in the peer-reviewed literature to undergo a rigorous review and evaluation process so that their suitability for a specific application in an HHRA can be determined.

To evaluate PBPK models, knowledge of the modeling process and approach as well as biology and the toxicity of the chemical in question are useful. The process for evaluating PBPK models for application in HHRA begins with extensive discussions with a scientist(s) knowledgeable about the biology and toxicology related to the compound. Specifically, these discussions aim to identify toxicity endpoints of concern, experimental data available for the endpoint of concern, and the choice of dose metric(s) and its relationship to mode of action. These discussions are critical for the review of the available PBPK models and the evaluation of their appropriateness for application in the risk assessment process. In general, publication of a PBPK model does not necessarily deem it usable for risk assessment if the extent or impact of its application is found to be problematic. For example, if a model is developed for one route of exposure while the risk assessment may be focused on a different route, modifications to the model may be needed. Other examples include when an older PBPK model does not include up-to-date information on metabolic pathways or includes fitted parameters where newer experimental data may provide different parameter values. In other situations, a review of the model may reveal a need for refinements or revisions to the published model to justify its

application for the HHRA. During this first stage of model review, the model reviewers and the scientist leading the HHRA for the chemical(s) in question may work together to identify the extent of modifications needed for the model to be applied, the resources needed to complete the modifications, and the duration needed for completion of these modifications to aid management in making a decision to move forward with additional model evaluation and potential application.

The second tier of model evaluation is the in-depth analysis the model. The focus of the in-depth evaluation is based on the applicability of the model for the specific risk assessment use rather than its scientific validity. For the in-depth analysis, in addition to published model simulations, the model code mathematical descriptions and parameters need to be publicly available to the evaluating agency.

In general, a PBPK model is made up of set of mathematical equations describing *in vivo* ADME of the chemical in question. Each mathematical equation (such as Equation 1 below) is based on the biology (e.g., first order uptake and/or removal, saturable metabolism) and quantitative parameter estimation (e.g., physiological, physiochemical, and biochemical).

$$\frac{dA_t}{dt} = Q_t \times C_{\text{art}} - Q_t \times \left(\frac{A_t}{V_t \times P_t}\right) - \left(\frac{V_{\max} \times C_t}{K_m + C_t}\right) \quad (1)$$

The form of the mathematical equation is based on understanding of the physiological behavior of the chemical. For example, a saturable Michaelis-Menten (MM) equation for metabolism is based on the knowledge of receptor-binding mechanisms. The MM equation can be modified to allow for biological processes of inhibition or induction of metabolism. Another example is a Hill equation, which can also be based on receptor-binding mechanism but includes parameters representing the extent of positive or negative biological cooperativity in the binding process. Both equations have parameters that need to be identified quantitatively so that the overall model can be used for simulating and predicting data.

The initial step in the PBPK model structure evaluation is reviewing the choice of model compartments. Questions such as number of compartments, type of tissue compartments, and flow-limited or diffusion-limited choice of compartments are assessed against knowledge of the physiochemical properties of the chemical and physiological determinants of its distribution. This is usually followed by in-depth analysis of the choice of equation form in relationship to knowledge of the mode of action of the chemical. For this reason, questions regarding metabolism pathways are raised and checked against the mathematical description of the model. Once the structure of the model is evaluated, the model parameterization and behavior are reviewed. Parameters that are obtained or calculated from literature are checked and ones that are fitted against available data are examined using original and more recent data if available.

TABLE 1
Information or Items That May Contribute to or Reduce Model Uncertainty

Contribute to model uncertainty	Reduce model uncertainty
Biological basis of model development is questionable	Model is based on ''known'' biological mechanisms
Flow/diffusion or active transport mechanisms, could all fit data	Toxic metabolites
Extrahepatic metabolism (other tissues or by other enzymes).	Route of exposure
Species differences	Physiological determinants of tissue dose
Parameter identifiability	Parameters have biological relevance: they can be tested or calculated
Calibration data are not sensitive to parameters	Calibration data are helpful in identifying parameters
Model behavior outside calibration data are questionable	Model is able to simulate data outside of calibration range
Acute versus chronic exposures	

A frequent problem with PBPK model parameterization is identifiability. This is a problem when more than one set of parameter values can be used to provide similar predictions. In this case, a judgment call can be made on model behavior based on the extent to which unique determination of a parameter has an impact on predicting the dose metric of interest. This call is supported by sensitivity analysis of the parameters. For instance, if the parameter of interest is not sensitive to the outcome but cannot be uniquely identified, then its individual impact on the dose metric may not be problematic. If the opposite case is true, i.e., a parameter is very sensitive to outcome but not uniquely identifiable, then a range of dose metric determinations are reported based on the range of biologically plausible values for this parameter. In such situations, formal statistical analysis, which is always appropriate but often difficult to perform, may be informative.

With the understanding that there is no complete PBPK model that can describe all relevant ADME biological process of a chemical accurately, the model reviewers are usually faced with judging and balancing the uncertainty inherent in every model against its possible application. Quantitative uncertainty analysis in the form of Bayesian analysis of models is time consuming and resource and data exhaustive. In many situations, a determination of uncertainty must be made based on qualitative criteria derived from in-depth evaluation of the structure and model parameterization. In some cases, demonstration of the model's ability to simulate data that were not used in its calibration/parameterization adds confidence in the model behavior. Table 1 contains some example information that can add to or subtract from the confidence of the application of a PBPK model to a health risk assessment.

Explicit PK Modeling: Tools for Documentation, Verification, and Portability

PBPK models vary in complexity, blending detail for biological subsystems for which there are rich data and/or need for mechanistic insight with abstraction for subsystems about which there are little data or influence upon the key systems. For example, the Blancato and Bischoff (1985) model for 2,5-hexanedione and its metabolite uses seven compartments to describe the brain and only two for the rest of the body, whereas the Timchalk *et al.* (2002) model for chlorpyrifos and its metabolite includes 20 compartments, albeit only two for the brain. Perhaps, because of this wide variation in complexity, PBPK models can be difficult to fully describe in publications. In general, the evaluator is often left wondering if all the parameters and their values were reported by the authors, whether the initial and dosing conditions were sufficiently described and was anything else (including equations) left out that would prevent the work from being reproduced. This can cause challenges and delays in the review and application of a PBPK model in an HHRA.

In addition, complex models are often interpreted by a wide community of scientists and policy makers. For this reason, it is important that the language used to communicate the model be precise and coherent. Formal ontologies provide a modern computer science mechanism to achieve this goal (Gruber, 1995). An ontology is a system explicitly describing the entities that exist (e.g., tissue compartments), how the entities can be related to each other (e.g., subcompartments for cellular space), how the entities can be grouped and subdivided (e.g., perfusion-limited vs. diffusion-limited compartments), and organizational hierarchies of these groups (both perfusion- and diffusion-limited compartments are physiologic compartments). Ontologies have been developed to describe many fields allied with toxicology, including chemistry (Ennis, 2004) and mathematical models for engineering (Gruber *et al.*, 1992).

Ontology for PBPK modeling should be sufficiently broad to describe all useful model structures. For instance, including a steady-state equation for gas exchange as opposed to a compartment model for the lung, or even more elaborately, considering dynamic breathing modes. Once a sufficiently broad ontology is established, a markup language can be prescribed for documenting the objects within the ontology. All models covered by the ontology can then be expressed with this language.

Coupled with ontologies, the extensible markup language (XML) approach is another modern computer science tool that could be used with PBPK models (Bray *et al.*, 1998). An XML code is long, precise, and very verbose—it is intended to be analyzed algorithmically. XML allows one to describe something in a language that is machine-readable and therefore machine translatable from one programming language to another. The ability to automatically translate the description into running code makes model documentation independent of specific hardware and software. Machine translation provides automatic checking of description completeness. An incomplete description is referred to as "junk"; DNA junk does not translate into a protein (Makalowski, 2000) and analogously an incomplete model description does not translate into a working model. Machine readability is the key advantage of XML. Although new languages and hardware inevitably replace what was used to develop the model, if there is sufficient information in an XML file to translate to one language, a translator could hypothetically be created for any computer or software package.

In systems biology publications, the systems biology markup language (SBML) provides a standard model description format that can be translated into working models (Hucka *et al.*, 2003). Both MATLAB (Schmidt and Jirstrand, 2006) and acslX (Aegis, 2008) have the ability to translate to and from SBML. In order to standardize the syntax of mathematics and documentation, allowing PK models to be disseminated as supplemental material in articles or through online databases, we propose a formal declarative description of PBPK models: PhLexicOn (Pharmacokinetics Lexicon and Ontology).

PhLexicOn consists of both a language and an ontology. The XML language for describing PK models is derived from a new ontology for PK concepts developed in the web ontology language (McGuinness and van Harmelen, 2004). As an XML-based language, functionality from other languages (e.g., SBML and MathML, Ausbrooks *et al.*, 2010) could be easily added. Likewise, functionality from PhLexicOn could be added to other XML descriptions.

Although demonstrating completeness of a model through machine translation would be a boon to model documentation, completeness of a model is not sufficient. A PBPK model must be plausible in terms of the relationship among tissue volumes, blood flows, and other fundamental biology. Plausibility is assessed though the ontology: it defines the appropriate constituents of physiologically based systems. First and foremost is the consideration of physical units—specifying an ontology allows some values to be required and, in this case, all parameter values are forced to have a unit. The machine translation step can then automatically check for unit consistency.

In addition to bounds on values (e.g., amounts must be positive, compartments must contain at least one compound), an ontology can specify additional rules for complete PBPK models; for instance, the sum of all arterial flows must equal cardiac output unless noncardiac flows (e.g., lymph) are included in the model. Mass balance can be strictly enforced (the rate of change of total amount of compound must equal its elimination and metabolism) and in a physiologic compartment, arterial flow must equal venous flow (again excepting alternative flows such as lymph). A final example rule would be to force all parameters tagged as "species specific" to match the species being simulated. Using an XML-based model description and proper ontology, all these rules can automatically be checked, allowing the modeler and/or model evaluator to immediately determine if rules have been violated. This does not prevent rule violations but does provide a tool to clearly flag them.

As important as software engineering is, the social engineering component cannot be overlooked, i.e., how to motivate researchers to document their models in a manner that benefits the community without an immediate benefit to the individual. Further complicating this is explicit, machine-interpretable documentation is not for humans (we have peer-reviewed journals)—the document produced is not well suited to human eyes. Although direct methods—such as convincing journals to require machine-readable model documentation to accompany any journal article based on those models—might work, incentivizing by taking advantage of the machine-interpretable format will also encourage wide acceptance. In particular, generation of publication-quality figures depicting the PBPK model schematic as well as compilation tables of relevant parameters could both performed algorithmically using a properly documented model.

The advantages of a machine-translatable language for assuring completeness and accuracy of PBPK models are many. Ongoing work is evaluating the structures that have been created for describing a model (the grammar) and developing rules to relate these structures and evaluate appropriateness (the semantics). The proof of concept will be to show that a complete PhLexicOn description can be automatically interpreted into a computable model. To generate PhLexicOn code, it could be an export option for modeling software such as acslX, and even Microsoft Word handles XML. PhLexicOn is a framework for documenting, verifying, and translating legacy and state-of-the-art models for reusability and efficient dosimetry estimation of chemicals for use in HHRAs.

Characterization of Population Distributions for Physiological Parameters

One of the more challenging issues that must be considered in performing an HHRA is the heterogeneity among humans. This heterogeneity is produced by interindividual variations in physiology, biochemistry, and molecular biology, reflecting both genetic and environmental factors. Heterogeneity in these characteristics results in differences among individuals in the biologically effective tissue dose associated with a given environmental exposure (PKs) as well as in the response to a given tissue dose (pharmacodynamics). It is useful in this context to consider the total variability among humans in terms of four contributing sources: (1) the variation across a population of "normal" individuals at roughly the same age, e.g., adults (Clewell and Andersen, 1996); (2) the variation across the population resulting from their different ages, e.g., infants or the elderly (Clewell *et al.*, 2004, 2002); (3) the variation resulting from the existence of subpopulations that differ in some way from the "normal" population, e.g., due to genetic polymorphisms in metabolizing enzymes (Gentry *et al.*, 2002; Haber *et al.*, 2002); and (4) the health status of a population. Health status should be considered, although it is frequently not characterized in HHRAs.

There has sometimes been a tendency in HHRA to use information on the variability of a specific parameter, such as inhalation rate or the *in vitro* activity of a particular enzyme, as the basis for expectations regarding the overall variability in dosimetry for *in vivo* exposures. However, whether or not the variation in a particular physiological or biochemical parameter will have a significant impact on *in vivo* dosimetry is a complex function of many interacting factors. In particular, the structures of physiological and biochemical systems frequently involve parallel processes, leading to compensation for the variation in a single factor. Moreover, physiological constraints may limit the *in vivo* impact of variability observed *in vitro* (Johanson *et al.*, 1999). For instance, high affinity intrinsic clearance can result in essentially complete metabolism of all the chemical reaching the liver in the blood; under these conditions, variability in amount metabolized *in vivo* would be more a function of variability in liver blood flow than variability in metabolism *in vitro* (Lipscomb *et al.*, 2003). Thus, it is often true that the whole (the *in vivo* variability in dosimetry) is less than the sum of its parts (the variability in each of the PK factors). Because the parameters in a PBPK model have a direct biological correspondence, they provide a useful framework for determining the impact of observed variations in physiological and biochemical factors on the population variability in dosimetry within the context of a risk assessment for a particular chemical (Clewell and Andersen, 1996; Price *et al.*, 2003).

The distinction between uncertainty and variability is important. Uncertainty represents imprecise or inadequate information and can be reduced through additional experimentation. Variability is an intrinsic property of a population that can be better characterized, but not reduced, by additional experimentation. Early attempts to distinguish the contributions of uncertainty and variability can be found in Bogen and Spear (1987) and Allen *et al.* (1996). Several studies have attempted to estimate the impact of parameter uncertainty and variability in PBPK models on risk assessment predictions using the Monte Carlo approach (Allen *et al.*, 1996; Clewell, 1995; Clewell and Andersen, 1996; Clewell *et al.*, 1999; Clewell and Jarnot, 1994; Portier and Kaplan, 1989).

Markov chain Monte Carlo (MCMC) simulation provides a computational method to perform a hierarchical Bayesian analysis by refining prior estimates of parameter uncertainty and variability based on experimental data. This approach is increasingly being used to refine and characterize PBPK models intended for use in risk assessments (Bois, 1999, 2000, 2001; Bois *et al.*, 1996a, 1996b; Chiu and Bois, 2006; Chiu and Ginsberg, 2011; Chiu *et al.*, 2009; Johanson *et al.*, 1999; Jonsson *et al.*, 2001a, 2001b; Jonsson and Johanson, 2001a, 2001b; Marino *et al.*, 2006; Qiu *et al.*, 2010).

Because analysis of PBPK models using MCMC is still a relatively new HHRA tool, using MCMC has many complicated practicalities involving much professional judgment in order to set up the model and interpret the results correctly. Thus, several important issues should be considered when

conducting or reviewing a Bayesian analysis performed via MCMC for use in a risk assessment. One important consideration is the necessity of evaluating whether the posterior parameter distributions obtained from the evaluation of specific datasets (those included in the MCMC simulation) should be used directly in the estimation of dose metrics. For example, data from a small number of young adult subjects in a controlled setting may not be reflective of the diversity of the population for which the risk assessment is being performed. Therefore, it is often necessary to substitute a suitable general population distribution for the posterior obtained from the experimental subjects.

A second consideration is whether there is adequate information content in the datasets used in the Bayesian analysis to identify a particular parameter. This is a more problematic issue because it is difficult to objectively evaluate the identifiability of multiple parameters estimated jointly from multiple datasets. One *ad hoc* way to evaluate the identifiability of a parameter from the data included in the Bayesian analysis is a posterior analysis of the correlation between each parameter and the model predictions for each data point. Another way to evaluate the identifiability of a parameter from the data in the Bayesian analysis is to compare the prior and posterior distributions. For example, a simple *t*-test can be performed to determine whether the difference between the prior and posterior means for a given parameter is statistically significant.

The third consideration is the difficulty of separating parameter variability/uncertainty from data and model error. Data error refers to measurement errors or bias arising from the limitations of instrumentation or techniques; model error is simply the result of less than perfect homeomorphic correspondence of the model structure to reality (which is always true). An important consequence of the existence of data and model error is that the parameter estimates obtained by the MCMC analysis may not be valid. That is, the algorithm may estimate a value for a model parameter that provides an adequate model prediction for the data, but due to data and model error, the estimated parameter may no longer represent the intended physicochemical entity. In particular, for a model in which a number of parameters are to some extent collinear, a combination of changes in parameters could compensate for data and model error, but the resulting parameter values would no longer represent the underlying physicochemical quantities (Vmax, Km, etc).

Approaches and Issues in Integrating PBPK With Benchmark Dose Modeling

Which to apply first: BMD or PBPK modeling? Once a PBPK model is reviewed and appropriately parameterized, a choice is needed regarding how to integrate it into a dose-response analysis and human extrapolation. A common animal-to-human dose-response extrapolation approach is to first select a POD, often the lower bound dose for an estimated incidence or level of response (U.S. EPA, 2011). The POD_{animal} is then extrapolated to estimate a human equivalent dose (HED) or concentration (HEC), based on known or assumed species differences in dosimetry. Before the BMD methodology and software became available, the POD_{animal} for noncancer effects was simply selected as the lowest no-observed adverse effect level (NOAEL) among all the dose-response datasets available; however, now, BMD analysis is the preferred approach for identifying a candidate POD from a given dose-response dataset. There are two approaches to integrate PBPK models with BMD analysis to estimate HECs or HEDs:

(1) Conduct the BMD modeling first, using the exposure concentration or applied dose as the dose metric (e.g., mg/kg/d by the oral route), to obtain an exposure-level POD_{animal}, then use the animal and human PBPK models to conduct the interspecies extrapolation;

(2) Use the animal PBPK model to estimate internal doses for each exposure or applied dose, then conduct BMD modeling using the internal dose metric to obtain an internal dose POD_{animal}, and finally use the human PBPK model to conduct the interspecies extrapolation.

Because dosimetry is often nonlinear due to metabolic saturation and the toxic response is expected to correlate better with an internal dose metric (e.g., the concentration of a toxic metabolite in the tissue where an effect occurs), the second approach (2) may be preferred. However, if the relationship between internal dose and exposure level is linear, then the HEC or HED will be the same whether BMD analysis is done first (1) or second (2).

The set of BMD models currently available in U.S. EPA's Benchmark Dose Software (BMDS; v2.1.2; http://www.epa.gov/NCEA/bmds/) provides a range of flexibility in fitting bioassay data, but if some of the dose-response nonlinearity arises from dosimetry and is accounted for by a PBPK model, use of internal doses for BMD modeling could significantly improve model fit. Use of the right internal dose metric could result in one or both of the following improvements: (1) a greater goodness of fit as quantified by a model-independent metric such as the sum of square errors (SSE) between model predictions and observations and a lower Akaike information criterion (AIC; Akaike, 1974) for the model, which best fits the external dose-response data and (2) A shorter 95% confidence interval on the BMD, quantified as a Benchmark Dose lower confidence limit (BMDL):BMD ratio closer to unity.

An example BMD analysis was conducted using a sample dataset (Supplementary table 1, where a second example is also shown). When a BMD analysis was run using BMDS (v2.1.2) with the internal dose metric from Supplementary table 1, the logistic model had the lowest AIC (although the SSE for the logistic model was higher than the log logistic) (Table 2). However, using the external metric, applied dose, the model with the lowest AIC was the log-logistic model (Table 2). Model fits to the data are illustrated in Supplementary figure 1.

In fact, for both models, both the SSE and the AIC were lower when using the internal compared with the same model using external dose metrics. However, the absolute difference in the

TABLE 2
BMD Analyses of Oral Ingestion Dataset[a]

	Logistic model		Log-logistic model	
BMD fit metric	Internal dose	External dose	Internal dose	External dose
AIC	183.6	186.3	184.8	185.4
SSE[b]	8.1	12.1	2.8	5.4
$BMDL_{10}$ (mg/l/d)[c]	65	(122)	35	(27)
$BMDL_{10}$/BMD	0.72	0.68	0.17	0.19

[a]The dataset used for this BMD analysis is shown in Supplementary table 1.
[b]Sum of squared errors between model predicted and observed total number of animals affected.
[c]The $BMDL_{10}$ was converted to the equivalent internal dose metric using the PBPK model; value shown in parentheses to indicate additional calculation.

SSE and AIC between internal and external metrics for the log-logistic model is less than for the logistic model. Furthermore, the choice of metric has little impact on the degree of uncertainty as quantified by the $BMDL_{10}$/BMD ratio for either model.

The most appropriate comparison to illustrate the impact of analyzing internal versus external dose would be to compare the best-model results when internal doses are used (logistic model) to the best-model results when external doses are used (log-logistic) (bolded columns in Table 2). This comparison still shows that use of the internal dose leads to a lower improved AIC (183.6 vs. 185.4). Furthermore, the internal dose equivalent of the $BMDL_{10}$ is lower when the log-logistic model is applied to external doses, whereas the $BMDL_{10}$/BMD ratio is almost four times lower. Thus, this comparison illustrates how use of the PBPK-derived internal dose metrics can improve statistical goodness of fit and confidence and result in a higher POD (i.e., the $BMDL_{10}$). If one has a low amount of biological information (i.e., in the absence of a PBPK model), one would like the BMD analysis to provide a greater margin of safety, hence a lower POD. In this case, that "ideal" scenario is actually produced.

This example illustrates that using internal doses from a PBPK model for BMD analysis can change the resulting BMDL and at least in some cases improve the level of confidence as quantified by the BMDL/BMD ratio. However, in the development of an HHRA, one drawback from applying BMD analysis to internal dosimetry is that the PBPK model may be revised multiple times throughout the iterative peer-review process, and each such change would subsequently require revisions to the BMD analysis. The added effort could be substantial, as compared with performing the BMD analysis once using applied or external doses.

Statistical issue: combined risk from cancer response in multiple tissues. When a PBPK model is to be integrated with a BMD analysis into a cancer risk assessment, a second challenge arises in estimating total cancer risk when the cancer response occurs in multiple tissues for which the best dose metric differs.

Unlike using PBPK-derived internal dose metrics for BMD modeling of single effects, there is no straightforward approach to calculating combined tumor risk from multiple tissues using internal dosimetry information in the BMD analysis. A relatively simple approach to estimate the combined cancer risk can be considered, but it involves assuming that the probability density of BMD values is normally distributed—an assumption which is questionable and difficult to test—and that both the exposure-dose and the dose-response relationship are linear below the HED or BMDL.

A more complex approach involves Monte Carlo sampling, which requires expertise in Bayesian methods and can be used to rigorously estimate the combined tumor risk from multiple tissues with differing internal doses. An outline of the method is provided here with additional details in the Supplementary Data. A key assumption for combining tumor risks is that except for correlation between exposure and internal doses, the tumor response in two tissues is independent. The National Research Council considered the issue of independence and concluded that this is a sound assumption (National Research Council (NRC), 1994). This approach also effectively assumes that cancer risk is directly proportional to internal dose below the BMDL values for each tissue. A human exposure level (E) must also be selected such that relationship between exposure level and internal dose is linear below E, but this can be lower than the human equivalent exposure level for the BMDLs, so the exposure-internal dose relationship can be nonlinear between the internal dose BMDLs and the internal dose equivalents of E. The procedure is as follows:

(1) Generate BMD sample distributions for the cancer slope factor (CSF) in each tissue (CSFi) using tissue-specific internal dose metrics:

a. These can be generated using a probabilistic PBPK model, if available

b. The distributions for BMD models (parameters) can be generated by the Bayesian approach described by Kopylev *et al*. (2007)

(2) Use the human PBPK model to generate a human internal dose (HID) for each tissue, i (HIDi), for a fixed, low exposure, E:

a. These can be generated using a probabilistic PBPK model, if available

b. E should be low enough that internal doses are linear with exposure below E

(3) Monte Carlo sampling from the distribution of CSFi values (and, independently from HIDi values) is then used to generate a distribution for the combined cancer slope factor:

$$CSF_{comb} = [(HID1 \times CSF1) + (HID2 \times CSF2)]/E.$$

(4) Calculate the 95% upper confidence limit of that sample

The U.S. EPA BMDS (v2.1.2) does not yet have the capability of estimating the combined tumor risk from tumors arising in multiple tissues "when a different internal dose metric is associated with each tissue-specific response." Simple

approaches to consider for calculating the combined tumor risk rely on assumptions about the distribution of BMD levels (given the underlying data) whose accuracy has not been determined. The limited dose-response data available for most chemicals are not sufficient to test these assumptions. The rigorous approach, which does not rely on these assumptions, requires significant computational effort and statistical expertise.

Application-Specific Considerations in Evaluation of PBPK Model Certainty

A risk assessor who is considering using an available PBPK model in an HHRA to develop toxicity reference values may ask, "Is this model acceptable?" Without any context, the answer is unlikely to be an unqualified "yes." More likely, the answer to the question will be another question: "Acceptable for what?" One of the criteria of PBPK model evaluation, as noted above, is model "purpose." Because a model may fulfill multiple purposes, evaluations should take this into consideration. The two case studies below illustrate some aspects of PBPK model evaluation and application in a risk assessment context.

Case study #1: ethylene dichloride (1,2-dichloroethane, EDC). A PBPK model describing the disposition of inhaled or orally dosed EDC in rats was published by Sweeney *et al.* (2008). As no human model was published, this model has no utility for interspecies extrapolation "as is." However, the purpose of this model was to meet a need for route-to-route extrapolation of existing and planned toxicology studies (U.S. EPA, 2003a); a similar approach was used for 1,1,2-trichloroethane (U.S. EPA, 2003b).

One of the model applications specified was to develop inhalation equivalents of NOAELs and lowest observed adverse effect levels (LOAELs) for the subchronic study of Daniel *et al.* (1994), which used gavage dosing in corn oil. Several potential PBPK-derived dose metrics were suggested as potential bases for route-to-route extrapolation: parent compound (peak, average, or area under the concentration vs. time curve [AUC]) and amount metabolized (U.S. EPA, 2003a). A change from earlier versions of the EDC model included an alteration in the location of extrahepatic metabolism from the lung (D'Souza *et al.*, 1987; D'Souza *et al.*, 1988) to a pooled venous blood compartment whose blood flow is collected from the kidney, slowly perfused and other well perfused tissues (Sweeney *et al.*, 2008). Metabolism in the lung could lead to a reduction of EDC that enters systemic circulation ("first pass" effect). Concerns existed over the effect of this on model application. When simulations were conducted with different placement of extrahepatic metabolism, it was observed that the location had essentially no impact at higher doses and concentrations and a minimal impact at lower doses and concentrations. Thus, the effect on route-to-route extrapolation is expected to be minimal (Supplementary tables 4 and 5) and the model is acceptable for route-to-route extrapolations of subchronic studies in this context.

Case study #2: perchloroethylene (tetrachloroethylene, Perc). Controversy exists as to cancer mode of action for perc and the structurally similar compound trichloroethylene, particularly the identity of the responsible metabolite(s) (Evans *et al.*, 2009; Sweeney *et al.*, 2009). Noncancer endpoints of concern for perc may include neurological/neurobehavioral effects that are likely related to levels of parent compound. Studies deemed to be candidates to serve as the basis of oral and inhalation toxicity reference values were identified by an epidemiologist (John Bukowski, personal communication). These studies relied on observations of workers exposed via inhalation. The candidate internal dose metrics to serve as the basis for extrapolating from LOAELs in occupational studies to toxicity reference values for continuous inhalation or episodic ingestion were peak and AUC for concentration of perc in the brain. The PBPK model used was by Covington *et al.* (2007), which provided good simulations of the available human datasets. The model was adapted to account for the exposure scenarios (continuous inhalation and episodic dietary exposure) useful for this assessment.

Sensitivity analysis can highlight key parameters for evaluation of model confidence. For the perc example (Table 3), the values of the internal dose metrics of interest for the noncancer assessment were generally sensitive only to parameters that would be expected to be well characterized. In particular, it should be noted that none of these dose metrics are sensitive to the rate of metabolism, a parameter that is frequently uncertain and/or variable within the human population. Model confidence for disposition of the parent compound is often greater than for metabolites. Based on the sensitivity evaluations, default uncertainty factors (UFs) for PK variability in the noncancer assessment could potentially be replaced with chemical-specific adjustment factors; at a minimum, this analysis indicates that there is no need to increase the UF based on concern for intraspecies variability.

CONCLUSIONS

Published PBPK models are very useful for HHRAs because there is rarely time to develop a model *de novo* for use in an assessment. Although a thorough review of the model is warranted prior to deciding upon its possible application in an HHRA, a continued dialogue between the model developer and the model evaluator is essential during this review process to ensure critical assumptions and parameters, which may not have been laid out in the publication, are well understood. In addition, when the model is being considered for use in HHRA, the review must include a complete quality assurance check of the computer code, which can be more demanding and require different information than the review process for publication in a scientific journal. This rigorous PBPK model review process is important to ensure the model used in the HHRA is well understood and documented is applicable to the specific needs of the HHRA. Ultimately, the final values from PBPK models and hence the dose-response assessments may be

TABLE 3
Sensitivity and Variability Analyses for Peak Concentration in Rapidly Perfused Tissues (CR) and Area Under the Rapidly Perfused Tissue Concentration Curve (AUCR) in Humans Exposed to Perchloroethylene at 2 ppm and 3 mg/kg/d

		Normalized sensitivity coefficients (NSC)			
		Oral		Inhalation	
Parameter	Coefficient of variation (CV)[a]	Peak CR	AUCR	Peak CR	AUCR
Body weight	0.3	0.21	0.24	—[b]	—
Daily dose or concentration	0	1.0	1.0	1.0	1.0
Absorption rate	0.3	0.12	—	—	—
Blood:air partition coefficient	0.3	0.81	0.93	—	0.93
Richly perfused tissue:air partition coefficient	0.3	0.98	1.0	1.0	1.0
Alveolar ventilation rate	0.3	−0.81	−0.93	—	0.068
Fractional blood flow to richly perfused tissues	0.3	−0.065	—	—	—
Model variance[c]		0.21	0.25	0.09	0.17
Model CV[c]		0.46	0.50	0.30	0.41
95th/50th percentile[c]		1.7	1.8	1.5	1.7
99th/50th percentile[c]		2.1	2.2	1.7	1.9

[a]Allen *et al*. (1996).
[b]|NSC| < 0.05.
[c]Model variance, CV, and percentile ratios estimated per Sweeney *et al*. (2003).

used in the completed HHRA to impact legislation. The use of a standardized syntax for mathematical and descriptive model documentation, such as PhLexicOn, could help to expedite the review process and provide an additional level of machine-conducted review, which would help reduce reliance on humans to check computer code and its implementation.

In conclusion, though not two PBPK models or HHRAs are alike, several steps are always required before a PBPK model may be used in such an assessment: (1) acquisition of publicly available PBPK model and computer code; (2) thorough review of model code (including computer implementation of equations, biological fidelity, parameterization, and sensitivity/uncertainty analysis); and (3) consideration of integration with other HHRA methods, including BMD analysis.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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