Background Paper for Breakout Group I: Model Specification

International Workshop on Uncertainty and Variability in Physiologically Based Pharmacokinetic (PBPK) Models

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Pharmacokinetics is the study of the time course for the absorption, distribution, metabolism, and excretion (ADME) of a chemical substance in a biological system. Implicit in any application of pharmacokinetics to toxicology or risk assessment is the assumption that the toxic effects in a particular tissue can be related in some way to the concentration time course of an active form of the substance in that tissue. Moreover, absent pharmacodynamic differences between animal species, it is expected that similar responses will be produced at equivalent tissue exposures regardless of animal species, exposure route, or experimental regimen (Andersen, 1981; Monro, 1992; Andersen et al., 1995a). Of course the actual nature of the relationship between tissue exposure and response, particularly across species, may be quite complex. With pharmacokinetic modeling, we employ established descriptions of chemical transport and metabolism to simulate observed kinetics in silico (Andersen et al., 1995b).

Classic compartmental modeling is largely an empirical exercise, where data on the time course of the chemical of interest in blood (and perhaps other tissues) are collected. Based on the behavior of the data, a mathematical model is selected which possesses a sufficient number of compartments (and therefore parameters) to describe the data. The compartments do not in general correspond to identifiable physiological entities but rather are described in abstract terms. The advantage of this modeling approach is that there is no limitation to fitting the model to the experimental data. If a particular model is unable to describe the behavior of a particular data set, additional compartments can be added until a successful fit is obtained. Since the model parameters do not possess any intrinsic meaning, they can be freely varied to obtain the best possible fit, and different parameter values can be used for each data set in a related series of experiments. Once developed, these models are useful for interpolation and limited extrapolation of the concentration profiles which can be expected as experimental conditions are varied. They are also useful for statistical evaluation of a chemical's apparent kinetic complexity

(O'Flaherty, 1987). However, since the compartmental model does not possess a physiological structure, it is often not possible to incorporate a description of these non-linear biochemical processes in a biologically appropriate context. Without a physiological structure it is not possible to correctly describe the interaction between blood-transport of the chemical to the metabolizing organ and the intrinsic clearance of the chemical by the organ.

Physiologically based pharmacokinetic (PBPK) models differ from the conventional compartmental pharmacokinetic models in that they are based to a large extent on the actual physiology of the organism (Teorell, 1937a,b). Figure 1 illustrates the structure of a PBPK model for a volatile, lipophilic compound.

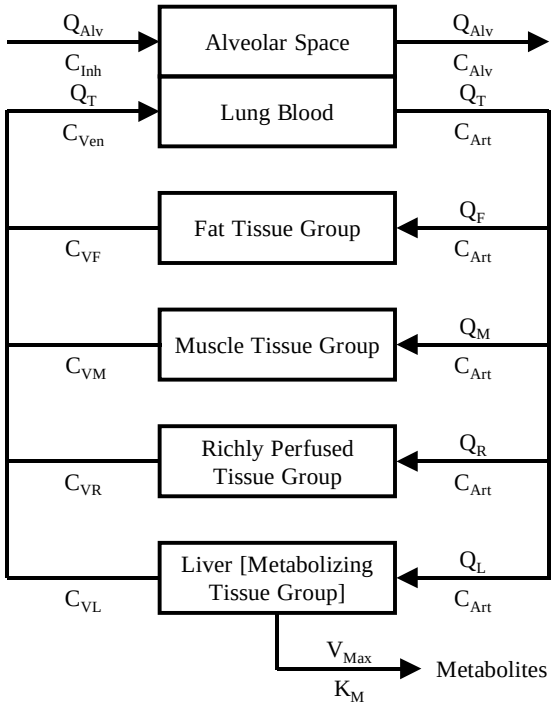

Figure 1. Diagram of a physiologically-based pharmacokinetic model for styrene (Ramsey and Andersen, 1984). In this description, groups of tissues are defined with respect to their volumes, blood flows (Q), and partition coefficients for the chemical. The uptake of vapor is determined by the alveolar ventilation (Q_{ALV}), cardiac output (Q_T), blood:air partition coefficient, and the concentration gradient between arterial and venous pulmonary blood (C_{ART} and C_{VEN}). Metabolism is described in the liver with a saturable pathway defined by a maximum velocity (V_{MAX}) and affinity (K_M). The mathematical description assumes equilibration between arterial blood and alveolar air as well as between each of the tissues and the venous blood exiting from that tissue.

Instead of compartments defined solely by mathematical analysis of the experimental kinetic data, compartments in a PBPK model are based on realistic organ and tissue groups, with weights and blood flows obtained from the literature. Moreover, instead of compartmental rate constants determined solely by fitting data, actual physical-chemical and biochemical properties of the compound can often be used to define parameters in the model. To the extent that the structure of the model reflects the important determinants of the kinetics of the chemical, the result of this approach is a model which can predict the qualitative behavior of an experimental time course without having been based directly on it. Refinement of the model to incorporate additional insights gained from comparison with experimental data yields a model which can be used for quantitative extrapolation well beyond the range of experimental conditions on which it was based. In particular, a properly validated PBPK model can be used to perform the high-to-low dose, dose-route, and interspecies extrapolations necessary for estimating human risk on the basis of animal toxicology studies (Clewell and Andersen, 1985; Andersen *et al.*, 1987;1991; O'Flaherty, 1989; Reitz et al., 1990; Gerrity and Henry, 1990; Johanson and Filser, 1993; Corley et al., 1994; el-Masri et al., 1995; Clewell *et al*., 1997; 2000; Fisher, 2000; Barton et al., 2000). The physiological structure of PBPK models is also useful for examining early life exposure (Fisher et al., 1989; 1991; Clewell et al., 2001; Corley et al., 2003; Sarangapani et al., 2003; Gentry et al., 2003; 2004; Clewell et al., 2004; Barton, 2005); however, the development and evaluation of PBPK models of the developmental period is beyond the scope of this paper.

The basic approach to PBPK model development is illustrated in Figure 2; a number of excellent reviews on the subject are available (Himmelstein and Lutz, 1979; Gerlowski and Jain, 1983; Fiserova-Bergerova, 1983; Bischoff, 1987; Leung, 1991). The process of model development begins with the definition of the chemical exposure and toxic effect of concern, as well as the species and target tissue in which it is observed. Literature evaluation involves the integration of available information about the mechanism of toxicity, the pathways of chemical metabolism, the nature of the toxic chemical species (i.e., whether the parent chemical, a stable metabolite, or a reactive intermediate produced during metabolism is responsible for the toxicity), the processes involved in absorption, transport and excretion, the tissue partitioning and binding characteristics of the chemical and its metabolites, and the physiological parameters (i.e., tissue weights and blood flow rates) for the species of concern (i.e., the experimental species and the human). Using this information, the investigator develops a PBPK model which

expresses mathematically a conception of the animal/chemical system. In the model, the various time-dependent biological processes are described as a system of simultaneous differential equations. The specific structure of the model is driven by the need to estimate the appropriate measure of tissue dose under the various exposure conditions of concern in both the experimental animal and the human. Before the model can be used in risk assessment it has to be validated against kinetic, metabolic, and toxicity information and, in many cases, refined based on comparison with the experimental results. The model itself can frequently be used to help design critical experiments to collect data needed for its own validation. Perhaps the most desirable feature of a PBPK model is that it provides a conceptual framework for employing the scientific method: hypotheses can be described in terms of biological processes, quantitative predictions can be made on the basis of the mathematical description, and the model (hypothesis) can be revised on the basis of comparison with targeted experimental data.

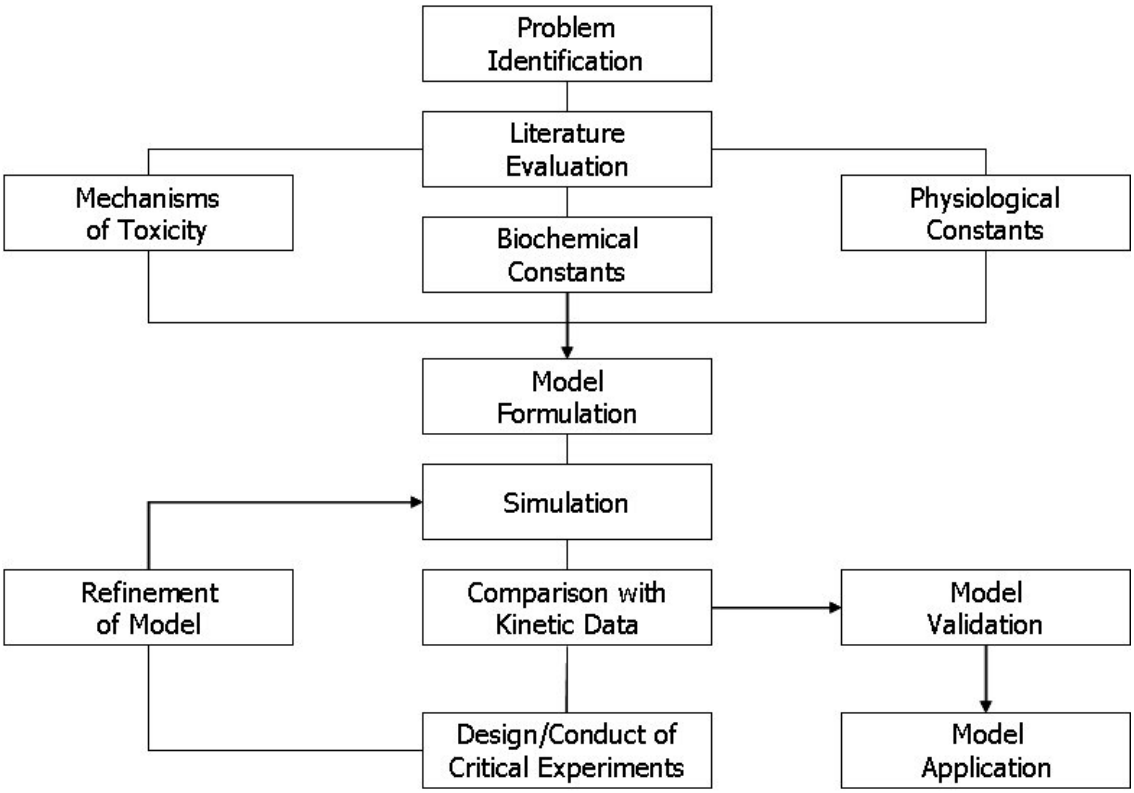

Figure 2. Flow-chart of the PBPK modeling process.

Specification of Model Structure

There is no easy rule for determining the structure and level of complexity needed in a particular modeling application. For example, model elements such as lung ventilation and fat storage, which are important for a volatile, lipophilic chemical such as styrene (Ramsey and Andersen, 1984), do not need to be considered in the case of a nonvolatile, water soluble compound such as methotrexate (Bischoff et al., 1971). Similarly, while kidney excretion and enterohepatic recirculation are important determinants of the kinetics of methotrexate, only metabolism and exhalation are significant for the water-soluble 2-butoxyethanol (Johanson, 1986). The decision of which elements to include in the model structure for a specific chemical and application involves finding a balance between two primary criteria: parsimony and plausibility.

The principle of parsimony simply states that a model should be as simple as possible for the intended application (but no simpler). That is, structures and parameters should not be included in the model unless they are needed to support the application for which the model is being designed. The desire for parsimony in model development is driven not only by the desire to minimize the number of parameters whose values must be identified, but also by the recognition that as the number of parameters increases, the potential for unintended interactions between parameters increases disproportionately. As a model becomes more complex, it becomes increasingly difficult to validate, even as the level of concern for the trustworthiness of the model should increase.

Countering the desire for model simplicity is the need for plausibility of the model structure. The credibility of a PBPK model's predictions of kinetic behavior under conditions different from those under which the model was validated rests on the correspondence of the model design to known physiological and biochemical structures and an accurate description of the chemical mode of action. In general, the ability of a model to adequately simulate the behavior of a physical system depends on the extent to which the model structure is homomorphic (having a one-to-one correspondence) with the essential features determining the behavior of that system. The trade-off against the greater predictive capability of physiologically-based models is the requirement for an increased number of parameters and equations.

The process of model identification is an iterative process that begins with the selection

of a model structure based on those elements that the modeler considers to be minimum essential determinants of the chemical's behavior in the animal system, from the viewpoint of the intended application of the model. Comparison with appropriate data can then provide insight into defects in the model which must be corrected either by re-parameterization or by changes to the model structure. Selection of a model structure can be broken down into a number of elements associated with the different aspects of uptake, distribution, metabolism, and elimination. These mechanistic aspects play a role in most aspects of the model development, including decisions on grouping and splitting tissue compartments, level of detail in describing chemical transport and metabolism, and inclusion of chemical exposure routes.

Tissue grouping is generally approached in one of two ways – by lumping or splitting model compartments. In the lumping approach, model development begins with information at the greatest level of detail that is practical, and decisions are made to combine tissue compartments based on the similarity of their physiological characteristics. The common grouping of tissues into richly perfused and poorly perfused on the basis of their blood perfusion rate is an example of this approach. In contrast, the splitting approach starts with the simplest reasonable model structure and increases the model's complexity only to the extent required to reproduce data on the chemical of concern for the application of interest. Lumping requires the greater initial investment in data collection and, if taken to the extreme, could paralyze model development. Splitting, on the other hand, is more efficient but runs a greater risk of overlooking chemical-specific determinants of chemical disposition. Tissues that are typically specifically defined in the model structure are the target tissues, those involved in storage, metabolism or clearance of the chemical, and those required to simulate chemical exposure depending on the dose routes used in simulated experiments.

Chemical transfer between the blood and tissue compartments may be governed by passive diffusion (flow- or diffusion-limited) or active transport. Many published PBPK models are flow-limited; that is, they assume that the rate of tissue uptake of the chemical is limited only by the flow of the chemical to the tissue in the blood. While this assumption is generally reasonable, for some chemicals and tissues uptake may instead be diffusion-limited. Examples of tissues for which diffusion-limited transport has often been described include the skin, placenta, mammary glands, brain, and fat (McDougal et al., 1986; Fisher et al., 1989; 1990; Andersen et al., 2001). If there is evidence that the movement of a chemical between the blood

and a tissue is limited by diffusion, a two-compartment description of the tissue can be used with a "shallow" exchange compartment in communication with the blood and a diffusion-limited "deep" compartment. Some chemicals may be transported against the concentration gradient through energy dependent processes. These processes are usually limited by the availability of transporter proteins, and such saturable processes are often well-described using Michealis-Menten type kinetics (Andersen et al., 2006).

The liver is frequently the primary site of metabolism, though other tissues such as the kidney, placenta, and adipose, may be important metabolism sites depending on the chemical. Metabolism may be described as occurring through a linear (first-order) pathway using a rate constant (k_F: hr^{-1}) or a saturable (Michealis-Menten) pathway with capacity V_{max} (mg/hr) and affinity K_m (mg/L). If desired, the pharmacokinetics of the resulting metabolite may also be explicity described in the model. The same considerations which drive decisions regarding the level of complexity of the PBPK model for the parent chemical must also be applied for each of its metabolites. As in the case of the parent chemical, the most important consideration is the purpose of the model. If the concern is direct parent chemical toxicity and the chemical is detoxified by metabolism, then there may be no need for a description of metabolism beyond its role in parent chemical clearance. If reactive intermediates produced during the metabolism are responsible for observed toxicity, a very simple description of the metabolic pathways might be adequate (Ramsey and Andersen, 1984; Andersen *et al*., 1987; Corley et al., 1990). On the other hand, if one or more of the metabolites are considered to be responsible for the toxicity of a chemical, it may be necessary to provide a more complete description of the kinetics of the metabolites themselves (Fisher *et al*., 1991; Gearhart *et al*., 1993; Clewell *et al*., 1997; 2000; Fisher, 2000).

Other processes that may have significant impact on the chemical kinetics include protein binding and excretion. Protein binding in the blood reduces the amount of free chemical available for distribution into the tissues or clearance via excretion. Binding within tissues may lead to dose- and time-dependent accumulation, and may be described as a saturable process. Clearance may occur through urinary or fecal excretion, exhaled air, or even through loss via hair. This loss is most often successfully described using first order clearance terms. However, more elaborate descriptions are sometimes required for chemicals that are substrates for transporters, which transfer chemicals against the concentration gradient. Some transporters in

the kidney and bile can increase clearance of xenobiotics, while others, such as those responsible for reabsorption, may increase residence time (Andersen et al., 2006).

Specification of Mean Parameters

Estimates of the various physiological parameters needed in PBPK models are available from a number of sources in the literature, particularly for the human, monkey, dog, rat, and mouse (Adolph, 1949; Bischoff and Brown, 1966; Astrand and Rodahl, 1970; ICRP, 1975; EPA, 1988; Davies and Morris, 1993; Brown *et al.*, 1997; Gentry et al., 2004). Table 1 shows typical values of a number of physiological parameters in adult animals.

TABLE 1: "Typical" Physiological Parameters for PBPK Models

Species		**Mouse**	**Rat**	**Monkey**	**Human**
Ventilation					
Alveolar	(L/hr-1kg)[a]	29.[b]	15.[b]	15.[b]	15.[b]
Blood Flows					
Total	(L/hr-1kg)[a]	16.5[c]	15.[c]	15.[c]	15.[c]
Muscle	(fraction)	.18	.18	.18	.18
Skin	"	.07	.08	.06	.06
Fat	"	.03	.06	.05	.05
Liver (Arterial)	"	.035	.03	.065	.07
Gut (Portal)	"	.165	.18	.185	.19
Other Organs	"	.52	.47	.46	.45
Tissue Volumes					
Body Weight	(kg)	.02	.3	4.	80.
Body Water	(fraction)	.65	.65	.65	.65
Plasma	"	.04	.04	.04	.04
RBCs	"	.03	.03	.03	.03
Muscle	"	.34	.36	.48	.33
Skin	"	.17	.195	.11	.11
Fat	"	.10[d]	.07[d]	.05[d]	.21
Liver	"	.046	.037	.027	.023
Gut Tissue	"	.031	.033	.045	.045
Other Organs	"	.049	.031	.039	.039
Intestinal Lumen	"	.054	.058	.053	.053

[a] Scaled allometrically: $QC = QCC*BW^{.75}$
[b] Varies significantly with activity level (range: 15 - 40)
[c] Varies with activity level (range: 15 - 20)
[d] Varies substantially (lower in young animals, higher in older animals)

Estimates for the same physiological parameter often vary widely, due both to experimental differences and to differences in the animals examined (age, strain, activity). Ventilation rates and blood flow rates are particularly sensitive to the level of activity (Astrand and Rodahl, 1970; EPA, 1988). Data on some important tissues is relatively poor, particularly in the case of fat tissue.

Many biochemical parameters may be measured directly from *in vitro* studies. For volatile chemicals, partition coefficients may be measured using a relatively simple *in vitro* technique known as vial equilibration (Fiserova-Bergerova, 1975; Sato and Nakajima, 1979a,b; Gargas *et al*., 1989). Partition coefficients for non-volatile compounds are not as easily measured *in vitro* (Jepson et al., 1994), and are therefore often estimated by comparing tissue:blood levels at steady state from *in vivo* studies (Lam et al., 1981; King et al., 1983). Metabolism parameters can be obtained from parent chemical disappearance (or metabolite formation) curves in intact cells, tissue homogenate, or microsomal fractions (Reitz et al., 1989; Kedderis and Lipscomb, 2001; Lipscomb and Kedderis, 2002; Lipscomb et al, 2004). Rapid *in vivo* approaches may also be used to estimate metabolic constants based on steady-state extraction (Andersen *et al*., 1984) or gas uptake experiments (Filser and Bolt, 1979; Andersen, *et al*., 1980; Gargas *et al*., 1986a, 1990; Gargas and Andersen, 1989), as well as information on the total amount of chemical metabolized in a particular exposure situation (Watanabe *et al*., 1976). Determination of stable end-product metabolites after exposure can also be useful in some cases (Gargas and Andersen, 1982; Gargas *et al*., 1986b).

In many cases, important parameters values needed for a PBPK model may not be available in the literature. In such cases it is necessary to measure them in new experiments, to estimate them by quantitative structure-activity relationship (QSAR) techniques (Gargas et al,, 1988; Poulin and Krishnan, 1999; Beliveau et al., 2005), or to identify them by optimizing the fit of the model to an informative data set. An example of a case where fitting the model to kinetic data is the only practical approach for parameter estimation is the attempt to describe enterohepatic recirculation (e.g., Clewell et al. 1997, 2000). The residence time of chemicals whose conjugation products are transferred into the bile and subsequently cleaved and re-absorbed in the intestine depend on a number of processes – such as biliary excretion into the duodenum, movement through the intestinal lumen, metabolism by intestinal bacteria, and resorption in the lower intestine – that are not easily measured *in vitro* or *in vivo*, and therefore

the parameters in such a description must be estimated by fitting the overall predictions of the model to kinetic data such as blood concentration time-courses as a function of dose.

Even in the case where an initial estimate of a particular parameter value can be obtained from other sources, it may be desirable to refine the estimate by optimization. For example, given the difficulty of obtaining accurate estimates of the fat volume in rodents, a more reliable estimate may be obtained by examining the impact of fat volume on the kinetic behavior of a lipophilic compound such as styrene. Of course, being able to uniquely identify a parameter from a kinetic data set rests on two key assumptions: (1) that the kinetic behavior of the compound under the conditions in which the data was collected is informative regarding the parameter being estimated, and (2) that other parameters in the model which could influence the observed kinetics have been determined by other means and are held fixed or otherwise constrained during the estimation process.

The actual approach for conducting a parameter optimization can range from simple visual fitting, where the model is run with different values of the parameter until the best correspondence appears to be achieved, or by a quantitative mathematical algorithm. The most common algorithm used in optimization is the least-squares minimization. To perform a least-squares optimization, the model is run to obtain a set of predictions at each of the times a data point was collected. The square of the difference between the model prediction and data point at each time is calculated and the results for all of the data points are summed. The parameter being estimated is then modified, and the sum of squares is recalculated. This process is repeated until the smallest possible sum of squares is obtained, representing the best possible fit of the model to the data.

In a variation on this approach, the square of the difference at each point is divided by the square of the prediction. This variation, known as relative least squares, is preferable in the case of data with an error structure which can be described by a constant coefficient of variation (that is, a constant ratio of the standard deviation to the mean). The former method, known as absolute least squares, is preferable in the case of data with a constant variance. From a practical viewpoint, the absolute least squares method tends to give greater weight to the data at higher concentrations and results in fits that look best when plotted on a linear scale, while the relative least squares method gives greater weight to the data at lower concentrations and results in fits that look best when plotted on a logarithmic scale.

When parameter estimation has been performed by optimizing model output to experimental data, the investigator must assure that the parameter is adequately identifiable from the data (Carson et al., 1983). Moreover, the practical reality of modeling biological systems is that regardless of the complexity of the model there will always be some level of "model error" (lack of homomorphism with the biological system) which can result in systematic discrepancies between the model and experimental data. This model structural deficiency interacts with deficiencies in the identifiability of the model parameters, potentially leading to mis-identification of the parameters. Due to the confounding effects of model error and parameter correlation, it is quite possible for an optimization algorithm to obtain a better fit to a particular data set by changing parameters to values that no longer correspond to the biological entity the parameter was intended to represent. Estimates of parameter uncertainty obtained from optimization routines should be viewed as lower bound estimates of true parameter uncertainty since only a local, linearized variance is typically calculated. In characterizing parameter uncertainty, it is probably more instructive to determine what ranges of parameter values are clearly inconsistent with the data than to accept a local, linearized variance estimate provided by an optimization algorithm.

As the number of fitted parameters in the PBPK model increases, the level of uncertainty in the accuracy of the individual values increases correspondingly. The ability to limit this uncertainty depends on the availability of data under conditions where the parameters being estimated would be expected to have an observable impact on the measured concentrations. Sensitivity analysis can sometimes be used to determine the appropriate conditions for such a comparison (Clewell et al., 1994). The demand that the PBPK fit a variety of data also restricts the parameter values that will give a satisfactory fit to experimental data.

Model Evaluation and Revision

Once an initial model has been developed, it must be evaluated on the basis of its conformance with experimental data. In some cases, the model may be exercised to predict conditions under which experimental data should be collected in order to verify or improve model performance. Model success in reproducing measured data supports the validity of the mechanistic assumptions, while model failure suggests that revision of the model is needed. In fact, model failure is often more informative to mechanistic investigations than success. PBPK

models can be used to test a variety of hypotheses quickly and inexpensively and, based on model results, we can design more efficient experiments to test key mechanistic assumptions. The following examples illustrate the role of model development, evaluation and refinement in gaining a better understanding of chemical kinetics. They also demonstrate the use of statistical methods (likelihood comparisons) to evaluate alternative model structures on the basis of their relative ability to conform with experimental data.

Suicide Inhibition in Trans-1,2-Dichloroethylene Metabolism. An effort to characterize the metabolism of trans-1,2-dichloroethylene (*t*DCE) provides an example of how PBPK model failure can aid the evaluation of mechanistic hypotheses and inform experimental design. In this case, a PBPK model structure that had been used successfully to describe the *in vivo* metabolism of several volatile chemicals failed to describe *t*DCE kinetics, and the investigation into the model behavior led to insights about the processes governing the chemical's metabolism. With the development of closed chamber metabolism studies (Gargas *et al*. 1986; Gargas *et al*. 1990), new and abundant data were made available describing the disappearance of VOCs after inhalation. For chemicals such as methylene chloride, where the metabolism occurred through parallel saturable and first-order pathways, this technique provided an efficient method for estimating metabolism parameters and the resulting models were able to describe blood time-course data from separate studies. However, when the same model structure was applied to *t*DCE (Lilly et al., 1998), it failed to predict the time-dependent behavior of the experimental data (Figure 3).

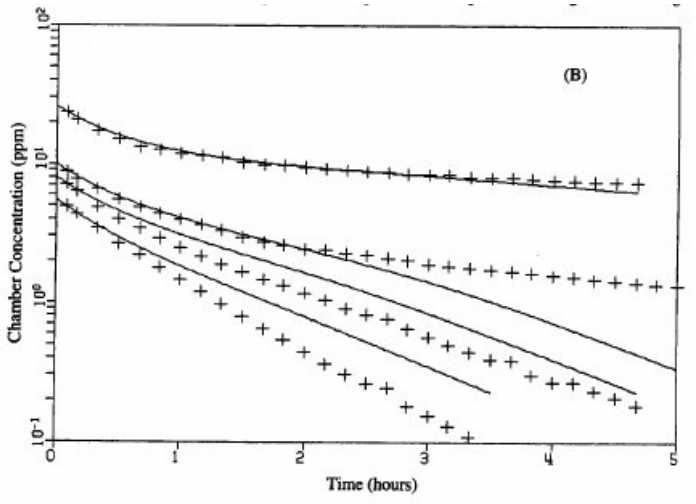

Figure 3. Failure of methylene chloride PBPK model structure to describe trans-1,2-Dichloroethylene gas chamber dose-response data in rats (Lilly et al., 1998).

This model failure suggested that the metabolic pathway was more complex than had been previously assumed. A revised hypothesis about the mechanism of *t*DCE metabolism was then developed based on the nature of the discrepancy between the predictions of the model and the observed data. Two important observations were made: 1) the decline in *t*DCE concentration slowed over time, and 2) the model consistently over-predicted that time-dependent decline in the lower doses. These observations suggested that the metabolism of the chemical might be resulting in the destruction of the metabolic enzyme, and that this decrease in enzyme capacity was less severe at lower doses. Based on these observations, the authors proposed four potential mechanisms of suicide inhibition which they incorporated into alternative versions of the model, and then tested each model against the existing data.

Since the equations describing the alternative mechanisms of inhibition each used the same number of parameters, the identification of the most succesful model could be accomplished by a direct comparison of likelihood estimates. For each of the alternative models, the parameters for metabolism were optimized against the same experimental data using the extended least squares method in Simusolv (Dow Chemical), and the resulting log-likelihoods were compared. The model that most successfully described the time-course data across doses (Figure 4) assumed that the reactive metabolite of *t*DCE disabled the enzyme-substrate complex. By ascertaining the most likely mechanism of enzyme inactivation, it was possible to tailor further experiments to test specifically for the occurrence of suicide inhibition. This hypothesis could then be confirmed experimentally (Lilly et al., 1998).

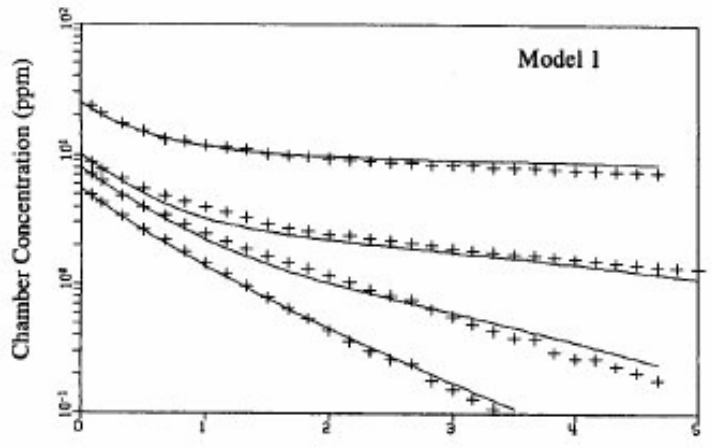

Figure 4. Revised PBPK model prediction of trans-1,2-Dichloroethylene gas chamber dose-response data in rats assuming suicide inhibition (Lilly et al., 1998).

Storage of Octamethylcyclotetrasiloxane in Tissue Lipids. Modeling of cyclic siloxane kinetics permitted the evaluation of lipid storage sites within tissues as well as lipid storage depots in blood that are not in communication with the free siloxanes circulating in blood (Andersen et al., 2001). Failure of the typical volatile chemical model to predict the timecourse data for the cyclic siloxanes led the authors to reexamine the assumption that lipophilic chemical behavior was determined only by metabolism, and describe additional processes that may play a role in the distribution of all lipophilic chemicals. Octamethylcyclotetrasiloxane (D4) is a common ingredient in a variety of consumer items and cleaning products. In addition to low-level consumer exposure, the volatility of this compound raised concerns about occupational exposure via inhalation. In order to aid in assessment of worker risk, Andersen and coauthors attempted to analyze the distribution data in rats after inhalation of D4 (Plotzke et al., 2000) using a PBPK model. It was originally assumed that the kinetic behavior of D4 would be similar to styrene. This is based on the fact that D4, like styrene, is a volatile chemical, and also like styrene, is dependent on a single, first-step metabolic pathway. Thus, the same structure that was successfully used other volatile chemicals was applied to D4. Initial model simulations of inhalation exposure showed good agreement with the time course data for the pulmonary exhalation rate, urinary excretion rate, and plasma concentration. However, similar data following oral and IV dosing were poorly predicted. In the case of IV dosing, model simulated plasma levels more than an order of magnitude lower than measured values.

The inability to describe D4 clearance led the authors to reexamine the underlying model assumptions. Firstly, in assuming that all the chemical in the blood was available for exhalation, the model was over-predicting the clearance rate as well as exhaled air concentrations. As opposed to the model behavior, measured data showed a slow loss of chemical from the blood and lower levels of D4 in the exhaled air, indicating that a portion of the blood D4 was somehow bound and therefore unavailable for exhalation. Additionally, this assumption that all serum D4 was free, coupled with the high fat:blood partition coefficient, was causing slow redistribution after dosing, which also helped to account for the under-predicted serum levels. Secondly, by assuming that the liver and lungs were well-mixed compartments, the authors were forced to use large values for the lung:blood and liver:blood partition coefficients in an effort to fit the measured data. Yet, the model still was not able to fit the kinetic behavior; it over-predicted

tissue concentrations at early times and under-predicted later time points.

The original hypothesis was eventually revised to account for the difference between the model predicted and experimentally observed values. The authors proposed that the lipophilic D4 was sequestered in tissue lipid stores so that only a portion of the chemical was freely available for transport. This would explain the two-phase clearance, including the initial, rapid drop due to loss of the free (unbound) chemical and the secondary, slower decrease resulting from the loss of the lipid-bound chemical. The existence of chylomicron-type transport of D4 between the liver and plasma lipid compartment was suggested as a biological basis for the proposed kinetic construct, based on the work of Roth and colleagues in nonvolatile chlorinated biphenyls and dioxins (Roth et al., 1993). Finally, the revised model structure included two-separate fat storage compartments in order to account for the multiphasic behavior of D4 in exhaled air. It was thought that the different phases in exhaled D4 concentrations could be due to the fact that D4 was stored in various fat depots, and that the rate of exchange between the fat and blood was dependent upon the characteristics of the individual fat stores. When these changes were applied to the model structure, they were able to successfully simulate data from all dosing routes in both single and repeated dose studies.

The elaboration of the D4 model was accomplished in such a way that the original and revised models were nested structures. Therefore it was possible to use a likelihood ratio test to demonstrate statistically that the additional features of the revised model significantly improved the ability of the model to describe the kinetic data (Andersen et al., 2001).

It is important to note that previous evaluations of both the human (Utell et al., 1998) and rat (Plotzke et al., 2000) inhalation data on D4 had not recognized any major discrepancies from previous data on other volatile chemicals. In fact, based on the blood time course curves and the exhalation data, the assumption had been that the *in vivo* kinetics of D4 were similar to those of other volatile hydrocarbons. But when a PBPK model was applied to the problem, it became clear that despite the similar shape of the time-course curves, the concentrations were actually different from previous expectations by an order of magnitude. Without a quantitative model that could account for the differences in blood:air partition coefficients and other kinetic differences (fat partitioning, tissue time course behavior), this discrepancy may well have continued to go unnoticed. Due to the insights obtained with the PBPK model, however, these siloxanes became a source of better understanding of the role of lipophilicity in chemical

transport and for unraveling pathways for lipid transport of chemicals in the body.

Model Verification and Validation

Model validation should consider the ability of the model to predict the kinetic behavior of the chemical under conditions which test the principal aspects of the underlying model structure. While quantitative tests of goodness of fit may often be a useful aspect of the validation process, the more important consideration may be the ability of the model to provide an accurate prediction of the general behavior of the data in the intended application (Clark et al. 2004).

The demand that the PBPK fit a variety of data with a consistent set of parameters limits its ability to provide an optimal fit to a specific set of experimental data. For example, a PBPK model of a compound with saturable metabolism is required to reproduce both the high and low concentration behaviors, which appear qualitatively different, using the same parameter values. If one were independently fitting single curves with a model, different parameter value might provide better fits at each concentration, but would be relatively uninformative for extrapolation.

Where only some aspects of the model can be validated, it is particularly important to assess the uncertainty associated with the aspects which are untested. For example, a model of a chemical and its metabolites which is intended for use in cross-species extrapolation to humans would preferably be verified using data in different species, including humans, for both the parent chemical and the metabolites. If only parent chemical data is available in the human, the correspondence of metabolite predictions with data in several animal species could be used as a surrogate, but this deficiency should be carefully considered when applying the model to predict human metabolism. One of the values of biologically based modeling is the identification of specific data which would improve the quantitative prediction of toxicity in humans from animal experiments.

Model validation is preferably carried out using data that was not used in the development of the model and the estimation of its parameters. In some cases, however, it may be considered necessary or preferable to use all of the available data to support model development and parameterization. Unfortunately, this type of modeling can easily become a form of self-fulfilling prophecy: models are logically strongest when they fail, but psychologically most appealing when they succeed (Yates, 1978). Under these conditions,

model validation can be particularly difficult, putting an additional burden on the investigators to substantiate the trustworthiness of the model for its intended purpose. Nevertheless, a combined model development and validation can often be successfully performed, particularly for models intended for interpolation, integration, and comparison of data rather than for true extrapolation.

Describing Distributions for Uncertainty and Variability

In addition to comparing model predictions to experimental data, model evaluation includes assessing the plausibility of the model input parameters, and the confidence which can be placed in extrapolations performed by the model. This aspect of model evaluation is particularly important in the case of applications in risk assessment, where it is necessary to assess the uncertainty associated with risk estimates calculated with the model. When used in the risk assessment process, the output from PBPK models has usually been considered to be an exact estimate of dose (i.e., Andersen et al. 1987). Thus, risk assessment predictions illustrate what will happen to an "average" member of the target species. However, when the results of the risk assessment are applied to an entire population, no measure of the effects of intrapopulation variability can be made without knowledge of the model output response to parameter variability. Equally important, it is not possible to determine which model parameters have the most influence on model predictions or what magnitude of prediction error is associated with model parameter errors.

It is important in this discussion to distinguish uncertainty from variability. As it relates to the issue of using PBPK modeling in risk assessment, uncertainty can be defined as the possible error in estimating the "true" value of a parameter for a representative ("average") animal. Variability, however, should only be considered to represent true interindividual differences. Understood in these terms, uncertainty is a defect in a particular approach that typically can be reduced by experimentation, and variability is a fact of life that must be considered regardless of the risk assessment methodology used (Allen et al., 1996). One of the attractive features of PBPK modeling is that it identifies important areas of uncertainty that deserve experimental determination. At the same time, PBPK modeling can be used to examine the effect of variability. The model can be run with different parameter values to simulate interindividual differences, such as weight or level of exertion or metabolic status, and the range of individual risks corresponding to a given population risk can be estimated (Clewell and Andersen, 1996).

Several investigators have attempted to estimate the impact of parameter uncertainty in PBPK models on risk assessment predictions using the Monte Carlo method (Fiserova-Bergerova et al., 1980; Farrer et al. 1989; Portier and Kaplan, 1989; Bois et al., 1990; Clewell and Jarnot, 1994; Clewell, 1995; Allen et al., 1996; Clewell et al., 1999). Briefly, in the Monte Carlo method a probability distribution for each of the model parameters is randomly sampled, and the model is run using the chosen set of parameter values. This process is repeated several times until the probability distribution for the desired model output is determined. The sensitivity of the model output to a given input parameter is then represented by the percentage contribution to the total model output variability.

The chief difficulty in all of these studies is the lack of experimental data on the variability of many of the model parameters. Typical ranges of parameter uncertainties are shown in Table 2 (Clewell, 1995). Physiological parameter variabilities are often based on estimates of standard error included in a review of the physiological literature originally performed by Lindstedt for the ILSI Risk Science Institute Physiological Parameters Working Group (Brown et al. 1997). Partition coefficient variability has been directly measured for perchloroethylene (Gearhart et al. 1993). Except for ventilation, the experimental data typically do not justify use of physiological parameter uncertainties of greater than 30% or of partition coefficient uncertainties of greater than 20%; however, variation in metabolism in the human can be much greater (Clewell and Andersen,1996).

Table 2: Typical Range of Coefficients of Variation for PBPK Model Input Parameters.

Parameters	CV (%)	Distribution
Tissue Volumes	6 – 30	Truncated normal
Blood Flows	8 – 30	Truncated normal
Ventilation	15 – 50	Truncated normal
Partitions	15 – 20	Truncated lognormal
Metabolism	30 – 70	Truncated lognormal

Table 2 also displays the distributional forms that are often used for the input parameters in PBPK models. Physiological parameters are usually described with a normal distribution,

which is consistent with the available data from the physiological literature. Partition coefficients are obtained as a ratio of the measured concentrations in two media; assuming the measurements themselves are normally distributed, the ratio would be expected to be lognormal. Finally, metabolism parameters are generally expected to be lognormally distributed, consistent with the results of analyses of enzyme activity measurements on the population of hospital patients. In every case, truncated distributions are recommended to avoid physiologically implausible values (negative or outside the range of physiological limitations). It is always imortant, however to determine the extent to which the truncation alters the sample distribution, particularly for assymetrical truncation (e.g., non-negative bounding of a normal distribution with a mean within a small number of standard deviations of zero).

There are several reasons why the actual impact of parameter variability on risk estimates is likely to be much less than that predicted by a typical simulation analysis. The most important is the high degree of correlation that exists between various parameters. For example, in the Monte Carlo sampling typically performed, the value for the fractional blood flow to a tissue is taken to be independent of the fractional tissue volume. Physiologically, these parameters are highly correlated, because their ratio, known as the perfusion ratio, is critical for oxygenation of tissues. Pairing a high blood flow with a low tissue volume (or vice-versa) would exaggerate the variation in kinetic behavior of the tissue. Other correlations that are likely to be important, but that the Monte Carlo analyses typically ignore, are between ventilation and perfusion (QPC and QCC), between the various partition coefficients, and between some metabolic parameters. These correlations can often be directly addressed during the execution of the Monte Carlo analysis (Allen et al., 1996). The impact of neglecting correlations may also be exacerbated by the use of lognormal distributions for the metabolic parameters, since the lognormal distribution has a significant "tail."

Model Documentation

In cases where a model previously developed by one investigator is being evaluated for use in a different application by another investigator, adequate model documentation is critical for evaluation of the model. The documentation for a PBPK model should include sufficient information about the model so that an experienced modeler could accurately reproduce its structure and parameterization. Usually the suitable documentation of a model will require a

combination of one or more "box and arrow" model diagrams together with any equations which cannot be unequivocally derived from the diagrams (i.e., Figure 1). Model diagrams should clearly differentiate blood flow from other transport (i.e., biliary excretion) or metabolism, and arrows should be used where the direction of transport could be ambiguous. All tissue compartments, metabolism pathways, routes of exposure, and routes of elimination should be clearly and accurately presented. All equations should be dimensionally consistent and in standard mathematical notation. Generic equations can help to keep the description brief but complete. The values used for all model parameters should be provided, with units. If any of the listed parameter values are based on allometric scaling (Dedrick, 1973; Dedrick and Bischoff, 1980; EPA, 1992), a footnote should provide the body weight used to obtain the allometric constant as well as the power of body weight used in the scaling.

"Best Modeling Practices"

The process of PBPK model development described in this paper is intentionally iterative. Physiological and biochemical systems are highly complex, and it is foolhardy to expect a successful description on the first try. Too often, model developers propose a single model structure and then struggle to parameterize it, without attempting to seriously consider alternative structures. The two examples given in this paper illustrate a process that consists of (1) envisioning and then specifying alternative model structures based on a combination of experimental inference and biochemical knowledge, (2) performing a quantitative evaluation using objective statistical methods (e.g., likelihood comparisons) and, when possible, (3) verifying the underlying biological hypothesis (e.g., suicide inhibition) by separate experiment. The development of a PBPK model strictly on the basis of existing data is more properly characterized as analysis rather than research, the key difference being the iterative nature of the latter. It has wisely been said, "If we knew when we started what we had to do to finish, they'd call it search, not research."

The most effective way to develop a PBPK model is to exercise the model to generate a quantitative hypothesis; that is, to predict the behavior of the system of interest under conditions "outside the envelope" of the data used to develop the model (at shorter/longer durations, higher/lower concentrations, different routes, different species, etc.). In particular, if there is an element of the model which remains in question, the model can be exercised to determine the

experimental design under which the specific model element can best be tested. For example, if there is uncertainty regarding whether uptake into a particular tissue is flow or diffusion limited, alternative forms of the model can be used to compare predicted tissue concentration time courses under each of the limiting assumptions under various experimental conditions. The experimental design and sampling time which maximizes the difference between the predicted tissue concentrations under the two assumptions can then serve as the basis for the actual experimental data collection.

Once the critical data has been collected, the same model can also be used to support a more quantitative experimental inference. In the case of the tissue uptake question just described, not only can the a priori model predictions be compared with the observed data to test the alternative hypotheses, but the model can also be used a posteriori to estimate the quantitative extent of any observed diffusion limitation (i.e., to estimate the relevant model parameter by fitting the data). If, on the other hand, the model is unable to reproduce the experimental data under either assumption, it may be necessary to re-evaluate other aspects of the model structure.

There is an unfortunate tendency in PBPK model development to rely heavily on previously published models for other chemicals. For example, recently published PBPK models are still sometimes described by the authors as being based on the original styrene model (Ramsey and Andersen, 1984), and make use of essentially the same physiological structure and parameters. However, a great deal of progress has taken place over the score of years since the publication of the original styrene model, including the convening of expert working groups to recommend physiological parameter values. Moreover, the structure of the original styrene model reflects an appropriate use of parsimony and pragmatism consistent with the purposes of that modeling effort; for example, the volume of the intestines is included in the richly perfused tissues compartment, while their blood flow is included in the liver compartment, and a non-physiological liver blood flow is used to account for extrahepatic metabolism. More recent descriptions of other volatile, lipophilic compounds have sometimes found it necessary to use a different physiological description (Clewell et al., 2000). Every aspect of the development of a new model should be subject to skeptical criticism and careful evaluation by experimental measurement and simulation.

Data Limitations

Current knowledge of physiological parameters is limited at best, with well characterized values only for the larger tissues and organs, and little data on skin, fat and the smaller organs. Available data are restricted primarily to humans, rats, and to a lesser extent, mice, dogs, and monkeys; there is almost no data on other species. Data is primarily on adult animals, with little information on the perinatal period other than tissue weights. There is even less data on the variability of physiological parameters, let alone their interdependencies.

Literature data on partitioning is limited primarily to the volatile lipophilic compounds. *In vitro* experimental methods exist for estimating thermodynamic partitioning (lipophilicity) in both volatile and nonvolatile compounds. QSAR methods for estimating partitioning have been demonstrated for volatile, lipophilic compounds, but not in general. For many compounds, the apparent distribution ratio between plasma and tissues is determined, at least in part, by specific or nonspecific binding to proteins or other cellular components; methods for estimating parameters in this case are not as well developed.

Literature data on metabolism is usually limited to measurements of "activity" (rate of metabolism under excess substrate conditions) rather than the multiple concentration studies that are necessary to separately determine enzyme affinity and capacity. There are a variety of *in vitro* experimental methods available for determining metabolism rate constants that can be used in a PBPK model, but these have been reliably demonstrated only in the liver. The collection of *in vitro* metabolism data from other tissues, such as kidney, lung, nose or testes is more problematic, and more reliable methods are needed. Often the key issue is the inability to detect metabolism in the human target tissue, which compromises the usefulness of the PBPK model to predict a metric of risk for that tissue.

Perhaps the most critical need is for the development of ethically acceptable approaches for conducting *in vivo* kinetic studies in humans for non-pharmaceuticals. While it is certainly arguable that it should be possible to develop a human PBPK model on the basis of a validated animal model together with human physiological data and *in vitro* metabolism data, there is no question that the reliability of the model would be in doubt in the absence of *in vivo* pharmacokinetic (ADME) validation data.

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