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Volume VI

DEVELOPMENT AND VALIDATION OF METHODS FOR APPLYING PHARMACOKINETIC DATA IN RISK ASSESSMENT

VOLUME VI OF VII: SENSITIVITY AND UNCERTAINTY ANALYSIS

Clement International Corporation
K.S. Crump Division
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HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
HUMAN SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Maj, USAF, BSC
Deputy Director, Toxic Hazards Division
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FOREWORD

This report has been prepared by Clement International Corporation, K.S. Crump Division, for the Department of the Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base in response to a request to investigate the incorporation of pharmacokinetic modeling into quantitative risk assessment. This report contains the results of this multiyear effort and reflects the changes in direction and priorities as this project has evolved. The Project Director was Dr. Kenny Crump and the Principal Investigator for this project was Mr. Bruce Allen; other investigators who provided technical support and internal peer review were Drs. Crump and Annette Shipp. Mr. Allen was assisted in the pharmacokinetic modeling and analyses primarily by Mr. Christopher Rambin and by Ms. Robinan Gentry. The sensitivity analyses were conducted by Mr. David Farrar, Dr. Crump, Dr. Richard Howe, and Mr. Allen. The software was developed by Ms. Cynthia Van Landingham, Mr. William Fuller, Mr. Eric Brooks, Dr. Howe, and Mr. Allen. The authors wish to acknowledge the support provided by Dr. Jeffery Fisher and Lt. Col. Harvey Clewell, who are at the Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base, and Drs. Melvin Andersen and Michael Gargas, formerly with the Harry G. Armstrong Aerospace Medical Research Laboratory and now with CIIT.

PREFACE

This volume describes analyses that were conducted in connection with the investigation of uncertainty and sensitivity of physiologically based pharmacokinetic (PBPK) models. In particular, interest was focussed on the impact of uncertainty about the values of model parameters and the sensitivity of PBPK models and dose response models to the values of the parameters.

Part 1 of this volume describes a sensitivity analysis conducted using a relatively simple PBPK model. Parameter values were varied one at a time by an arbitrary, small percentage. The percentage change in PBPK model output (dose surrogate estimates) was recorded for each parameter change.

Also presented in Part 1 are the preliminary considerations and experimental data relevant to the conduct of an uncertainty analysis. In such an analysis, the parameters are allowed to vary in biologically and experimentally meaningful ways to a degree consistent with the observed uncertainty and variability associated with the parameter values. Then, a distribution of output values (dose surrogate estimates or risk estimates) derived from those varying parameters is available for subsequent use. The output distribution reflects all the parameter uncertainties.

Part 2 presents some additional analyses related to the uncertainty analysis just described. The contribution of an individual parameter (or set of interrelated parameters) to the overall output uncertainty is examined by allowing only that parameter (or set of parameters) to vary. The variation in the output values, relative to the variation in the output when all parameters are allowed to vary, is taken as an indicator of the contribution of that parameter (or set of parameters).

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SENSITIVITY/UNCERTAINTY ANALYSIS

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SENSITIVITY ANALYSIS;

PRELIMINARY CONSIDERATIONS FOR UNCERTAINTY ANALYSES

A. INTRODUCTION

An analysis of PBPK models and their use in risk assessment should attempt to characterize the uncertainties that are associated with that use. Some uncertainties described in the Introduction to this document (Volume I, Part 1) relate to the more or less general, conceptual problems that must be resolved in order to make the use of PBPK modeling in risk assessment less uncertain. This volume describes an investigation of a more specific source of uncertainty: the uncertainty associated with the estimation of parameters for the PBPK model and the sensitivity of the desired dose surrogates to changes in those parameters. The investigation focussed on the simple PERC model discussed in Volume III, Part 1. The goal was to relate the extent of knowledge about the input parameters to the range of dose surrogate values, and ultimately risk estimates, that are consistent with that knowledge.

The investigation of PBPK model uncertainty was divided into two phases, a sensitivity analysis and an uncertainty analysis. The former was concerned with the degree to which the model results (dose surrogate estimates) depended on the values of the input parameters and the extent of change in those estimates associated with changes in the parameter values. The uncertainty analysis examined uncertainty in the parameter values *per se* and the associated distribution of dose surrogate values that resulted from the simultaneous consideration of all the uncertainties. Of course, the resulting distribution of the dose surrogate values depended not only on the degree of uncertainty associated with a parameter but also on the sensitivity of the model to changes in that parameter. Thus, the sensitivity analysis was the basis for both phases of this investigation and is described first.

B. SENSITIVITY

The sensitivity analysis consisted of an examination of the sensitivity of the PERC model to changes in single parameter values. Three dose surrogates were used to measure sensitivity: area under the liver concentration curve of the parent (AUCL), area under the arterial blood concentration curve of the parent (AUCA), and virtual concentration of the metabolite in the liver. The average daily values of these dose surrogates were used. Three sets of "preferred" parameter values were selected, one for mice, one for rats, and one for humans (Table VI-1-1). In addition, because it is likely that route of exposure, pattern, and level of exposure may modify the sensitivity of the model to a particular parameter, dosing variations were defined by combining several options with respect to such aspects. The routes and patterns used were:

- inhalation, exposure for 8 hours per day
- inhalation, continuous exposure
- gavage, once per day
- intravenous injection, once per day.

For each of the four route/pattern combinations three separate dose levels were chosen. A low level of exposure was selected such that the metabolic pathway was far from saturation. An intermediate dose level at which the metabolic pathway was changing from first-order to zero-order and a saturating dose provided the other two doses. Preliminary runs of the model with the parameter sets listed in Table VI-1-1 allowed estimation of the appropriate dose levels (Table VI-1-2).

For the gavage, intravenous, and 8-hour inhalation exposure scenarios, the model was run to simulate 24 hours starting at the beginning of exposure. For the continuous inhalation scenario, an initial time was determined such that at that time the system was close to steady-state. The model was then allowed to simulate another 24 hours. It was that 24-hour period over which the PERC liver concentration and the arterial blood concentration were integrated and over which the metabolism was summed to yield the dose surrogates of interest. The times at which the appropriate periods began are listed in Table VI-1-2.

For each of the sets of "preferred" parameter values and for each selected combination of route, pattern, and level of dosing, the model was run to obtain baseline values for each of the dose surrogates. Then, each parameter (one at a time) was increased by 1%: when examining the sensitivity to parameter X, all other parameters remained fixed at their "preferred" values. A minor exception to this rule related to the handling of blood flow rates. The flows to the tissue compartments must sum to the total cardiac output. Thus when one compartment flow rate was increased by 1%, the total cardiac output was increased by the same amount. The other individual compartment flow rates were not changed. Thus the effect of this treatment was to change slightly the percentage of the cardiac output that reaches any particular compartment.

Sensitivity was expressed in terms of the percent change in the values of the three dose surrogates. If DS was one of the dose surrogates, DS_0 was its baseline value, and DS_x was its value obtained by increasing the value of parameter X by 1%, then $100*(DS_x - DS_0)/DS_0$ was the percent change recorded. Tables VI-1-3 through V-1-14 present the results of the sensitivity

investigation of the PERC model. [In those tables, percent changes less than 10^{-5} have been uniformly designated by asterisks to indicate changes at or below the accuracy of the model.]

Some patterns were evident from examination of the values reported in the tables. Not surprisingly, the AUCL dose surrogate was most sensitive to pl for almost all routes of exposure, dose levels, or species. The liver/blood partition coefficient, pl, determines the extent to which PERC concentrates in the liver. For some inhalation exposures at high enough dose (Table VI-1-9), the sensitivity of AUCL to pb exceeded that of AUCL to pl. This must be because the blood becomes so laden with PERC that a large amount of the chemical will partition to the liver for both pl values examined. For intravenous administration, AUCL was more sensitive to the parameter tiv (the duration of an iv dose administration) than to pl in rats and humans at medium doses (Tables VI-1-8 and VI-1-12).

For AUCA, pb and qpc (which, in general, were very similar for gavage or intravenous dosing in the magnitude of the sensitivity values but differed in sign) were the most important parameters. [Note: in this discussion, parameters to which a dose surrogate is more highly sensitive are referred to as "important" parameters. To a considerable degree this designation is relative, depending as it does on the sensitivity of the dose surrogate to other parameters.] For virtual concentration of metabolite (CM), vlc and, especially for the human parameter set, vmaxc were most important.

The body weight parameter (bw) displayed an interesting pattern. AUCL and AUCA were more sensitive to bw than was CM when dose was given by gavage or iv but these two dose surrogates were less sensitive than was CM when inhalation exposures were considered. This pattern was especially evident for

the mouse parameter set. It is suspected that this pattern was due, at least in part, to the manner in which doses were expressed. The iv and gavage scenarios were defined in terms of doses given in mg/kg body weight. Thus, changes in body weight also changed the dose administered by these two routes but not for the inhalation exposures. The sensitivity of CM in the latter cases is most likely a reflection of the differential scaling of vmaxc (scaled according to $bw^{0.7}$) and vl (scaled according to $bw^{1.0}$). Since all the flows and compartment volumes are scaled proportionally to $bw^{1.0}$, the effects on blood and liver PERC concentrations due to a change in body weight were not as substantial.

As mentioned above, vmaxc was important for CM. It was substantially less important to AUCL and AUCA, although for mice, particularly at low doses, its effect on these two dose surrogates can be relatively important and even exceeded the effect on CM (Tables VI-1-3 through VI-1-6). It was also the case that the sensitivity of CM to vmaxc increased as dose increased. However, the sensitivity of AUCA and AUCL to vmaxc decreased as dose level increased. As doses increased and as metabolism became saturated, entailing relatively less PERC being metabolized, the effect of metabolism on the parent concentrations became less important. Note that, of the three parameter sets considered, the mouse set had the largest vmaxc, followed by the rat set. As vmaxc decreased, the sensitivity of CM to vmaxc increased while the sensitivity of AUCL and AUCA to vmaxc decreased.

The other metabolic parameter, km, became less important as dose level increased, as expected. This was especially true for the dose surrogates AUCL and AUCA. Those two dose surrogates were more sensitive to km for mice than for rats or humans. On the other hand, at low to medium dose levels, CM was

less sensitive to km when the parameters were equal to the values in the mouse set than when they took the rat or human values. The km value for rats and humans was about two times larger than the value for mice.

All the dose surrogates examined were relatively insensitive to changes in the partition coefficients corresponding to compartments other than the liver, regardless of route or level of exposure. In fact, pl was important only for the AUCL surrogate. Conversely, pb was relatively important for all routes, species, and dose levels. The sensitivity of the dose surrogates to pb tended to increase with dose level, although the differences across dose levels were generally not great. An exception to this observation was noted for the CM surrogate, for which the sensitivity to pb tended to decrease at high doses, especially for continuous inhalation exposures. In those cases, the high doses entailed high enough blood concentrations that the limiting step was metabolism, not delivery of PERC to the liver.

For a compartment other than the liver, the sensitivity of the dose surrogates to the partition coefficient and to the compartment volume were almost indistinguishable. This relationship was strongest at the high doses but was evident also at medium and, to a lesser extent, low dose levels. (This observation may have important implications for the optimization of parameters in the face of uncertainty about both compartment volumes and partition coefficients. Generally, it will be the case that a partition coefficient value is more uncertain than the corresponding compartment volume.) The observation that partition coefficients other than pb and pl were relatively unimportant extends to compartment volumes as well. Indeed, dose surrogates other than CM were not very sensitive to liver volume changes,

certainly no more so than to changes in the other compartment volumes. The importance of vlc to the CM surrogate was noted above.

The ventilation rate parameter was relatively important for all dose surrogates and all routes of exposure at low to medium dose levels. The sensitivity of the dose surrogates to ventilation rate (qpc) varied somewhat with the level of dose. Moreover, the dose trend that was observed depended on the route of exposure. For inhalation exposures, the surrogates became less sensitive to qpc as dose increased (at high enough doses the concentration in the blood was high no matter how fast the animals took in the chemical). For gavage and intravenous dosing, the sensitivity increased as dose level increased (high iv or gavage doses saturated the blood and exhalation was a prime means of eliminating the parent but not, as in the case of inhalation, of taking in PERC). As might be expected, increasing qpc increased the dose surrogate estimates when dose was administered via inhalation whereas an increase in qpc reduced the surrogate estimates when breathing rate did not influence the absorption of the chemical, i.e., when PERC was given via gavage or iv.

As noted above, qpc was a more important parameter for AUCA than for the other surrogates. This was not due to qpc sensitivity values that were substantially greater for AUCA than for the other surrogates. Rather, the other parameters that were important for AUCL and CM (pl, vlc, and vmaxc, for example) were much less important for AUCA so that the relative importance of qpc to AUCA increased.

The blood flow rates were among the least important parameters for any dose surrogate. It is interesting to note the changes in the direction of change of a dose surrogate induced by an increase in a parameter value. For

increases in the blood flow rates, it was frequently the case that the signs on the sensitivity values differed for different dose levels, parameter sets, and dose surrogates. No consistent pattern was apparent. This effect was not anticipated.

Another way to examine the sensitivity issue is to rank-order the parameters with respect to the degree of sensitivity of a dose surrogate to those parameters. Tables VI-1-15 and VI-1-16 display the orderings obtained for the CM surrogate when two routes of exposure and two species (parameter sets) were considered. The importance of vlc and vmaxc to CM is underscored in these representations. Several other observations are relevant.

- The interaction of dose route and dose level in defining sensitivities was seen, for example, in the increasing importance of qpc as iv dose increased and as inhalation dose decreased for the rat parameter set.
- The distinction between important and unimportant parameters was much clearer when a high inhalation dose was administered, whereas a more continuous variation in sensitivity was seen at lower doses or for other routes.
- Blood flow rates to the compartments were consistently unimportant (with the possible exception of qlc).

There was little difference between the two parameter sets with respect to the most sensitive parameters, although the particular ordering differed somewhat between the species. This was a reflection of the difference in the values assigned to the parameters. It was not the case, however, that the sole determinant of the importance of a parameter was the value of that parameter. Consider the parameters defining the percentage of cardiac output directed to the compartments. Even though these parameters were the same in the rat and mouse parameter sets, the ranks that these parameters received

differed across species. So too did the ranking based solely on those blood flow parameters, with qsc ranked higher than qfc in one species or for one dose level and vice versa for the other parameter set and for other dose levels.

As stated above, the sensitivity of the dose surrogates to the parameters is an important factor in the determination of uncertainty propagation. Those parameters for which uncertainty with respect to their real value is great may not necessarily lead to great uncertainty in the dose surrogates if the surrogates are not particularly sensitive to those parameters. On the other hand, even relatively minor uncertainty regarding the value of a parameter may entail large uncertainty in a dose surrogate (and ultimately in risk estimates based on dose surrogate estimates) if the surrogates of interest are extremely sensitive to the parameter in question. Uncertainty is discussed more completely in the next section.

C. UNCERTAINTY

The values of the parameters that define a PBPK model are uncertain. The uncertainty associated with a parameter estimate may be due to lack of adequate data, differences in methods used to estimate the parameter value, and/or recognition of the fact that a single value may not be adequate to characterize a parameter that varies over the population of interest. The goal of the uncertainty analysis described here was to relate the parameter uncertainties to the distribution of the results of the PBPK models, i.e., to the dose surrogates estimated by those models. In addition, that analysis included computation of distributions of risk estimates, which depended on the

values estimated for the dose surrogates, and thus were also related to parameter uncertainty. We refer to the distribution of dose surrogates attributable to parameter uncertainty as dose surrogate uncertainty. Similarly, the distribution of risk estimates attributable to parameter uncertainty is referred to as risk uncertainty. The definition of risk uncertainty is extended to include effects of uncertainty about the true rate of response for any group of animals tested (observed for tumor response) and used for fitting of a dose-response model.

The first task in this uncertainty analysis was the definition of the joint uncertainty distribution for the parameters. The description of that work for the PERC model parameters comprises the bulk of this section. As described below, the parameter uncertainty distribution takes into account as many of the features of the estimation of parameter values as possible. The emphasis in this section is on development of methods and conceptual approaches to the issue of parameter uncertainty estimation.

Given estimated uncertainty distributions for the parameters, the dose surrogate uncertainty distributions were estimated by Monte Carlo simulation. For each simulation, a value was sampled from each of the distributions defined for the parameters. These values were then used in the PBPK model to determine the corresponding values of the dose surrogates. A large number of simulations allowed estimation of the uncertainty distribution of the dose surrogates.

Dose surrogate uncertainty was then extended to estimate the uncertainty in risk estimates due to uncertainties in model parameters. The uncertainty distributions for the animal surrogate doses were estimated as described in the preceding paragraph, for each of the dose patterns used in one or more

carcinogenicity bioassay. Similarly, uncertainty distributions for human surrogate doses were estimated for (the usually low) levels of exposure that are of concern; this was accomplished by sampling from the parameter uncertainty distributions corresponding to the human parameter values and running the PBPK model many times exactly as described above for the animal parameters and simulations.

Given the distributions of the dose surrogate estimates (for each animal dosing pattern and for the human exposure scenario of interest), it was possible to estimate risk uncertainty. The methods by which this was accomplished for one carcinogenicity bioassay are described as follows.

1. A bioassay was selected. Carcinogenic responses appropriate for use in estimation of human risk were chosen.

2. For the doses employed in the bioassay, the dose surrogate values corresponding to the preferred PBPK model parameter values were used to fit a dose-response curve.

3. In order to incorporate the uncertainty about the true response rate at the doses tested, the probabilities of response estimated by the dose-response curve fit in step 2 were used to randomly regenerate response rates (from binomial distributions with their parameters defined by the number of animals tested at each dose and the estimated probabilities of response).

4. The dose surrogate distributions (previously estimated) were sampled once for each dose group and a new dose-response function was fit using the just-sampled dose surrogate values and the randomly generated tumor response rates.

5. A value from the uncertainty distribution of the human dose surrogates (also determined previously) was sampled; it determined the value for which risk was estimated, which was accomplished using the newly fitted dose-response curve.

Repetition of this procedure a large number of times allowed estimation of the uncertainty distribution of the human risk estimates.

Several results are available from an uncertainty analysis as described:

- Uncertainty distributions for each of the parameters: the uncertainty distribution for each parameter or set of parameters is represented by a statistical probability distribution. Several of the parameters for the PERC model are represented in this manner below.

- Uncertainty distributions for dose surrogates: the empirical distributions resulting from the Monte Carlo sampling of the parameter uncertainty distributions can be displayed graphically as cumulative frequency plots, histograms, or displayed in tabular form, listing, for example, the 2.5, 5, 10, 90, 95, and 97.5 percentiles.

- Uncertainty distributions for the risk estimates: the empirical distributions resulting from Monte Carlo sampling of the parameter uncertainty distribution, PBPK modeling for each bioassay dose level and human exposure level, and dose-response analysis.

The remainder of this section reviews the considerations and data relevant to estimation of uncertainty distributions for the parameters of the PERC PBPK model considered in Volume III, Part 1 (and used for the sensitivity analysis in this volume). The focus has been on the development of the methodological approaches and identification of difficulties that may arise in the specification of uncertainty distributions.

Appendix VI-1-A contains a published analysis that has completed the task of deriving dose surrogate and risk estimate uncertainty distributions. The example in that document is also for PERC, and uses results from a female mouse carcinogenicity bioassay to get risk estimates.

1. Parameter Uncertainty Estimation

The uncertainty associated with a parameter value depends on the type of data available for estimating the parameter and the way that the data are used to arrive at an estimate. Frequently more complex and indirect estimates (e.g., estimates based on numerous assumptions) will be attended by greater

uncertainty than relatively simple and direct estimates. For example, if one attributes to humans a value measured directly only in rats, then the uncertainty for the human value is affected by the degree of variation in the value from species to species, as well as whatever uncertainties are associated with the direct measurement in rats.

In an uncertainty analysis as described here, it is necessary to have "preferred" values for parameters. In general, a preferred value for a parameter is an estimate that has been determined by one or more users of the specific PBPK model and has been validated to some extent. The preferred value roughly corresponds to the center of the uncertainty distribution that is to be estimated. For the PERC model parameters, for example, the preferred values may be assumed to be those given by Reitz and Nolan (1986) (cf. Table III-1-1).

The manner in which Reitz and Nolan derived estimates for the parameters of their PERC model is representative of the considerations that typically apply to the issue of parameter estimation. The necessity of attributing to humans parameter values based on measurements in animals is but one example. Thus, the approaches that they have used are convenient starting points for this investigation of parameter uncertainty estimation and are examined in some detail here. The approaches of Reitz and Nolan do not necessarily provide the most reliable estimates possible for PBPK model parameters; however, by examining their approaches, one can evaluate the uncertainties that will arise in many situations. One nice feature of the approach to uncertainty estimation described below is that, as more reliable estimates of parameter values become available, the uncertainties associated with the

parameters will be reduced because of the considerations given to the sources for and assumptions inherent in the estimation procedure.

Probability Models for Uncertainty. The first assumption underlying this approach to uncertainty analysis is that uncertainties are naturally expressed in terms of probability. For example, if one is fairly certain that a parameter value must lie within a specified interval then that interval will be assumed to contain the true parameter value with high probability. (Note: for continuous variables the probability of any single value is zero, while the probability density of the value may be greater than zero. A probability density may be integrated over an interval to yield the probability associated with the interval.) Given this basic assumption, the formal treatment of uncertainties involves specification of a joint probability distribution for all the parameters that are subject to uncertainty.

Consider an example for a parameter that is assumed to be independent of the other parameters. A parameter is independent of another parameter when information regarding the value of the other parameter is uninformative with respect to the value of the first parameter. In this case, the preferred value of the parameter might be assigned the highest probability density, and values at increasing distances (both above and below the preferred value) are associated with lower density. If it is further assumed that values equidistant from the preferred value have equal probability density and that every value has nonzero (positive) density, then a natural representation of the uncertainty distribution is given by the normal distribution. The mean of the distribution is equated with the preferred value and the variance (the only other parameter defining a normal curve) is estimated on the basis of the

data used to derive the parameter estimates. The variance would be the probability parameter reflecting uncertainty.

The probability distribution for a single parameter is called the marginal probability distribution for that parameter. A set of marginal probability distributions (one for each parameter) specifies the joint probability distribution when the parameters are independent. When the parameters are not assumed to be independent, additional assumptions or parameters may be required to specify the nature of the dependencies and to characterize the joint distribution.

The assumptions discussed above in relation to the representation of the uncertainty distribution of a parameter by a normal curve are generally not appropriate for characterization of PBPK model parameter uncertainty. This is true because of the known, logical bounds on the values of the parameters. Obviously, all the parameters are constrained to be nonnegative. Moreover, certain of the parameters (such as percentage of body weight that is occupied by a compartment and proportions of cardiac output that flow to a compartment) are bounded above by unity (or 100%). The parameters that are proportions or percentages are also constrained by the values of the other parameters that describe proportions associated with other compartments. Some distributions that may be considered for defining parameter uncertainty are described below; they all are expressed in terms of explicit probability models that have a finite mean (generally equated with the preferred value of the corresponding parameter) and additional parameters specifying the distribution of probability about the mean.

Two cases are recognized. For parameters that are logically bounded below, one may use the lognormal probability distribution, i.e., one assumes

that uncertainty follows a normal distribution with a specified mean and variance when parameter values are expressed in the log scale. The lognormal is flexible in the sense that different selections of log-scale mean and variance may lead to distributions with widely different shapes, although all assign zero probability density to values zero or smaller. If the variance is small relative to the mean, then the shape of the density function is similar to that of the normal distribution; relatively larger values of the variance lead to J-shaped distributions.

For lognormal distributions, one can assume that the mean of the distribution, in the log scale, is the log of the preferred value. Then for a preferred value X and any positive number K, the values X/K and X*K have equal probability density (i.e., the probability density function is symmetric in the log scale about the log of the preferred value.) It is convenient to represent uncertainty using an "uncertainty factor" (UF) not smaller than unity such that for the preferred value X, the interval X/UF to X*UF is considered to contain the true value of the parameter with probability at least 95%. More about uncertainty factors and their estimation is given below.

In the second case, for parameters that are proportions (e.g. compartment volumes as percentage of total body volume), a joint distribution must be specified for each set of proportions, since the observed proportions cannot be varied independently. One can use a Dirichlet distribution for each set of proportions (Johnson and Kotz, 1972), which is a multivariate generalization of the beta distribution. (The beta distribution is the most commonly encountered continuous distribution for a single proportion: Iman and Shortencarier (1984) have previously discussed the use of the beta

distribution in the contexts of uncertainty analysis.) For each proportion or percentage, the Dirichlet distribution permits only values between 0 and 1. Subject to this constraint, the Dirichlet distribution is extremely flexible in terms of the diversity of shapes which may result from selection of various values for the parameters. Given a preferred value for each proportion, the variances and covariances of the proportions are determined by a parameter THETA. A brief discussion of the Dirichlet distribution is provided here.

For purposes of discussion, assume that the parameters are proportions, i.e., that their sum is constrained to equal 1. For K proportions, it is necessary to specify K parameters for the Dirichlet distribution. There are two common ways of expressing the parameters of a Dirichlet distribution. One representation is given in terms of the parameters p(1),p(2),...,p(K-1) (where p(i) is the expected value of the ith proportion) and the parameter THETA (which can be no smaller than zero). Note that the Kth expectation is known if the first K-1 are specified. A second common representation is in terms of a(i) = p(i)*THETA, for i = 1,...,K. The first representation is more natural to PBPK modeling applications. The preferred value for the ith proportion ie set equal to p(i); the parameter THETA must be estimated from experimental data.

It is clear that two proportions (such as proportion of body weight that is the liver and proportion that is fat) must be negatively correlated. That is, as the proportion of body weight that is liver increases, the proportion that is fat must tend to decrease. In fact, the covariance of two proportions described jointly by a Dirichlet distribution is given by

$$(1) \qquad COV(i,j) = -p(i)*p(j)/(THETA + 1).$$

Thus the uncertainties for members of a set of proportions assumed to be Dirichlet-distributed are not independent. They are completely specified by the preferred values and the parameter THETA.

As an example, consider volumes of tissue compartments. These can be represented by a Dirichlet-distribution when they are expressed as proportions of the total body weight. In fact, the proportions may change with changes in body weight. For instance, it appears reasonable that the volume of the fat compartment will be correlated with body weight. A relatively simple representation of the dependence of fat volume on body weight is given by

$$(2a) \qquad pF(BW) = pF*BW/BWp \qquad \text{for } BW \leq BWp;$$

$$(2b) \qquad pF(BW) = 1 - (BWp/BW)*(1 - pF) \qquad \text{for } BW \geq BWp,$$

where pF is the preferred value for proportion that is fat at the preferred body weight, BWp. This formula implies that the expected value of fat volume goes down quadratically with body weight for body weight less than BWp, and that body weight in excess of BWp consists of fat. The preferred values of the remaining (non-fat) proportions, p(i), can be adjusted by multiplying each by (1-pF(BW))/(1-p(i)), so that compartment proportions other than that for the fat compartment retain the same relationship among themselves that they have at the preferred body weight. The pF(BW) and other adjusted p(i)'s are the parameters that, along with THETA, now define the Dirichlet distribution for compartment proportions in an animal of weight BW.

In summary, then, PBPK model parameter uncertainty can be modeled on the basis of probability distributions. For parameters other than those that are expressed in terms of proportions, log-normal distributions can be assumed. For proportions, the Dirichlet distribution provides a convenient definition of the relationship among the proportions. As illustrated in the case of compartment volumes as proportions of body weight, inter-relationships among parameters of a PBPK model can be incorporated so as to represent biological realities.

What remains is to specify the relevant sources of uncertainty and the manner in which they can be used to estimate the other parameters of the probability distributions (such as the log-variance or log-standard deviation in the case of log-normal distributions), the ones that actually define the uncertainty about preferred values.

Sources of Uncertainty. Two general types of uncertainty appear to be relevant to the definition of the uncertainty distributions discussed above. The first, which will be denoted as source I, is uncertainty about the value of a parameter for a random individual in a population that is due to individual variability about a population mean. This uncertainty may be reflected in the variation in parameter estimates among individual subjects within a study and expressed in terms of the standard deviation or coefficient of variation. The second source of uncertainty is identified with uncertainty regarding the population mean value of a parameter; this source is denoted as source M. Data from different studies that suggest different mean values are relevant to this type of uncertainty. Both sources are pertinent to the estimation of total uncertainty and can be incorporated by considering the following framework.

The total uncertainty is defined as the uncertainty regarding the true value for an individual, selected at random from the population. This total uncertainty may be evaluated by the following sort of two-step Monte Carlo approach. (In practice the two steps can often be telescoped into one.) First, pick a value at random to represent the population mean, from a distribution with mean equal to the preferred value, and other parameters (e.g., variance) determined by the variation that we observe among studies (reflecting source M uncertainty). Second, randomly select a value to represent the parameter value for an individual in the population, from a distribution with mean equal to the simulated population mean from the first step, and with other parameters determined by the variation observed among individuals within studies (reflecting source I uncertainty). When the complete process is performed an indefinite number of times, performing each step precisely once for each final value generated, the distribution of the final values (from the second step) describes the distribution of "total uncertainty."

Note that of the two sources of uncertainty, source M is relatively more subjective in nature, ideally depending on the scientific process of reviewing the relative reliability of each study, and weighting studies appropriately in their contribution to the preferred value. (Uncertainty may be reduced if the more extreme values are determined by independent arguments to be based on relatively unreliable procedures and are therefore given less weight.) In view of this process, source M may be regarded as more legitimately the subject of expert opinion than source I.

The two basic sources of uncertainty may be subdivided further for particular parameters, depending upon the method by which parameter estimates

were determined. For example, if one assumes for humans a value measured directly for animals, then a component of source M uncertainty is due to variation among the values typical for different species. Such considerations can be incorporated into the estimation of uncertainty distributions.

In many instances, it is convenient to identify and estimate source-specific uncertainty. Uncertainty factors corresponding to the sources can be denoted either as UF_I or UF_M, as appropriate. These source-specific uncertainty factors would represent the residual uncertainty, if uncertainty from other sources could be eliminated. Presented below are methods for combining source-specific uncertainty factors into a total uncertainty factor.

In the context of sources of uncertainty, it is valuable to distinguish between reducible and irreducible sources of uncertainty. To a large extent, uncertainty about the population mean (source M) is reducible uncertainty, in principle. Mechanisms for reducing this uncertainty include scrutiny of methods used in particular studies, possibly leading to identification of extreme estimates that are based on faulty procedures. Also, reduction of uncertainty may be achieved by averaging estimates that are considered comparable in reliability. The studies that are properly conducted and equally reliable should all be able to contribute to the estimation of a population average.

In contrast, individual variation as a source of uncertainty is more or less irreducible. This does depend on the context of application however. If the goal of an assessment is to describe the distribution of risk levels among individuals in a specified population, then some consideration of individual variation in parameter values is appropriate and should be incorporated into the analysis. In that case the object is not to reduce the source I

uncertainty, but to incorporate a reliable estimate of that uncertainty. On the other hand, if an assessment is desired for a particular individual, then individual variation is a relevant source of uncertainty when attributes of the individual are unknown. (In this case we equate uncertainty regarding the individual's parameter values to the frequency distribution of values observed for the population.) Obviously the uncertainty may be reduced if relevant characteristics of the individual, such as sex, weight, or breathing rate, are measured and taken into account.

Methods for Estimation of Uncertainty Factors. The discussion above has indicated sources of uncertainty and the manner in which uncertainty about the values of a parameter can be modeled, i.e., either by a lognormal distribution about a preferred value or by a Dirichlet distribution with expected values for proportions set equal to the preferred values of those proportions. What remains is to specify the methods that can be used to estimate the other parameters of those distributions, the parameters that actually correspond to uncertainty in the sense of defining the "spread" of the distribution.

In the case of the lognormally distributed uncertainties, several statistics that are computed directly from available data are relevant to the estimation of uncertainty factors (Table VI-1-17). Recall that for a lognormal distribution, the uncertainty factor (UF) that is desired is one such that the interval (X/UF, X*UF) contains approximately 95% of the total probability of the distribution, i.e., that specifies with about 95% certainty the interval that contains the true value of the parameter, where X is the preferred value of the parameter. Three cases can be identified that may lead to estimates of UF based on different statistics shown in Table VI-1-17; all are intended to yield UF estimates with the desired "95%" property.

In the first case, suppose that data on the variation of a particular parameter is given only in terms of a plausible range, perhaps specified by a relevant expert. Assume that this range represents a "95%" interval in the sense given above. Then it is natural to equate the uncertainty factor to the statistic S1 = $(UB/LB)^{0.5}$, where LB is the lower bound of the interval and UB is the upper bound. This representation can be derived by equating LB to GM/UF, and UB to GM*UF, where GM is the geometric mean of the range.

The second case arises when individual measurements (or relevant summary statistics of those measurements) are available and it is assumed that the measurements are independent and identically distributed (iid). The relevant summary statistics are estimates of the log-scale standard deviation (SDL); the primary ones considered are SSDL and CV (cf. Table VI-1-17). [As indicated in Table VI-1-17, SSDL is the preferred estimate of SDL but it is frequently the case that individual measurements are not provided, so that SSDL can not be computed, and that SSDL itself is also not reported. Use of CV, the coefficient of variation, is then sufficient. Its approximation of SDL is based on a first-order Taylor's series expansion. Our experience indicates that the approximation is accurate to one to three significant digits.] Recall that, for a normal distribution, approximately 95% of the probability is contained within two standard deviations of the mean. Thus for the log-normally distributed uncertainty distribution, the interval (X/exp(2*SDL), X*exp(2*SDL)) contains approximately 95% of the probability. When the uncertainty in question is irreducible, or when no steps are taken to reduce a reducible uncertainty, these considerations imply a choice for UF of S2 (Table VI-1-17). When a reducible uncertainty has been reduced by averaging the iid observations that underlie these statistics, so that the

preferred value is the geometric mean of the observations, then the statistic S3 (Table VI-1-17) appropriately reflects the reduction in uncertainty attained by the averaging.

The third case involves observations that are likely to deviate strongly from the iid assumption, with the nature of the deviation not fully understood or specified. This case is obviously problematic. This type of situation may hold frequently in evaluating source M: the available studies may have used different methods to estimate the value of a parameter and particular studies may have associated with them measurement biases or levels of measurement error which are more or less specific to the study and are of unknown magnitude. If some measure of relative reliability can be attached to each available measurement, and if the relationships between the different measurements can also be fully described, then in principle it may be possible to develop a formal assessment of uncertainty using a probability model. In the absence of such a complete analysis there is no guarantee that any formula will produce a better UF than a guess from an experienced researcher. In some cases one may have to apply methods based on iid assumptions, leading to evaluations of uncertainty that may be relatively questionable. These cases should be documented when they occur.

It was argued above that when the technique used to reduce a reducible uncertainty is averaging of the relevant iid observations, the statistic S3 is an appropriate estimate of the uncertainty factor for the preferred value that is the geometric mean of the observations. However, not all of the preferred values are geometric means of observations and, moreover, observations from different studies (pertinent to the estimation of source M uncertainty) are frequently not iid. A development of estimates of uncertainty for other

uncertainty-reduction mechanisms would require a formal model of such mechanisms that have operated in producing the preferred values, including, perhaps, the impressions of experts regarding the relative merits of different studies. This is not a trivial undertaking. However, it may be the case that an adjustment similar to that seen in statistic S3 (i.e., division by the number of measurements) may be considered, replacing the number of measurements by some number not larger than the number of study-specific estimates considered in arriving at the preferred value. Again, source M appears to be more legitimately evaluated on the basis of expert opinion than source I.

The statistics such as those in Table VI-1-17 that are computed to estimate source M uncertainty are all "impure" estimates. The value reported in each study is affected to some degree by the variation of individuals within studies, depending on the number of individuals measured. Consequently, to an extent, individual variation is included twice in such an evaluation of total uncertainty. Similar redundancy occurs in evaluating various other sources of uncertainty. In principle a correction is possible based on a components-of-variance approach, but such an approach has not been investigated here.

For those parameters that are expressed as proportions and whose uncertainty is modeled by a Dirichlet distribution, the uncertainty parameter that must be estimated is THETA. For proportions described by the Dirichlet distribution, the variance of an observed proportion f(i) is given by

$$V = p(i)*(1-p(i))/(THETA + 1), \quad (3)$$

where, as discussed above, p(i) is the expected value of the proportion. Therefore a "quick and dirty" estimate of THETA can be obtained by equating the variance of a sample of measurements of a proportion to V, substituting the mean proportion for p(i). For example, Caster et al. (1956) reported that the mean liver volume of rats, as a proportion of total body volume, was 0.0477, with a variance of $2.8*10^{-5}$. Thus, the approximation of THETA can be determined from

$$(4) \qquad 2.8*10^{-5} = 0.0477*(1 - 0.0477)/(\text{THETA} + 1),$$

so that THETA is estimated to be 1621. Other sample variances may suggest a different value for THETA, in which case some sort of average of the estimates may be used. Figure VI-1-1 shows the implied probability density function for liver as a proportion of body volume given by the equation above. (Each of the f(i) will follow a beta distribution with parameters p(i) and THETA.)

In the discussion above, source-specific uncertainties (corresponding to source M or source I) were discussed. The methods for estimating uncertainty factors just presented were also specific to one source of uncertainty. Methods for combining the source-specific uncertainty factor estimates to obtain a total uncertainty factor are discussed here.

In practice, when the distributions are simple, the distributions associated with the two sources can be combined into one. Where the source-M distribution is normal with mean M1 and variance V1, and the source-I distribution is normal with mean 0 and variance V2, then the distribution generated by the two-step algorithm is normal with mean M1 and variance V1+V2. This distribution approximately describes total uncertainty for parameters

whose uncertainty distribution is assumed to be log-normal, provided that the means and variances refer to the log scale.

In the case of log-normally distributed uncertainty, if uncertainty is evaluated for K independent sources (e.g. K=2 for source M and source I) then we may estimate a UF specific to each source (UF_i for source i, i = 1,...,K.). Corresponding to UF_i is a log-scale standard deviation $SDL_i = \ln(UF_i)/2$ for the assumed lognormal probability distribution. If the sources of uncertainty are assumed to be independent, then the appropriate value of SDL representing our total uncertainty (SDL_T) is

$$(5) \qquad SDL_T = [\Sigma_i \; SDL_i^{\,2}\;]^{0.5}.$$

Consequently an appropriate UF for total uncertainty is

$$(6) \qquad UF_T = \exp[\{\Sigma_i(\ln UF_i)2\;\}^{0.5}].$$

Thus, for example, any UFi equal to unity (i.e. any SDLi equal to zero) makes no contribution to the overall UF.

These arguments apply to uncertainty associated with use of direct measurements of a quantity of interest. In some cases, for example when a measurement of a parameter for a rodent is used for humans, special treatment may be required. In any case, uncertainty can be evaluated by constructing an algorithm such as the "two-step" algorithm above, where the first step is to pick a population mean value from a distribution with mean equal to the preferred value and variance reflecting the source M uncertainty, and the second step is to pick a value for the parameter from a distribution having a

mean equal to the value sampled in step one and a variance reflective of individual variation. For the example just cited, the variance for the first step must include consideration of the differences in the parameter observed for different species.

It is sometimes the case that a PBPK model parameter is not measured directly but is a function of other variables that are themselves subject to uncertainty. Let $f(\mathbf{h})$ be a function of the vector of variables $\mathbf{h}' = (h_1,\ldots,h_K)$, each subject to uncertainty. For example, a tissue/blood partition coefficient (PC) for a given compound is generally estimated by the ratio of an estimate of the tissue/gas partition coefficient (here denoted h_1) to an estimate of the blood/gas partition coefficient (here denoted h_2), i.e., $PC = f(\mathbf{h}) = h_1/h_2$. It is necessary to evaluate uncertainty in the function f implied by uncertainty in its arguments h_1 and h_2.

The simplest case is when the function is linear. For example, in the example of partition coefficients, it is convenient to deal with uncertainty in the natural log of the tissue/blood PC, since we will assume that the PC's are normally distributed in the log scale, with specified variance SDL_2. Therefore we evaluate uncertainty for the function

$$\ln PC = \ln h_1 - \ln h_2. \qquad (7)$$

A further simplication is to consider the uncertainties in the arguments to be independent. In this case the variance of a linear function is simply the same linear function of the variances of the arguments, but with the coefficients squared (again, assuming that the arguments are distributed independently.) If we assume independent lognormal distributions for h_1 and

h_2, with log-scale standard deviations SDL_1 and SDL_2, respectively, then the log-scale standard deviation for the function f is

$$(7) \qquad SDL_f = [\ (SDL_1^2 + SDL_2^2)\ ]^{0.5}.$$

This is essentially formula (5). The appropriate UF is simply $\exp(2*SDL_f)$.

For nonlinear functions of uncertain arguments, simple Taylor-series approximations are available; also, non-independent uncertainties in the arguments can be handled in a simple manner, provided that the dependencies are well described by correlations or covariances.

Uncertainty Evaluation for PERC Partition Coefficients. The first example of how uncertainty from various sources, or arising from various assumptions, can be combined into an assessment of total uncertainty for PERC PBPK model parameters was applied to the partition coefficients. A partition coefficient (PC) is the ratio of concentrations in two compartments at equilibrium, and so is a dimensionless quantity that is logically bounded below by zero but not logically bounded above. In accordance with the discussions above, the uncertainty was modeled using a lognormal distribution with log-scale mean equal to the log of the preferred value.

The examples of uncertainty estimation provided here illustrate four different scenarios. First were those PC's that were measured directly, i.e. the blood/air PC's, and which may have been measured by several investigators. Second, for the rat tissue/blood PC's, the estimates were derived as functions (ratios) of tissue/air and blood/air PC's and thus their uncertainty was evaluated using equation (7). Third, human tissue/air PC's were not measured directly so that rodent values had to be attributed to humans, illustrating

the importance of considering species-to-species variation in the estimation. Fourth were the PC's such as rapidly perfused/air that were not measured in any species but were equated with specific other PC's, in this case the liver/air PC. The derivation of uncertainty estimates is illustrated for each case.

For all of these "estimation scenarios" source M uncertainty was visualized as shown in Figure VI-1-2. For each tissue group, there is a (hypothetical) super-distribution of values of PC's for that group over mammalian species. The species of greatest interest in the present context were rats, mice, and humans. Each species has for each tissue group its own distribution of values of PC's and an associated true mean value. This distribution is manifested in the measurements that are obtained from individual animals and the mean of those measurements from each study. Source I uncertainty can be visualized in terms of additional branches added to the top of Figure VI-1-2, branches emanating from the means of the measured values to the measurements taken from individual animals.

The representation given by Figure VI-1-2 has associated with it certain assumptions. The first is that a tissue group in one species is more similar to that tissue group in another species than that tissue group is to other tissue groups in the same species. This appears to be supported by the observations of Fiserova-Bergerova (1983) on species differences in partition coefficients. She stated that species differences in PC's appear to be haphazard and most likely without biological significance. If this is realistic, then perhaps it is appropriate to treat species-to-species variation in PC's as independent for different compartments. This is the second assumption underlying the representation given in Figure VI-1-2.

As indicated in the figure, the estimation of the true mean of a species-specific distribution requires, at least, the estimation of that mean from the measured mean values. This has associated with it uncertainty represented in the figure by SM1(x,i), a log-scale standard deviation for species x and tissue group i that reflects the variation one would expect to see in mean values given the distribution around the true mean PC for that species and tissue group. Other components of source M uncertainty are displayed in the figure as SM2(i) and SM3(i,j), log-scale standard deviations representing variation across species of mean values of PC_i and variability in the relationship between PC_i and PC_j, respectively. Specific estimates for each of the standard deviations are discussed below, but it is perhaps appropriate to note here that, given those estimates, the overall source M uncertainty (expressed in terms of a lognormal variance) can be determined by summing the squared estimates of the standard deviations that are associated with each branch that must be traversed to get from measured means to the true mean desired.

Estimates of uncertainties for the directly measured PC's, the blood/air coefficients, were considered first. In terms of Figure VI-1-2, the uncertainty involved is associated with the single branch from the measured values to the true mean. Tables VI-1-18 and VI-1-19 summarize blood/air partition coefficient estimates that have been obtained for various chemicals, including PERC. Source M uncertainty in this case is identified solely with variation across studies. Thus, as indicated in Table VI-1-19, if no uncertainty reduction is attempted, the value 0.32 is the estimate of SM1(h,b) and the statistic S2, equal to 1.9 for PERC in humans, is the estimate for UF_M. However, if the geometric mean of the four PERC human blood/air PC

estimates is used as the preferred value, and if the measurements from different studies can be assumed to be iid (a questionable assumption), then the estimate of SM1(h,b) (for the mean of the measured values) is $0.32/(4)^{0.5}$ = 0.16 and UF_M may be equated to S3, which in this case is equal to 1.4.

No direct information is available on the individual variation in the human PERC blood/air PC for estimation of UF_I. However, Table VI-1-20 displays some information on individual variation for other volatile lipophilic compounds that may be used in the absence of more pertinent data. That table indicates that inter-individual variation of the blood/air PC is at a maximum for trichloroethylene in humans. Thus, let us assign the SDLE estimate for that case to the SDLE that will be used to characterize inter-individual variability for the PERC blood/air PC in humans. Using statistic S2 to estimate UF_I, one obtains the value 1.8 (=exp(2*0.3)). Finally, combining UF_M and UF_I via equation (6), the estimate for UF_T is

$$(8) \qquad UF_T = \exp[\{\ln(1.4)^2 + \ln(1.8)^2\}^{0.5}] = 2.0.$$

If the same considerations were applied to rats and mice, then the first immediate problem would be that there are no data regarding study-to-study differences in blood/air PC's for these two species. The data in Tables VI-1-18 and VI-1-19 were used to address this issue. We tentatively based evaluations for various tissue/air PC's on data for blood/air PC's, assuming similar levels of relative variation between studies for different tissue/air PC's. Also, we know of no reason to suspect different levels of relative variation between studies for different blood/air PC's, provided that the values of the PC's themselves are not too different in magnitude. (Based on

preliminary investigations, very small PC's were expected to be accompanied by somewhat larger SDL's, representing measurement error; consequently, we ignored the large values of S2 in Table VI-1-19 which correspond to small values of the GM, e.g., 9.8 for carbon tetrachloride and 3.4 for ethane.) Based on the S2 column in Table VI-1-19, it appeared that a UF of 2 was a reasonable (or slightly inflated) estimate for source M, for many tissue/air PC's, if uncertainty-reducing procedures are not performed or taken into account. In particular, a UF_M of 2.0 for rat or mouse blood/air PC's was assumed. Expressed in terms of the standard deviations displayed in Figure VI-1-2, that estimate of UF_M corresponds to SM1(r,b) = SM1(m,b) = 0.347.

Similarly, the data in Table VI-1-20 were used in the absence of data on inter-individual variation in blood/air PC values in rodents. The maximal SDLE for the blood/air PC from that table was 0.3, the same value used in the derivation of the UF_I for human blood/air PC. Thus, the same UF_I value was estimated for the rodent blood/air PC's as for the human PC, 1.8. Combining UF_M and UF_I via equation (6), one obtains a total uncertainty factor for rodent blood/air PC's as follows:

$$(9) \qquad UF_T = \exp[\{\ln(2.0)^2 + \ln(1.8)^2\}^{0.5}] = 2.5.$$

The second case involved those rodent PC's that are ratios of tissue/air and blood/air PC's. The derivation of an uncertainty factor in these instances involved the use of equation (7). An example for liver/blood PC in rats is provided.

Once again, no study-to-study variability data were available for rats for this PC. As argued above, the data in Table VI-1-19 suggested that UF_M =

2.0 was not unreasonable, in this case both for liver/air and blood/air PC's. The application of equation (7) to the two (identical) UF_M values yields a value of 2.7 for UF_M for rat liver/blood PC.

Table VI-1-20 presents data on the individual variation in the value of liver/air partition coefficients. No data on rats were available, but the maximum value for SDLE corresponding to the liver/air PC was 1.0. The use of the maximum SDLE is recommended for all of those cases in which the particular species of interest is not measured directly. In this instance, the maximum value was from the study of Webb and Weaver (1981) using horses. The inter-individual variation for those horses is likely to be greater than that for carefully bred laboratory rats. The SDLE estimate of 1.0 corresponds to a UF_I estimate for the liver/air PC of

$$(10) \qquad \exp(2*1.0) = 7.4.$$

Using equation (7) to combine Source I uncertainties for the liver/air and blood/air PC's, one obtains $UF_I = 8.1$ for the rat liver/blood PC. Combining UF_M and UF_I using equation (6), the resulting UF_T was

$$(11) \qquad UF_T = \exp[\{\ln(2.7)^2 + \ln(8.1)^2\}^{0.5}] = 10.1.$$

A completely analogous situation applied to the derivation of rat fat/blood and slowly perfused/blood PC uncertainty factors (if the slowly perfused tissues are equated with muscle).

The third case for PC uncertainty estimation involved those PC's that were not measured in the species for which estimates are desired. Human

tissue/air PC's, and often mouse tissue/air PC's, have not been measured directly for tissues other than the blood. The use of the PC's estimated from rat tissues to approximate the human or mouse values added uncertainty to the human or mouse estimates that was related to the variation in PC's seen across species. In terms of Figure VI-1-2, this uncertainty involved the branches connecting the measured species-specific mean values and the two branches connecting the tissue group to the specific species of interest, the one for which measurements were available and the one for which an estimate of the population mean was desired. An example using the human liver/blood PC is presented.

The liver/air PC value measured for rats was to be attributed to humans. It was shown above that the source M uncertainty associated with the rat estimate itself yields $UF_M = 2.0$ (equivalently, SM1(r,1) = 0.347). This uncertainty had to be combined with the uncertainty due solely to the need to extrapolate the estimate across species. That is, SM2(1) had to be estimated also. The resulting equation for combined uncertainty, expressed in terms of the standard deviation is as follows:

$$(12) \qquad SDLE = (2*SM2(1)^2 + SM1(r,1)^2)^{0.5},$$

where the branches corresponding to the need to go from the true mean rat liver/air PC's to the true mean human liver/air PC contributed the associated standard deviation term, SM2(1), twice.

Table VI-1-21 gives data from Fiserova-Bergerova (1983) on variation in tissue/air PC's among four species (human, monkey, dog, and rat) for isoflurane and methylene chloride. Some statistics based on this data are

reported in Table VI-1-22. Since the estimate used by Reitz and Nolan (1986; the source of preferred values considered here) involved attributing rodent estimates to humans, the most relevant data from Table VI-1-21 appeared to be that for rats and humans. Therefore, included in Table VI-1-22 were estimates of the error to be expected in extrapolations of this type ("human/rat error"). It was reassuring that the uncertainty factors estimated by statistic S2 were at least as large as the human/rat error term. Some additional conservatism may be warranted since the iid assumption involved in equating a UF to S2 may be doubtful in this case.

Based on the values of S2 shown in Table VI-1-22, use of a UF of 1.8 appeared reasonable or slightly conservative for most tissues. (An exception was muscle, for which S2 was 2.3 for isoflurane and 2.0 for methylene chloride.) Thus, an SM2(1) based on a UF of 1.8 for the liver compartment was used; in this case SM2(1) was estimated to be $\ln(1.8)/2 = 0.29$. Combining this SM2(1) with SM1(r,1) (=0.34) via equation (12) yielded a standard deviation of 0.54 or an uncertainty factor of 2.92.

The calculations just presented were for liver/air partitioning. To get the liver/blood PC for humans, equation (7) was used to combine UF_M's for liver/air and blood/air. Recall that for humans the blood/air PC UF_M was equal to 1.4. Thus equation (7) yielded

$$(13) \qquad UF_M = \exp[\{\ln(2.9)^2 + \ln(1.4)^2\}^{0.5}] = 3.1$$

for the human liver/blood source M uncertainty factor.

For source I, the data on PERC PC's in humans were as scarce as those for PERC PC's in rats. Thus the same procedure as described above for the rat

UF_I calculations was followed. The maximum inter-individual variation was used (cf. Table VI-1-20). The SDLE of 1.0 was consistent with $UF_I = 8.1$. Total uncertainty was reflected in the value $UF_T = 10.8$ derived using equation (6) with the indicated values of UF_M and UF_I.

The uncertainties associated with the human fat/blood and slowly perfused/blood PC's were estimated in an analogous manner.

The final situation that arises in the estimation of partition coefficients is illustrated by the rapidly perfused/blood PC (also referred to as the rapid/blood PC) in rats and humans. In this case, no direct measurements have been taken for this tissue group; instead, the value of its PC was inferred from measurements on other tissue, i.e., the liver. Once again, this is an uncertainty associated with source M, i.e., with the estimation of a population mean value.

The estimation of a PC for one group of tissues using PC measurements from another tissue is problematic. Although the liver may be representative of rapidly perfused tissue in many respects, it is not at all certain that a PC for liver is representative of a PC characterizing the entire tissue group. Indeed, as the data in Table VI-1-20 show, other rapidly perfused tissues such as the brain, kidney, or lung may have PC's that differ substantially from that of the liver. If one represents the estimation of the rapid/air PC from the liver PC by

$$(14) \qquad PC_r = \alpha * PC_l,$$

where α is a coefficient that must be estimated, then the uncertainty arises in the estimation of that coefficient. Reitz and Nolan (1986) assumed that α

was equal to 1. The data in Table VI-1-20 indicate that a value for α of around 0.5 to 0.7 may be reasonable for some of the chemicals shown there. However, PERC itself is not represented in that table, and the variation seen in that table is substantial.

Based solely on the information available in that table, an uncertainty factor for this aspect of the estimation of the rapid/air PC of 2 appeared justified and was used. If the rapid/air PC is set equal to half of the liver/air PC, then this uncertainty factor has the effect of making the liver/air PC the 95% upper bound (of the uncertainty distribution) for the rapid/air PC.

This cross-tissue extrapolative uncertainty is reflected in Figure VI-1-2 by the dotted line connecting the different tissue groups. It must be added to the other uncertainties that are pertinent to the estimation of the rapid/air PC, those that are reflected in the SM1 and SM2 standard deviation terms. The formula that combines the branch-specific variance estimates pertinent to this estimation problem is

$$(15) \qquad SDLE = [SM1(r,1)^2 + SM2(1)^2 + SM2(r)^2 + SM3(1,r)^2]^{0.5},$$

where SM3(1,r) is the standard deviation associated with the extrapolation across tissue groups just discussed. Both SM2 values were set equal to 0.29 on the basis of the data in Tables VI-1-21 and VI-1-22 (discussed above). The value of SM3(1,r) was given by

$$(16) \qquad SM3(1,r) = \ln(2.0)/2 = 0.347,$$

where the value 2.0 was the uncertainty factor postulated for the cross-tissue extrapolation. Thus, equation (15) yielded an estimate of 0.64 for the SDLE of interest. This corresponds to an uncertainty factor, UF_M, of 3.6 for rapid/air partitioning. Combining this with the source M uncertainty for blood/air PC's (2.0 for rats, 1.4 for humans) using equation (7), the UF_M's for rapid/blood PC's were 4.3 and 3.8 for rats and humans, respectively.

Source I uncertainty was again estimated on the basis of the data shown in Table VI-1-20. The brain and the kidney were assumed to be representative of rapidly perfused tissues in general with respect to inter-individual variation in PC values. The brain SDLE was 0.5; the SDLE for kidney was only slightly smaller, 0.4. We assumed that the tissue group as a whole is not more variable than the most variable component, in this case the brain. An SDLE of 0.5 corresponds to $UF_I = 2.7$ for the rapid/air PC. Using equation (7) to combine this uncertainty with that for blood/air source I uncertainty ($UF_I = 1.8$) the resulting UF_I was

$$(17) \qquad UF_I = \exp[\{\ln(2.7)^2 + \ln(1.8)^2\}^{0.5}] = 3.2.$$

Finally, combining the source M and source I uncertainties via equation (6) gave the total uncertainty factors, $UF_T = 6.5$ for rats and $UF_T = 5.9$ for humans.

This concludes the examples of uncertainty estimation for the partition coefficients of the PERC model. The other coefficients were analyzed in the same manner as the examples just presented. The entire process is summarized in Table VI-1-23.

The entire set of partition coefficient estimates (preferred values) and their estimated uncertainty factors are displayed in Table VI-1-24. Note that the preferred values for human i/blood PC's (where i is one of the compartments used in the model) were based on an average of mouse and rat i/air PC's. The uncertainty factors derived did not consider this averaging and any corresponding reduction in uncertainty. That is, as the examples above illustrated, the human PC uncertainty factors were derived as if only one rodent species formed the basis of the human coefficients. This is probably not too bad. Even in the iid case, the log-scale standard deviations that would correspond to the averages would be the unaveraged standard deviations divided by the square root of 2 (the number of terms contributing to the average). That would be a small change in and of itself. Moreover, since the iid assumption is at best questionable in this case of two sets of rodent PC's, the appropriate adjustment should probably fall between 1 and the square root of 2. Because the exact value of the appropriate adjustment factor is not known, and because it will make little difference in the estimation of uncertainty factors, the unadjusted uncertainty estimates are presented and are considered adequate.

Estimation of Uncertainty for PERC Metabolic Constants. The parameters vmax and km define metabolism for the PERC PBPK model. Values for these parameters were reported by Reitz and Nolan (1986) and Hattis et al. (1986). Reitz and Nolan employed an optimization technique to estimate the values appropriate for mice, rats, and humans. They used the data of Schumann et al. (1980) and gas uptake data obtained from WPAFB to derive two sets of mouse values. The data of Pegg et al. (1979) were used to optimize the rat values

and the data of Monster et al. (1979) and Fernandez et al. (1976) were used for human optimization.

Hattis et al. did not employ a formal optimization procedure. They used the same data sets for rat and mouse parameter estimation as used by Reitz and Nolan. Hattis et al. also referred to the results presented by Mitoma et al. (1985) for parameter estimation in the two rodent species. For their human metabolic constant estimates, Hattis et al. adjusted vmax and km based on Ohtsuji et al. (1983) and Ikeda et al. (1972). Described here are considerations of uncertainty related to the metabolic parameter estimates.

First, some comment on the use of optimization to derive parameter estimates is warranted. Typically, a subset of PBPK parameters is estimated by optimizing the fit of model predictions to data, while the remaining parameters are fixed at constant values. In general the "fixed" subset will include parameters for which *in vitro* measurements are available; the *in vitro* values are assumed equal to *in vivo* values. The choice of which parameters will be in each subset is not necessarily clear-cut. For example, Reitz and Nolan fixed values of partition coefficients for their "Mouse 1" estimates, but optimized those values for their "Mouse 2" estimates; vmax and km were fitted for both Mouse 1 and Mouse 2 sets.

Estimates for the fitted parameters depend to some degree on the values assumed for the fixed set. Uncertainty for the fitted set will be determined by uncertainty in the fixed set, as well as by experimental error in the data to which the model is fit, by variations in the results of experiments used in optimization, and by uncertainty regarding model structure. In principle, uncertainty regarding the values of the fixed parameters can be evaluated by a Monte Carlo approach in which values are selected randomly for the fixed set,

re-fitting the model with each selection. That approach would be highly computer-intensive. For each selection of values for the fixed parameters (a selection that would have to be repeated many times to get an accurate picture of the variation) an optimization would have to be performed; the optimizations themselves require many simulation runs.

Rather than adopt such a complex procedure, we obtained a tentative evaluation of uncertainty by treating the available estimates of the fitted parameters as a representative sample of the values that were reasonable. Implicitly, the vectors of fixed parameters associated with the various *fitted* estimates were also treated as a representative sample.

The units used to report vmax and km values in Hattis et al. (1986) were not the same as those used by Reitz and Nolan (1986). The Hattis et al. units were converted to those used by Reitz and Nolan, using the standard body weights assumed by Hattis et al. (0.025, 0.25, and 70 kg for mouse, rat, and human, respectively). Also, since Hattis et al. defined metabolism in terms of liver concentration (as opposed to the use of concentration in the venous blood leaving the liver, the formulation employed by Reitz and Nolan) their km values were converted to units comparable to those of Reitz and Nolan by dividing by pl, the liver/blood partition coefficient. (This follows from the fact that, according to the models, liver concentration equals hepatic portal venous concentration times pl.) In making the conversion, the "best estimates" of pl reported by Hattis et al. were used (4.73 for humans, 3.72 for rodents). Vmax was scaled, as was done in Reitz and Nolan, by estimating vmaxc such that

$$(18) \qquad vmax = vmaxc * BW^{0.74}.$$

All uncertainty estimates were estimated for vmaxc and km as defined by Reitz and Nolan.

Tables VI-1-25 and VI-1-26 present the estimates reported in Reitz and Nolan (1986) and Hattis et al. (1986). Hattis et al. gave two estimates for each species, based on different data sets, for both km and vmaxc. The estimates were named according to the sources of data used to estimate the parameters. Note that the "Mouse 1" and human estimates of km (4.56) reported in Reitz and Nolan were actually extrapolated from rats, so they were not used for variance estimation in mice or humans.

For the different estimates of metabolic parameters reported in Tables VI-1-25 and VI-1-26, the iid assumption clearly seemed to be violated. Consequently, methods based on iid assumptions were applied only because of a lack of additional information.

For vmaxc, values of the sample standard deviation of logs (SSDL) are 0.105 for humans, 0.414 for rats, and 0.721 for mice. These differences do not necessarily reflect real differences between species in the uncertainty involved in measuring vmaxc, since SSDL is itself a statistic subject to some measurement error. Tentatively we based an uncertainty factor for vmaxc on an SSDL of 0.532, the weighted average of SSDL's from different species. Some reduction of the uncertainty may be appropriate if one used the average of several estimates as the preferred value for each species; however, proper application of the statistic S3, which accomplishes such a reduction in a formal manner, is based on the iid assumption. Some conservatism (implying

greater uncertainty) was introduced by using S2 rather than S3. Accordingly, the derived uncertainty factor was 2.9 (=exp[2*0.532]) for mice, rats, and humans. (Uncertainty could still be reduced when S2 is used, but by a different mechanism: if the spread of values that were considered reasonable estimates was lowered by further study of estimation procedures, this would be reflected in a smaller value of S2.)

Uncertainty for km was evaluated in a similar manner, arriving at an uncertainty factor of 13 (=exp[2*1.28]) for all three species.

Tables VI-1-25 and VI-1-26 also display the geometric means of vmaxc and km for all three species. In the absence of a reliable analysis of the relative merits of the different estimates, we adopted the geometric means as preferred values for the Monte Carlo simulations. Note the differences between the geometric means and the estimates of Reitz and Nolan.

The uncertainty factors for vmaxc and km appeared to be representing source M. We suspected that individual variation (source I) was small in proportion to the source M uncertainty represented by factors of 2.9 for vmaxc and 13 for km. In any case, quantitative information suitable for evaluating individual variation for vmaxc and km was not available. Thus, tentatively, we set the UF_T's, representing total uncertainty, equal to the UF_M's of 2.9 for vmaxc and 13 for km.

The statistical procedure for evaluating individual variation in metabolic parameters estimated by optimization depends on whether the available data consist of multiple measurements at different times for the same individuals, or (e.g., Young and Wagner, 1979) different animals measured at each time. In the first case, the optimization may be performed separately for each subject, and the variation of subject-specific estimates summarized.

In the second case the model may be fitted once to the combined data from all individuals; then information on individual variation is represented by the distribution of observed values at given times about the values predicted by the model. A statistic summarizing this variation, and potentially useful in describing individual variation, is the mean square error, which is generally reported by least-squares optimization routines.

Uncertainties in parameters fitted simultaneously by an optimization program are not independent, since for a given vector of fitted parameters a change in the value of one parameter can be compensated for by changes in the other fitted parameters. For example, since low-dose, first-order metabolic rate is approximated by vmax/km, it may be the case that among combinations of vmax and km providing similar fits to a given data set, a positive correlation exists between the values of vmax and km. Such a relationship can be modeled approximately by assuming a bivariate lognormal distribution for vmax and km with an appropriate correlation parameter. If the fitting routine employed in an optimization provides an estimated covariance matrix for the parameter estimates, this matrix is potentially useful in estimating a value of the correlation parameter. At this time we have no basis for estimating the correlation between vmax and km; they have tentatively been treated as if they were independent.

Estimation of Uncertainty for PERC Circulatory and Ventilatory Flow Rates. This category of parameters included ventilation rate (qp) and cardiac output (qc) (both in liters/hour), and percentages of cardiac output directed to specific compartments: qlc to liver, qrc to "rapidly perfused," qsc to "slowly perfused," and qfc to fat. (In some cases it was more convenient to treat absolute compartment flows, in liters/hr: ql for liver, qr for rapidly

perfused, etc.) Because for physiological reasons these parameters are correlated (and also probably correlated with compartment volumes), it was convenient to treat them together.

A complete accounting of uncertainty might involve detailed assumptions regarding physiological mechanisms regulating the relationships among the parameters. The description here is sufficiently complete to demonstrate the sort of physiological considerations that are relevant, along with possibilities for quantifying the way that these considerations may determine uncertainty.

The formal description of uncertainty involved the three following assumptions. (1) Uncertainty with respect to compartment flows (ql, qr, etc.) was related to uncertainty with respect to corresponding compartment volumes. Volume-specific perfusion rates (e.g., liters blood/hr/liters tissue) were assumed to be constant so that the rate of delivery of blood to a compartment varies as the compartment volume varied. (2) Additional uncertainty was incorporated for the flows in humans. This added uncertainty related primarily to variation in the levels of physical exertion in daily activities and primarily affected qc, qp, qs, and qf. (3) Ventilation rate (qp) was related linearly to cardiac flow rate (qc).

Support for assumption 1 came from consideration of the relationship between individual variation in compartment volumes and individual variation in corresponding blood flow rates. Increase in a compartment volume should be accompanied by an increase in blood supply, depending on the relationship between compartment volume and oxygen demand per unit volume. We had no data relevant to the latter relationship, and so we assumed constant weight-specific perfusion rates for each compartment. Some uncertainty is associated

with the values of the weight-specific perfusion rates. We did not quantify this uncertainty; we assumed that perfusion rates were constant and were given by the ratios of preferred compartment flows (in liters blood/hr) to corresponding preferred compartment volumes (in liters tissue).

The assumption of constant perfusion rates accounted for some individual variation in compartment flow rates. That is, as compartment volumes varied, according to the scheme for incorporating uncertainty in the volume estimates (see the discussion of the Dirichlet distribution modeling of volume uncertainties given above), the flows varied, but the relationship between flow rate and compartment volume was maintained.

Additional uncertainty for humans with given compartment volumes may relate primarily to the level of physical exertion required in their daily activities. Increasing activity levels result in higher values of qc and therefore increasing values for one or more compartment flows. There is evidence that the increased cardiac output is directed almost entirely to the muscle. Folkow and Neil (1971) reported that a change in activity level from resting to heavy exercise resulted in approximately equivalent increases in qc and flow to muscle (qs), for average subjects and top athletes. Data summarized by Hattis et al. (1986) were roughly consistent with this conclusion (Table VI-1-27); the discrepancies may be due to the fact that different entries in that table were taken from different primary sources.

Hattis et al. (1986) assumed that perfusion of fat and muscle was related to activity level, and that perfusion of other tissues was independent of activity level. (The conclusion that fat perfusion is related to activity level is based on Astrand (1983).) Since fat is typically a smaller proportion of body weight than muscle, this does not contradict the conclusion

that activity-related increase in cardiac output is allocated primarily to muscle. We assumed simply that for an activity-related increase in qc of dqc liters/hour, qs increased by dqs = dqc*vs/(vs+vf) and qf increased by dqc-dqs, where vs was the volume assumed for the slowly perfused (muscle) compartment and vf was the volume assumed for fat. It was convenient to assume that dqc was distributed lognormally with log-scale mean equal to zero and with an uncertainty factor to be estimated.

Regarding the third assumption, some relationship between qp and qc is to be expected on physiological grounds. Both parameters are related to the oxygen demand of tissues, and both are reported to follow an allometric relationship with body weight across species, with exponent about 0.8 (Stahl, 1967). Reitz and Nolan (1986) assumed that the two parameters were equal in all species; data from various sources summarized by Hattis et al. (1986) (cf. Table VI-1-27) suggested that such an equality may hold approximately for individuals at rest but that with increasing activity levels, qp increases more rapidly than does qc, so that the parameters differ by a factor of 1.6 with light exercise and by a factor of 2.4 with heavy exercise. Regarding the linearity of the relationship, the data in Table VI-1-27 suggested that the true relationship over activity levels may be convex: as activity level changed from resting to "light exercise," qp increased by 540 liters/hour while qc increased by 180 liters/hour, a ratio of increments of 3; going from rest to heavy exercise, the ratio of increments had a value of 4. However, the range of activity levels represented in Table VI-1-27 appears to be much wider than the range of activity levels characteristic of usual daily activities, which might be generally between sitting and light exercise. In this range the assumption of a linear relationship may provide a good

approximation. We approximated the relationship by a line with slope 3 connecting the "sitting" values (qp = 420; qc = 440) to the "light exercise" values (qp = 960; qc = 582). An expression of the assumption that was convenient for application in different species is

$$(19) \qquad qp - qp_0 = 3 * (qc - qc_0),$$

where qp_0 and qc_0 are the "resting values" of qp and qc, respectively, for a given species. For rodents we tentatively equated resting values with preferred values in Reitz and Nolan (1986); for humans we used the "sitting" values from Table VI-1-27.

A summary of the uncertainty considerations associated with the flow parameters is provided here. This is expressed in terms of the algorithm that was implemented to generate flow parameter values (as well as other parameter values, especially the compartment volumes) when randomly selected parameter values were needed as input for dose surrogate uncertainty estimation. Note that the volumes were generated by the appropriate Dirichlet distribution, as discussed above, and the algorithm then maintained the relationship between volume and flow rate discussed in the preceding paragraphs. The steps taken were:

1. Generate compartment volumes from the Dirichlet distribution describing their uncertainty. Denote their values by vi (i = 1,r,s,f).

2. Calculate a compartment flow rate, qi, for each compartment (i = 1,r,s,f) according to the equation

$$qi = vi * (qi_p/vi_p), \quad (20)$$

where qi_p and vi_p are the preferred values for compartment i flow rate and volume, respectively. Calculate qc = ql + qr + qs + qf.

3. For humans only. Randomly select the value of dqc from its lognormal uncertainty distribution (with mean zero and estimated uncertainty factor) and increment cardiac output and flow rates to the fat and slowly perfused tissues:

$$qc = qc + dqc; \quad (21a)$$

$$qs = qs + dqc*(vs/(vs+vf)); \quad (21b)$$

$$qf = qf + dqc*(vf/(vs+vf)). \quad (21c)$$

4. Recalculate percentage of cardiac output flowing to the compartments:

$$qic = qi/qc, \; i = l,r,s,f. \quad (22)$$

5. Compute ventilation rate according to formula (19).

In the implementation of this procedure, preferred values for flows and volumes were equated to the values suggested by Reitz and Nolan (1986) with the exception that, for humans, the "shoeworker" values from Table VI-1-27 were used. A value for the uncertainty factor associated with dqc was also required. It was assumed that usual daily activities vary between resting and

light exercise, so the qc values associated with those levels of activity were taken as upper and lower bounds for a range of acceptable values. Following the suggestions given above for estimation of uncertainty factors (cf. Table VI-1-17), the uncertainty factor was equated to statistic S1, which in this case took the value $(582/400)^{0.5} = 1.2$, where 582 and 400 are the appropriate qc values from Table VI-1-27.

2. Propagation of Parameter Uncertainties; Dose Surrogate and Risk Uncertainty

Having estimated uncertainty distributions appropriate for all the PBPK model parameters (for mice, rats, and humans) one can determine the effects of the parameter uncertainties on estimates of dose surrogates and risks. Monte Carlo simulation provides the basis for estimating dose surrogate and risk uncertainties.

Appendix VI-1-A is a reprint of a published document describing an example of this approach to risk uncertainty estimation. The example extends the work presented above regarding estimation of PERC model parameter uncertainties. Bioassay results for female mice exposed to PERC are used for illustration.

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Table VI-1-1

Parameter Sets Used in Sensitivity Analyses

Parameter	Mouse	Rat	Human
bw	0.025	0.25	70.0
tiv	0.003	0.003	0.003
tgav			
vmaxc	3.6	1.27	0.346
km	2.22	4.56	4.56
pb	24.43	18.8	10.3
pl	4.05	3.74	5.88
pf	96.6	87.1	119.0
ps	5.2	1.06	3.10
pr	4.61	3.74	5.88
vlc	0.04	0.04	0.0314
vfc	0.05	0.07	0.231
vsc	0.78	0.75	0.62
vrc	0.05	0.05	0.0371
qpc	28.0	15.0	15.0
qcc	28.0	15.0	15.0
qlc	0.25	0.25	0.24
qfc	0.05	0.05	0.05
qsc	0.19	0.19	0.19
qrc	0.51	0.51	0.52

Table VI-1-2

Doses Employed in Sensitivity Analyses

Dose Pattern	Species	Low	Medium	High
Gavage	Mouse	0.30	30	2000
(doses in mg/kg)	Rat	0.15	15	1400
	Human	0.05	6.0	600
Intravenous injection	Mouse	0.01	2.0	200
(doses in mg/kg)	Rat	0.03	3.0	350
	Human	0.04	4.0	450
Inhalation, 8 hrs/day	Mouse	0.30	30	1600
(doses in ppm)	Rat	0.60	60	5000
	Human	1.0	115	11500
Inhalation, continuous	Mouse	0.30 (24)	24 (24)	1400 (48)
(doses in ppm[a])	Rat	0.50 (48)	44 (48)	3800 (48)
	Human	0.90 (24)	90 (24)	9000 (48)

[a]In parentheses are the times (in hours) that mark the beginning of the 24-hour period used to calculated daily dose surrogates. The times are those at which the system is close to steady state.

Table VI-1-3
PERC Model Sensitivity
Mouse Parameter Set: Gavage Administration

	Dose Surrogate								
	AUCL			Virtual Concentration of Metabolite			AUCA		
Parameter	Low	Medium	High	Low	Medium	High	Low	Medium	High
bw	2.60E-01	3.93E-01	4.67E-01	-4.15E-04	6.40E-02	4.22E-02	2.60E-01	3.93E-01	4.67E-01
tgov	-3.87E-02	-1.23E-01	1.01E-01	-3.52E-02	8.13E-02	1.08E-01	-3.89E-02	-1.23E-01	1.01E-01
vmaxc	-7.05E-01	-6.06E-01	-5.92E-02	2.90E-01	4.59E-01	8.51E-01	-7.05E-01	-6.06E-01	-5.91E-02
km	6.17E-01	3.48E-01	8.48E-03	3.77E-01	-3.35E-01	-1.25E-01	6.16E-01	3.48E-01	8.43E-03
pb	2.36E-01	3.97E-01	8.07E-01	2.36E-01	3.84E-01	6.67E-01	3.79E-01	5.37E-01	9.47E-01
pl	1.05E+00	1.02E+00	1.23E+00	4.38E-02	1.71E-02	8.70E-02	4.37E-02	1.55E-02	2.36E-01
pf	********	-3.18E-02	-2.90E-02	********	3.00E-02	3.83E-01	********	-3.18E-02	-2.94E-02
pa	-8.70E-04	-8.26E-02	-1.63E-02	3.80E-04	2.24E-02	2.20E-01	-1.37E-03	-8.27E-02	-1.64E-02
pr	********	1.73E-01	2.36E-01	1.98E-05	1.51E-01	8.96E-02	0.00E+00	1.73E-01	2.36E-01
vlc	4.38E-02	1.55E-02	2.36E-01	-9.47E-01	-9.73E-01	-9.03E-01	4.38E-02	1.55E-02	2.36E-01
vfc	-6.71E-04	-3.18E-02	-2.90E-02	1.60E-05	3.00E-02	3.83E-01	-7.10E-04	-3.19E-02	-2.94E-02
vac	-7.83E-04	-8.26E-02	-1.63E-02	3.79E-0[illegible]	2.24E-02	2.20E-01	-8.20E-04	-8.27E-02	-1.64E-02
vrc	-4.18E-05	1.73E-01	2.36E-01	2.19E-05	1.51E-01	8.96E-02	-3.94E-05	1.73E-01	2.36E-01
qpc	-3.25E-01	-4.28E-01	-8.00E-01	-3.24E-01	-4.10E-01	-6.63E-01	-4.64E-01	-5.68E-01	-9.39E-01
qcc	1.36E-01	-8.49E-03	4.67E-01	1.35E-01	9.42E-02	1.19E-01	2.72E-01	1.31E-01	6.08E-01
qlc	-2.76E-01	2.16E-01	4.33E-01	-2.75E-01	2.51E-01	2.00E-01	-1.37E-01	3.56E-01	5.73E-01
qfc	-1.67E-04	-4.85E-02	6.27E-03	2.66E-04	-9.98E-03	-7.81E-02	-1.18E-04	-4.85E-02	6.40E-03
qac	-1.06E-05	-3.24E-02	9.14E-04	1.51E-05	-2.58E-02	-1.21E-02	********	-3.24E-02	9.25E-04
qrc	-8.77E-02	-2.07E-01	3.38E-01	-8.76E-02	-1.75E-01	1.12E-01	-8.77E-02	-2.07E-01	3.38E-01

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-4
PERC Model Sensitivity
Mouse Parameter Set: Intravenous Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Virtual Concentration of Metabolite Medium	Virtual Concentration of Metabolite High	AUCA Low	AUCA Medium	AUCA High
bw	2.61E-01	2.59E-01	2.58E-01	-7.40E-05	-3.53E-04	-4.63E-04	2.61E-01	2.59E-01	2.58E-01
tiv	-9.98E-01	-1.43E-01	-4.90E-01	-9.97E-01	-1.27E-01	-2.42E-01	-9.98E-01	-1.40E-01	-4.78E-01
vmaxc	-6.21E-01	-5.64E-01	-2.49E-01	3.79E-01	3.96E-01	6.70E-01	-4.46E-01	-4.03E-01	-2.04E-01
km	6.25E-01	5.06E-01	9.03E-02	-3.79E-01	-3.55E-01	-2.43E-01	4.34E-01	3.62E-01	7.37E-02
pb	4.84E-01	4.72E-01	7.84E-01	4.66E-01	5.05E-01	7.07E-01	4.78E-01	4.78E-01	7.80E-01
pl	1.00E+00	9.73E-01	9.94E-01	3.08E-04	1.72E-02	8.49E-03	********	-1.75E-02	-2.59E-03
pf	********	-3.46E-03	-8.08E-02	********	2.22E-03	2.15E-01	********	-2.92E-03	-6.61E-02
ps	********	-1.19E-02	-7.14E-02	********	8.23E-03	1.91E-01	********	-8.47E-03	-5.84E-02
pr	********	-1.81E-02	-4.09E-03	********	1.27E-02	1.10E-02	********	-1.29E-02	-3.34E-03
vlc	-3.17E-04	-2.45E-02	-3.17E-03	-9.97E-01	-9.71E-01	-9.80E-01	-2.61E-04	-1.75E-02	-2.59E-03
vfc	-1.98E-04	-3.69E-03	-8.08E-02	-2.05E-04	2.20E-03	2.15E-01	-3.26E-04	-2.71E-03	-6.61E-02
vsc	********	-1.19E-02	-7.14E-02	********	8.23E-03	1.91E-01	********	-8.52E-03	-5.84E-02
vrc	-1.98E-04	-1.81E-02	-4.09E-03	1.25E-04	1.27E-02	1.10E-02	********	-1.30E-02	-3.34E-03
qpc	-4.44E-01	-4.73E-01	-7.79E-01	-4.68E-01	-5.05E-01	-7.04E-01	-5.47E-01	-4.78E-01	-7.75E-01
qcc	1.11E-01	4.21E-02	3.81E-02	8.72E-02	1.10E-01	3.77E-02	-1.82E-01	-1.12E-01	-1.11E-02
qlc	8.77E-02	1.00E-01	4.15E-02	8.72E-02	6.88E-02	2.77E-02	-9.94E-02	-7.01E-02	-8.39E-03
qfc	********	-1.57E-02	-3.70E-03	1.78E-04	1.12E-02	1.08E-02	********	-1.12E-02	-2.98E-03
qsc	-3.76E-04	-4.19E-02	2.57E-04	2.45E-04	2.94E-02	-6.38E-04	********	-3.00E-02	2.13E-04
qrc	********	-7.62E-04	1.83E-05	3.87E-05	5.33E-04	-4.92E-05	********	-5.39E-04	1.50E-05

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-5
PERC Model Sensitivity
Mouse Parameter Set: 8 Hour Inhalation Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Virtual Concentration of Metabolite Medium	Virtual Concentration of Metabolite High	AUCA Low	AUCA Medium	AUCA High
bw	-6.31E-02	-3.58E-02	-9.60E-03	-3.21E-01	-2.45E-01	-1.77E-01	-6.30E-02	-3.03E-02	-9.11E-03
vmaxc	-6.51E-01	-5.38E-01	-3.20E-02	3.46E-01	6.28E-01	9.54E-01	-4.65E-01	-4.08E-01	-2.68E-02
km	5.91E-01	3.20E-01	2.31E-03	-4.02E-01	-3.29E-01	-8.05E-02	4.05E-01	2.48E-01	1.88E-03
pb	4.65E-01	7.26E-01	9.69E-01	4.63E-01	4.95E-01	3.52E-01	4.65E-01	7.00E-01	9.66E-01
pl	1.01E+00	9.92E-01	1.02E+00	1.03E-02	-5.12E-03	1.00E-02	1.05E-02	-7.29E-03	2.12E-02
pf	4.89E-02	-5.07E-02	-2.14E-02	4.99E-02	5.26E-02	1.94E-01	4.67E-02	-3.91E-02	-2.03E-02
ps	-7.64E-04	2.14E-02	-1.10E-02	-1.05E-04	8.34E-02	1.12E-01	-5.59E-04	2.83E-02	-1.03E-02
pr	3.61E-03	5.27E-02	-4.66E-02	3.61E-03	4.68E-02	-7.03E-03	3.47E-03	5.21E-02	-4.64E-02
vlc	1.03E-02	-7.56E-03	2.12E-02	-9.80E-01	-9.95E-01	-9.80E-01	1.03E-02	-7.29E-03	2.12E-02
vfc	4.90E-02	-5.07E-02	-2.14E-02	4.99E-02	5.26E-02	1.94E-01	4.91E-02	-3.91E-02	-2.03E-02
vsc	-7.77E-04	2.14E-02	-1.10E-02	-1.06E-04	8.34E-02	1.12E-01	-6.71E-04	2.83E-02	-1.03E-02
vrc	3.59E-03	5.27E-02	-4.66E-02	3.61E-03	4.68E-02	-7.03E-03	3.59E-03	5.21E-02	-4.64E-02
qpc	5.34E-01	5.47E-01	6.06E-02	5.30E-01	2.49E-01	-2.30E-01	5.33E-01	5.13E-01	5.90E-02
qcc	5.58E-02	1.01E-01	7.83E-02	5.49E-02	4.47E-02	-2.26E-02	-1.29E-01	-1.59E-02	7.25E-02
qlc	1.88E-02	1.08E-01	2.95E-02	1.82E-02	6.57E-02	1.17E-02	-1.67E-01	-8.18E-03	2.42E-02
qfc	6.32E-04	5.49E-03	-2.64E-03	5.19E-04	-4.41E-03	-4.19E-02	6.10E-04	4.38E-03	-2.85E-03
qsc	-1.55E-03	5.93E-02	-7.13E-03	-1.63E-03	4.25E-02	-8.50E-03	-1.56E-03	5.74E-02	-7.14E-03
qrc	5.16E-03	8.28E-02	2.87E-02	5.15E-03	6.90E-02	7.88E-03	5.15E-03	8.13E-02	2.86E-02

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-8
PERC Model Sensitivity
Mouse Parameter Set: Continuous Inhalation Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Virtual Concentration of Metabolite Medium	Virtual Concentration of Metabolite High	AUCA Low	AUCA Medium	AUCA High
bw	********	-1.20E-04	********	-2.58E-01	-2.58E-01	-2.58E-01	********	-1.14E-04	********
vmaxc	-6.20E-01	-5.83E-01	-1.58E-02	3.80E-01	7.02E-01	1.00E+00	-4.33E-01	-4.49E-01	-1.36E-02
km	6.16E-01	2.94E-01	1.52E-04	-3.77E-01	-3.54E-01	-9.60E-03	4.30E-01	2.26E-01	1.30E-04
pb	4.67E-01	7.85E-01	1.00E+00	4.63E-01	3.95E-01	9.53E-03	4.67E-01	7.44E-01	1.00E+00
pl	1.00E+00	1.00E+00	1.00E+00	********	********	********	********	********	********
pf	********	-3.29E-04	********	-4.05E-05	-1.67E-04	********	********	-3.15E-04	********
ps	-2.83E-05	-1.31E-04	********	********	-6.59E-05	********	********	-1.23E-04	********
pr	********	********	********	********	********	********	********	********	********
vlc	********	********	********	-9.90E-01	-9.90E-01	-9.90E-01	********	********	********
vfc	-2.50E-05	-3.30E-04	********	-2.47E-05	-1.66E-04	********	-2.42E-05	-3.12E-04	********
vsc	********	-1.30E-04	********	********	-6.59E-05	********	********	-1.24E-04	********
vrc	********	********	********	********	********	********	********	********	********
qpc	5.33E-01	5.00E-01	1.35E-02	5.29E-01	2.52E-01	1.29E-04	5.32E-01	4.74E-01	1.34E-02
qac	8.71E-02	8.17E-02	2.20E-03	8.62E-02	4.13E-02	2.12E-05	-9.82E-02	-2.62E-02	********
qlc	8.69E-02	8.16E-02	2.20E-03	8.62E-02	4.12E-02	2.12E-05	-9.83E-02	-2.63E-02	********
qfc	1.17E-05	1.15E-04	********	1.14E-05	5.80E-05	********	1.07E-05	1.09E-04	********
qsc	********	1.07E-05	********	********	********	********	********	1.01E-05	********
qrc	********	********	********	********	********	********	********	********	********

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-7
PERC Model Sensitivity
Rat Parameter Set: Gavage Administration

	Dose Surrogate								
	AUCL			Virtual Concentration of Metabolite			AUCA		
Parameter	Low	Medium	High	Low	Medium	High	Low	Medium	High
bw	2.37E-01	2.46E-01	2.31E-01	-2.24E 02	-6.41E-02	-1 94E-01	2.30E-01	2.39E-01	2.24E-01
tgav	-2.94E-01	-3.35E-01	-4.38E-01	-2.90E-01	-1.44E-01	-6.94E-02	-2.95E-01	-3.35E-01	-4.39E-01
vmaxc	-2.79E-01	-2.10E-01	-2.22E-02	7.16E-01	7.88E-01	9.83E-01	-2.77E-01	-2.08E-01	-2.15E-02
km	2.76E-01	1.50E-01	3.59E-03	-7.10E-01	-5.71E-01	-1 73E-01	2.75E-01	1.48E-01	3.40E-03
pb	5.26E-01	5.72E-01	7.10E-01	5.26E-01	5.91E-01	2 98E-01	6.92E-01	7.39E-01	8.74E-01
pl	9.97E-01	9.94E-01	9.99E-01	-7.33E-04	8.04E-03	3 80E-03	-1.27E-03	-3.64E-03	-1.97E-03
pf	-7.77E-02	-8.56E-02	-1.15E-01	-7.72E-02	-8.36E-02	3 40E-02	-9.89E-02	-1.10E-01	-1.41E-01
ps	-4.62E-03	-1.61E-02	-8.02E-03	-3.69E-03	3.62E-02	1 63E-02	-5.90E-03	-1.75E-02	-9.74E-03
pr	-1.02E-03	-3.48E-03	-1.84E-03	-8.37E-04	7.51E-03	3.56E-03	-1.27E-03	-3.79E-03	-2.23E-03
vlc	-9.22E-04	-3.48E-03	-1.76E-03	-9 89E-01	-9.80E 01	9 86E-01	-1.07E-03	-3.64E-03	-1.97E-03
vfc	-7.75E-02	-8.56E-02	-1.15E-01	-7.75E-02	-8.36E-02	3.40E-02	-1.02E-01	-1.10E-01	-1.41E-01
vsc	-4.63E-03	-1.61E-02	-8.02E-03	-3.69E-03	3.62E-02	1 63E-02	-5.88E-03	-1.75E-02	-9.74E-03
vrc	-1.03E-03	-3.48E-03	-1.84E-03	-8.64E-04	7.51E-03	3.56E-03	-1.34E-03	-3.79E-03	-2.23E-03
qpc	-8.24E-01	-5.70E-01	-7.05E-01	-5.24E-01	-5.88E-01	-3.01E-01	-6.90E-01	-7.36E-01	-8.69E-01
qcc	-1.07E-01	-1.62E-01	-1.58E-01	-1.04E-01	5.01E-02	6.72E-02	8.35E-02	2.76E-02	3.30E-02
qlc	-1.30E-01	-1.65E-01	-1.84E-01	-1.29E-01	-6.02E-02	1.53E-03	5.22E-02	1.74E-02	1.48E-04
qfc	2.33E-02	2.13E-03	2.63E-02	2.49E-02	1.12E-01	6.63E-02	3.08E-02	9.62E-03	3.27E-02
qsc	7.63E-05	8.45E-04	1.15E-04	4.08E-05	-1.69E-03	-6.25E-04	8.63E-05	5.63E-04	1.38E-04
qrc	********	4.16E-05	********	********	-1.45E-04	-1.61E-05	********	4.17E-05	********

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-8
PERC Model Sensitivity
Rat Parameter Set: Intravenous Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Medium	High	AUCA Low	AUCA Medium	AUCA High
bw	2.34E-01	2.31E-01	2.30E-01	-2.70E-02	-3.11E-02	-1.11E-01	2.34E-01	2.31E-01	2.29E-01
tiv	-9.98E-01	1.57E+00	-4.40E-01	-9.96E-01	1.39E+00	-2.00E-01	-9.98E-01	1.56E+00	-4.36E-01
vmaxc	-2.81E-01	-2.43E-01	-8.72E-02	7.23E-01	7.37E-01	9.13E-01	-2.12E-01	-.85E-01	-5.27E-02
km	2.80E-01	2.12E-01	2.69E-02	-7.18E-01	-6.44E-01	-3.85E-01	2.12E-01	1.61E-01	2.08E-02
pb	7.04E-01	7.07E-01	8.56E-01	7.04E-01	7.46E-01	6.73E-01	7.03E-01	7.10E-01	8.53E-01
pl	1.01E+00	9.82E-01	9.95E-01	-2.16E-04	2.80E-02	9.62E-03	-2.00E-03	-8.49E-03	-2.02E-03
pf	-9.38E-02	-9.88E-02	-1.34E-01	-9.54E-02	-1.05E-01	3.01E-02	-9.31E-02	-9.71E-02	-1.31E-01
pa	-5.37E-03	-1.65E-02	-1.18E-02	-4.83E-03	2.72E-02	4.64E-02	-5.10E-03	-1.39E-02	-1.09E-02
pr	-1.15E-03	-8.13E-03	-2.64E-03	-8.62E-04	1.89E-02	9.90E-03	-2.00E-03	-6.53E-03	-2.45E-03
vlc	-1.22E-03	-1.09E-02	-2.32E-03	-9.98E-01	-9.57E-01	-9.79E-01	-1.05E-03	-8.50E-03	-2.02E-03
vfc	-9.68E-02	-9.68E-02	-1.34E-01	-9.70E-02	-1.05E-01	3.01E-02	-9.67E-02	-9.71E-02	-1.31E-01
vac	-5.41E-03	-1.65E-02	-1.18E-02	-4.80E-03	2.72E-02	4.64E-02	-5.21E-03	-1.39E-02	-1.09E-02
vrc	-1.36E-03	-8.13E-03	-2.64E-03	-7.78E-04	1.89E-02	9.90E-03	-1.31E-03	-8.52E-03	-2.45E-03
qpc	-7.00E-01	-7.05E-01	-8.50E-01	-7.01E-01	-7.43E-01	-6.74E-01	-6.97E-01	-7.07E-01	-8.48E-01
qcc	8.29E-02	*.10E-02	3.69E-02	8.47E-02	1.29E-01	1.90E-01	1.60E-02	6.41E-03	2.43E-02
qlc	5.34E-02	5.21E-02	1.41E-02	5.28E-02	3.11E-02	1.31E-02	-1.56E-02	-7.65E-03	-7.04E-04
qfc	3.08E-02	1.63E-02	2.25E-02	3.14E-02	7.53E-02	1.79E-01	3.06E-02	1.98E-02	2.48E-02
qac	-1.17E-04	-7.15E-03	2.98E-04	5.58E-04	2.11E-02	-2.33E-03	-1.02E-04	-5.47E-03	2.57E-04
qrc	********	-3.48E-04	********	5.88E-05	1.01E-03	-5.48E-05	********	-2.65E-04	********

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-9
PERC Model Sensitivity
Rat Parameter Set: 8 Hour Inhalation Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Virtual Concentration of Metabolite Medium	Virtual Concentration of Metabolite High	AUCA Low	AUCA Medium	AUCA High
bw	1.81E-01	-2.99E-01	-2.79E-01	-9.78E-02	-4.83E-01	-2.71E-01	1.61E-01	-2.96E-01	-2.79E-01
vmaxc	-1.03E-01	-1.93E-01	-7.84E-03	8.95E-01	8.47E-01	9.99E-01	-3.44E-02	-1.48E-01	-6.14E-03
km	4.75E-01	1.20E-01	2.84E-04	-5.14E-01	-5.37E-01	-4.02E-02	4.07E-01	9.14E-02	2.17E-04
pb	6.78E-01	7.72E-01	8.53E-01	6.75E-01	5.95E-01	5.89E-02	6.78E-01	7.64E-01	8.52E-01
pl	9.67E-01	7.56E-01	8.43E-01	-3.09E-02	-1.94E-01	-8.86E-03	-3.08E-02	-2.40E-01	-1.56E-01
pf	8.23E-02	-1.47E-01	-1.61E-01	8.29E-02	-7.55E-02	2.29E-03	8.22E-02	-1.43E-01	-1.61E-01
ps	-7.46E-03	-1.28E-02	-1.22E-02	-7.12E-03	9.22E-03	2.42E-03	-7.42E-03	-1.18E-02	-1.22E-02
pr	-3.13E-02	-2.43E-01	-8.77E-02	-3.12E-02	-1.94E-01	-4.53E-03	-3.13E-02	-2.41E-01	-8.76E-02
vlc	-3.10E-02	-2.43E-01	-1.57E-01	-1.02E+00	-1.18E+00	-9.99E-01	-3.08E-02	-2.40E-01	-1.56E-01
vfc	8.24E-02	-1.47E-01	-1.61E-01	8.30E-02	-7.55E-02	2.29E-03	8.26E-02	-1.43E-01	-1.61E-01
vsc	-7.47E-03	-1.28E-02	-1.22E-02	-7.12E-03	9.22E-03	2.42E-03	-7.42E-03	-1.18E-02	-1.22E-02
vrc	-3.13E-02	-2.43E-01	-8.77E-02	-3.12E-02	-1.94E-01	-4.53E-03	-3.13E-02	-2.41E-01	-8.76E-02
qpc	3.18E-01	2.97E-01	1.63E-01	3.14E-01	9.33E-02	-1.86E-02	3.17E-01	2.88E-01	1.52E-01
qcc	2.82E-01	6.82E-02	8.57E-02	2.83E-01	1.20E-01	1.68E-02	2.14E-01	1.78E-02	8.37E-02
qlc	1.64E-01	-1.47E-01	-7.55E-02	1.64E-01	-1.24E-01	-4.22E-03	9.60E-02	-1.89E-01	-7.73E-02
qfc	1.40E-01	1.80E-02	3.03E-02	1.41E-01	9.49E-02	1.37E-02	1.40E-01	2.13E-02	3.03E-02
qsc	1.09E-01	8.45E-04	1.81E-04	1.09E-01	-1.67E-03	-2.46E-04	1.09E-01	4.48E-04	1.80E-04
qrc	1.11E-01	-8.78E-02	-3.17E-01	1.11E-01	-7.14E-02	-1.86E-02	1.11E-01	-8.72E-02	-3.16E-01

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-8.

Table VI-1-10
PERC Model Sensitivity
Rat Parameter Set: Continuous Inhalation Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Virtual Concentration of Metabolite Medium	Virtual Concentration of Metabolite High	AUCA Low	AUCA Medium	AUCA High
bw	-2.09E-03	-3.65E-03	-4.38E-03	-2.60E-01	-2.60E-01	-2.58E-01	-2.08E-03	-3.48E-03	-4.36E-03
vmaxc	-2.94E-01	-1.89E-01	-3.95E-03	7.06E-01	9.05E-01	1.00E+00	-2.26E-01	-1.50E-01	-3.26E-03
km	2.90E-01	9.35E-02	3.73E-05	-6.96E-01	-4.47E-01	-9.37E-03	2.23E-01	7.43E-02	3.08E-05
pb	7.53E-01	9.31E-01	9.90E-01	7.46E-01	4.59E-01	9.18E-03	7.53E-01	9.14E-01	9.89E-01
pl	1.00E+00	1.00E+00	1.00E+00	-5.26E-05	-5.32E-05	********	-6.46E-05	-9.85E-05	-1.40E-04
pf	-7.77E-03	-1.30E-02	-1.60E-02	-7.67E-03	-8.47E-03	-1.50E-04	-8.27E-03	-1.28E-02	-1.59E-02
ps	-2.89E-04	-5.75E-04	-7.66E-04	-2.85E-04	-2.86E-04	********	-2.84E-04	-5.64E-04	-7.64E-04
pr	-6.49E-05	-1.31E-04	-1.76E-04	-6.31E-05	-6.51E-05	********	-6.46E-05	-1.29E-04	-1.75E-04
vlc	-4.98E-05	-1.07E-04	-1.49E-04	-9.90E-01	-9.90E-01	-9.90E-01	-4.52E-05	-9.84E-05	-1.40E-04
vfc	-7.76E-03	-1.30E-02	-1.60E-02	-7.68E-03	-8.47E-03	-1.50E-04	-7.74E-03	-1.28E-02	-1.59E-02
vsc	-2.88E-04	-5.75E-04	-7.66E-04	-2.85E-04	-2.86E-04	********	-2.91E-04	-5.64E-04	-7.64E-04
vrc	-6.59E-05	-1.31E-04	-1.76E-04	-6.53E-05	-6.51E-05	********	-6.56E-05	-1.29E-04	-1.75E-04
qpc	2.46E-01	1.63E-01	1.37E-02	2.43E-01	8.08E-02	1.28E-04	2.46E-01	1.60E-01	1.37E-02
qoc	5.50E-02	3.83E-02	6.76E-03	5.45E-02	1.90E-02	8.35E-05	-1.29E-02	2.40E-03	6.05E-03
qlc	5.16E-02	3.30E-02	6.97E-04	5.10E-02	1.64E-02	********	-1.63E-02	-2.74E-03	********
qfc	3.48E-03	5.23E-03	6.05E-03	3.43E-03	2.60E-03	5.68E-05	3.45E-03	5.13E-03	6.04E-03
qsc	4.71E-06	9.07E-06	1.08E-05	********	********	********	********	********	1.09E-05
qrc	********	1.98E-07	********	********	********	********	********	********	********

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-8.

Table VI-1-11
PERC Model Sensitivity
Human Parameter Set: Gavage Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite: Low	Medium	High	AUCA Low	AUCA Medium	AUCA High
bw	2.48E-01	2.46E-01	2.44E-01	-1.84E-02	-5.84E-02	-1.55E-01	2.39E-01	2.38E-01	2.36E-01
tgav	7.37E-01	-1.30E-01	2.32E-01	7.39E-01	3.77E-02	9.70E-02	7.35E-01	-1.31E-01	2.30E-01
vmaxc	-5.29E-02	-3.59E-02	-3.08E-03	9.46E-01	9.61E-01	9.94E-01	-5.25E-02	-3.55E-02	-2.96E-03
km	5.22E-02	2.54E-02	6.36E-04	-9.33E-01	-6.83E-01	-2.22E-01	5.20E-02	2.50E-02	5.83E-04
pb	3.97E-01	4.01E-01	4.16E-01	3.97E-01	4.61E-01	3.24E-01	6.44E-01	6.48E-01	6.62E-01
pl	9.97E-01	9.93E-01	9.94E-01	-1.38E-03	4.05E-02	2.39E-02	-2.61E-03	-4.40E-03	-3.09E-03
pf	-2.64E-02	-2.57E-02	-2.68E-02	-2.79E-02	-3.59E-02	-6.26E-02	-3.53E-02	-4.19E-02	-4.27E-02
pa	-2.24E-02	-2.45E-02	-2.50E-02	-2.18E-02	2.52E-02	1.95E-01	-3.58E-02	-3.78E-02	-3.87E-02
pr	-1.68E-03	-2.39E-03	-1.87E-03	-1.38E-03	1.70E-02	1.60E-02	-2.61E-03	-3.39E-03	-2.90E-03
vlc	-2.09E-03	-3.71E-03	-2.37E-03	-9.91E-01	-9.47E-01	-9.66E-01	-2.77E-03	-4.40E-03	-3.09E-03
vfc	-2.57E-02	-2.57E-02	-2.66E-02	-2.58E-02	-3.59E-02	-6.26E-02	-4.20E-02	-4.19E-02	-4.27E-02
vac	-2.24E-02	-2.45E-02	-2.50E-02	-2.18E-02	2.52E-02	1.95E-01	-3.57E-02	-3.78E-02	-3.87E-02
vrc	-1.67E-03	-2.40E-03	-1.87E-03	-1.37E-03	1.70E-02	1.60E-02	-2.67E-03	-3.39E-03	-2.90E-03
qpc	-3.96E-01	-4.00E-01	-4.15E-01	-3.97E-01	-4.49E-01	-3.26E-01	-6.42E-01	-6.46E-01	-6.60E-01
qac	-4.92E-01	-5.08E-01	-5.18E-01	-4.89E-01	-2.82E-01	-6.92E-02	-2.16E-01	-2.33E-01	-2.43E-01
qlc	-3.46E-01	-3.58E-01	-3.65E-01	-3.44E-01	-1.83E-01	-9.96E-03	2.00E-02	7.50E-03	6.33E-04
qfc	-1.50E-01	-1.53E-01	-1.58E-01	-1.50E-01	-1.56E-01	-3.40E-02	-2.44E-01	-2.49E-01	-2.51E-01
qac	4.47E-03	2.55E-03	4.93E-03	5.36E-03	5.82E-02	-2.52E-02	7.09E-03	5.18E-03	7.61E-03
qrc	********	2.89E-05	1.23E-05	********	-4.72E-04	-2.08E-04	********	3.47E-05	1.86E-05

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-8.

Table VI-1-12
PERC Model Sensitivity
Human Parameter Set: Intravenous Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite: Low	Medium	High	AUCA Low	AUCA Medium	AUCA High
bw	2.41E-01	2.36E-01	2.37E-01	-1.84E-02	-2.61E-02	-1.30E-01	2.44E-01	2.37E-01	2.37E-01
tlv	-9.98E-01	1.54E+00	-4.24E-01	-9.94E-01	1.28E+00	-1.23E-01	-9.97E-01	1.53E+00	-4.24E-01
vmaxc	-5.32E-02	-4.21E-02	-5.80E-03	9.53E-01	9.50E-01	9.92E-01	-3.25E-02	-2.58E-02	-3.51E-03
km	5.26E-02	3.47E-02	1.55E-03	-9.42E-01	-7.86E-01	-2.82E-01	3.18E-02	2.12E-02	9.03E-04
pb	6.53E-01	6.47E-01	6.67E-01	6.53E-01	6.72E-01	4.12E-01	6.53E-01	6.48E-01	6.67E-01
pl	1.00E+00	9.88E-01	9.95E-01	-1.38E-03	6.68E-02	2.45E-02	-2.16E-03	-3.95E-03	-2.30E-03
pf	-4.55E-02	-4.36E-02	-4.47E-02	-5.58E-02	-5.26E-02	-8.13E-02	-4.36E-02	-4.42E-02	-4.51E-02
pa	-3.25E-02	-3.40E-02	-3.59E-02	-3.20E-02	7.79E-03	2.41E-01	-3.18E-02	-3.29E-02	-3.47E-02
pr	-2.43E-03	-4.12E-03	-2.76E-03	-1.38E-03	3.41E-02	2.04E-02	-2.16E-03	-3.46E-03	-2.68E-03
vlc	-2.66E-03	-5.68E-03	-2.92E-03	-9.98E-01	-9.19E-01	-9.65E-01	-2.07E-03	-3.95E-03	-2.30E-03
vfc	-4.43E-02	-4.36E-02	-4.47E-02	-4.44E-02	-5.26E-02	-8.13E-02	-4.46E-02	-4.41E-02	-4.51E-02
vac	-3.24E-02	-3.40E-02	-3.59E-02	-3.20E-02	7.79E-03	2.41E-01	-3.19E-02	-3.29E-02	-3.47E-02
vrc	-2.52E-03	-4.12E-03	-2.76E-03	-1.66E-03	3.41E-02	2.04E-02	-2.48E-03	-3.46E-03	-2.68E-03
qpc	-6.61E-01	-6.45E-01	-6.65E-01	-6.61E-01	-6.70E-01	-4.14E-01	-6.52E-01	-6.46E-01	-6.65E-01
qac	-2.16E-01	-2.21E-01	-2.40E-01	-2.15E-01	-1.75E-01	-7.26E-02	-2.36E-01	-2.36E-01	-2.42E-01
qlc	2.04E-02	1.80E-02	2.86E-03	1.92E-02	-2.31E-02	-4.74E-03	-7.04E-04	6.27E-04	2.53E-05
qfc	-2.43E-01	-2.41E-01	-2.49E-01	-2.43E-01	-2.35E-01	-3.90E-02	-2.42E-01	-2.41E-01	-2.48E-01
qac	6.10E-03	2.73E-03	8.65E-03	7.67E-03	8.15E-02	-2.88E-02	5.99E-03	3.88E-03	6.43E-03
qrc	1.88E-05	-8.58E-05	1.70E-05	1.22E-04	1.55E-03	-2.82E-04	********	-2.98E-05	1.59E-05

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-5.

Table VI-1-13
PERC Model Sensitivity
Human Parameter Set: 8 Hour Inhalation Administration

	Dose Surrogate								
	AUCL			Virtual Concentration of Metabolite			AUCA		
Parameter	Low	Medium	High	Low	Medium	High	Low	Medium	High
bw	-2.45E-02	-2.60E-02	-2.57E-02	-2.81E-01	-2.49E-01	-2.49E-01	-2.47E-02	-2.52E-02	-2.53E-02
vmaxc	-5.17E-02	-3.32E-02	-1.22E-03	9.45E-01	9.71E-01	9.99E-01	-3.13E-02	-2.01E-02	-7.25E-04
km	5.10E-02	2.12E-02	8.81E-05	-9.31E-01	-8.29E-01	-5.37E-02	3.09E-02	1.28E-02	5.24E-05
pb	6.30E-01	6.41E-01	6.47E-01	6.27E-01	5.05E-01	7.18E-02	6.30E-01	6.40E-01	6.48E-01
pl	9.93E-01	9.92E-01	9.90E-01	-3.49E-03	6.17E-03	2.92E-03	-2.88E-03	-3.22E-03	-3.27E-03
pf	-3.60E-02	-3.59E-02	-3.64E-02	-3.74E-02	-4.04E-02	-1.24E-02	-3.56E-02	-3.63E-02	-3.67E-02
ps	-5.14E-02	-5.62E-02	-5.48E-02	-4.96E-02	6.01E-02	4.29E-02	-5.05E-02	-5.38E-02	-5.38E-02
pr	-3.58E-03	-4.01E-03	-3.88E-03	-3.49E-03	6.23E-03	3.17E-03	-3.52E-03	-3.82E-03	-3.81E-03
vlc	-3.70E-03	-4.15E-03	-4.11E-03	-9.90E-01	-9.81E-01	-9.87E-01	-2.91E-03	-3.22E-03	-3.27E-03
vfc	-3.57E-02	-3.59E-02	-3.64E-02	-3.57E-02	-4.04E-02	-1.24E-02	-3.60E-02	-3.63E-02	-3.67E-02
vsc	-5.14E-02	-5.62E-02	-5.48E-02	-4.97E-02	6.01E-02	4.29E-02	-5.05E-02	-5.38E-02	-5.38E-02
vrc	-3.59E-03	-4.01E-03	-3.88E-03	-3.43E-03	6.23E-03	3.17E-03	-3.53E-03	-3.82E-03	-3.81E-03
qpc	3.62E-01	3.62E-01	3.43E-01	3.69E-01	1.49E-01	-1.88E-02	3.62E-01	3.59E-01	3.42E-01
qcc	-2.18E-01	-2.30E-01	-2.44E-01	-2.17E-01	-1.58E-01	-1.80E-02	-2.39E-01	-2.43E-01	-2.45E-01
qlc	2.03E-02	1.35E-02	1.32E-03	2.01E-02	7.99E-03	-2.40E-04	-7.24E-04	-1.89E-04	4.21E-05
qfc	-2.50E-01	-2.55E-01	-2.57E-01	-2.48E-01	-1.76E-01	-1.10E-02	-2.49E-01	-2.54E-01	-2.57E-01
qsc	1.14E-02	1.16E-02	1.18E-02	1.14E-02	1.06E-02	-6.42E-03	1.12E-02	1.13E-02	1.16E-02
qrc	2.95E-08	4.87E-05	3.09E-05	1.81E-05	-5.27E-04	-3.19E-04	2.83E-05	4.06E-05	3.00E-05

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-8.

Table VI-1-14
PERC Model Sensitivity
Human Parameter Set: Continuous Inhalation Administration

Parameter	Dose Surrogate: AUCL Low	AUCL Medium	AUCL High	Virtual Concentration of Metabolite Low	Virtual Concentration of Metabolite Medium	Virtual Concentration of Metabolite High	AUCA Low	AUCA Medium	AUCA High
bw	-2.24E-02	-2.31E-02	-3.01E-02	-2.80E-01	-2.70E-01	-2.59E-01	-2.22E-02	-2.29E-02	-3.01E-02
vmaxc	-5.39E-02	-2.91E-02	-5.75E-04	9.46E-01	9.85E-01	1.00E+00	-3.35E-02	-1.83E-02	-3.73E-04
km	5.29E-02	1.49E-02	********	-9.29E-01	-5.01E-01	-9.54E-03	3.29E-02	9.37E-03	********
pb	6.74E-01	6.94E-01	7.35E-01	6.6HE-01	3.53E-01	6.96E-03	6.74E-01	6.91E-01	7.36E-01
pl	9.98E-01	9.98E-01	9.99E-01	-1.67E-03	-9.30E-04	-1.35E-05	-1.30E-03	-1.41E-03	-1.08E-03
pf	-6.58E-02	-6.75E-02	-1.02E-01	-6.61E-02	-3.44E-02	-9.60E-04	-6.62E-02	-6.74E-02	-1.02E-01
pa	-1.69E-02	-1.79E-02	-1.15E-02	-1.67E-02	-9.20E-03	-1.10E-04	-1.67E-02	-1.77E-02	-1.15E-02
pr	-1.61E-03	-1.70E-03	-1.28E-03	-1.60E-03	-8.71E-04	-1.22E-05	-1.64E-03	-1.69E-03	-1.27E-03
vlc	-1.68E-03	-1.82E-03	-1.38E-03	-9.92E-01	-9.91E-01	-9.90E-01	-1.30E-03	-1.41E-03	-1.08E-03
vfc	-6.58E-02	-6.75E-02	-1.02E-01	-6.52E-02	-3.44E-02	-9.68E-04	-6.61E-02	-6.74E-02	-1.02E-01
vac	-1.69E-02	-1.79E-02	-1.15E-02	-1.67E-02	-9.20E-03	-1.10E-04	-1.67E-02	-1.77E-02	-1.15E-02
vrc	-1.61E-03	-1.70E-03	-1.28E-03	-1.59E-03	-8.72E-04	-1.22E-05	-1.60E-03	-1.69E-03	-1.27E-03
qpc	3.22E-01	3.15E-01	2.61E-01	3.19E-01	1.61E-01	2.49E-03	3.21E-01	3.13E-01	2.61E-01
qac	-1.83E-01	-1.98E-01	-1.45E-01	-1.81E-01	-1.02E-01	-1.39E-03	-2.03E-01	-2.08E-01	-1.45E-01
qlc	1.99E-02	1.09E-02	4.99E-04	1.97E-02	5.55E-03	********	-7.34E-04	-1.18E-04	********
qfc	-2.04E-01	-2.11E-01	-1.46E-01	-2.02E-01	-1.08E-01	-1.40E-03	-2.04E-01	-2.09E-01	-1.45E-01
qac	1.58E-03	1.69E-03	1.26E-04	1.58E-03	8.74E-04	********	1.54E-03	1.64E-03	1.26E-04
qrc	********	********	********	********	********	********	********	********	********

Sensitivity is expressed as percent change in the dose surrogate induced by a 1% change in the indicated parameter.
The asterisks signify an absolute magnitude less than 1.00E-8.

Table VI-1-15

Relative Ranking of Parameter Sensitivity of
Virtual Concentration of Metabolite; Mouse Parameter Set

Range of Percent Changes	IV Dosing			Continuous Inhalation Exposure		
	L	M	H	L	M	H
$\geq 10^{-1}$	vlc-[a] qpc- pb+ km- vmaxc+	vlc- qpc- pb+ vmaxc+ km- qcc-	vlc- pb+ qpc- vmaxc+ km- pf+ vfc+ ps+ vsc+	vlc- qpc+ pb+ vmaxc+ km- bw-	vlc- vmaxc+ pb+ km- bw- qpc+	vmaxc+ vlc- bw-
10^{-2} - 10^{-1}	qcc+ qlc+	qlc+ qsc+ pl+ pr+ vrc+ qfc+	qcc+ qlc+ pr+ vrc+ qfc+	qcc+ qlc+	qcc+ qlc+	
10^{-3} - 10^{-2}		vsc+ ps+ pf+ vfc+	pl+			km- pb+
10^{-4} - 10^{-3}	pl+ qsc+ vfc- qfc+ vrc+	qrc- bw-	qsc- bw-		pf- vfc-	qpc+
10^{-5} - 10^{-4}	bw- qrc+		qrc-	pf- vfc- qfc+	ps- vsc- qfc+	qcc+ qlc+

Table VI-1-15 (continued)

Relative Ranking of Parameter Sensitivity of
Virtual Concentration of Metabolite; Mouse Parameter Set

Range of Percent Changes	IV Dosing			Continuous Inhalation Exposure		
	L	M	H	L	M	H
$<10^{-5}$	pf+			ps-	qsc+	pf-
	ps+			vsc-	pr-	vfc-
	pr+			qsc+	vrc-	ps-
	vsc+			vrc+	pl-	vsc-
				pl+	qrc+	qfc+
				pr+		qsc+
				qrc+		vrc-
						qrc-
						pl+
						pr+

[a]The plus or minus sign following the parameter indicates the direction of change of CM given 1% increase in the parameter value.

Table VI-1-16

Relative Ranking of Parameter Sensitivity of
Virtual Concentration of Metabolite; Rat Parameter Set

Range of Percent Changes	IV Dosing			Continuous Inhalation Exposure		
	L	M	H	L	M	H
$>10^0$						vmaxc+[a]
10^{-1} - 10^0	vlc- vmaxc+ km- pb+ qpc-	vlc- pb+ qpc- vmaxc+ km- qcc+ vfc- pf-	vlc- vmaxc+ qpc- pb+ km- qcc+ qfc+ bw-	vlc- pb+ vmaxc+ km- bw- qpc+	vlc- vmaxc+ pb+ km- bw- qpc+	vlc- bw-
10^{-2} - 10^{-1}	vfc- pf- qcc+ qlc+ qfc+ bw-	qfc+ qlc+ bw- pl+ vsc+ ps+ qsc+ pr+ vrc+	vsc+ ps+ vfc+ pf+ qlc+	qcc+ qlc+	qcc+ qlc+	
10^{-3} - 10^{-2}	ps- vsc-	qrc+	vrc+ pr+ pl+ qsc+	vfc- pf- qfc+	pf- vfc- qfc+	km- pb+
10^{-4} - 10^{-3}	pr- vrc- qsc+ pl-			vsc- ps-	vsc- ps-	vfc- pf- qpc+
10^{-5} - 10^{-4}	qrc+		qrc-	vrc- pr- pl-	pr- vrc- pl-	qcc+ qfc+

Table VI-1-16 (continued)

Relative Ranking of Parameter Sensitivity of
Virtual Concentration of Metabolite; Rat Parameter Set

Range of Percent Changes	IV Dosing			Continuous Inhalation Exposure		
	L	M	H	L	M	H
$<10^{-5}$				qsc+ qrc	qsc+ qrc+	vsc- ps- qlc+ vrc- pr- pl- qsc+ qrc

[a]The plus or minus sign following the parameter indicates the direction of change of CM given 1% increase in the parameter value.

Table VI-1-17

Statistics Relevant to Uncertainty Estimation

GM	Geometric mean: antilog of mean log.
SSDL	Sample standard deviation of natural logs. The maximum likelihood and preferred estimator for SDL, the log-scale standard deviation.
CV	Sample coefficient of variation - standard deviation/mean. Used here often to approximate the log-scale standard deviation.
S1 - $(UB/LB)^{0.5}$	Use as an estimate of a UF based on a reference interval LB to UB (usually representing expert opinion.)
S2 - exp(2*SDLE)	for SDLE an estimate of the log-scale standard deviation, (either SSDL or CV.) Use as a direct estimate of a UF for an irreducible uncertainty source based on data assumed iid[a].
S3 - exp(2*SDLE/SQRT(#M))	When the prefered value is the GM of #M iid measurements, use S3 as an estimator for the UF.

[a] iid - independent, identically distributed. (Generated independently from the same probability distribution.)

Table VI-1-18

Data Compiled by Fiserova-Bergerova (1983) Regarding Study-to-Study Variation in Estimates of Blood/Gas Partition Coefficients at 37°C

Chemical	Species	Estimate (Reference[a])
Acetone	man	245(1), 341(2), 313(3), 302(4)
Acetone	dog	363(2), 376(3), 291(4)
Benzene	man	7.8(1), 6.5(5), 9.0(6), 7.8(7), 7.7(8)
Carbon Tetrachloride	man	2.4(2), 0.6(9), 5.8(6)
Chloroform	man	10.3(10), 9.0(9), 8.2(5), 11.0(6)
Cyclopropane	man	0.58(2), 0.35(3), 0.43(11), 0.51(4)
1,1-Dichloroethane	man	4.7(10), 4.5(9), 6.0(6)
1,2-Dichloroethane	man	19.5(10), 20(9), 19.7(6)
Diethylether	man	12.5(2), 11.6(3), 12.3(4), 12.8(6), 11.9(11)
Diethylether	dog	12.7(2), 10.7(4), 11.8(3)
Methylene Chloride	man	9.7(10), 7.0(9), 9.4(6)
Ethane	man	0.1(2), 0.05(3), 0.08(4)
Ethane	dog	0.13(2), 0.04(3), 0.10(4)
Fluroxene	man	1.3(12), 1.5(2), 1.4(11)
Halothane	man	2.6(12), 2.5(2), 2.6(13), 2.8(3), 2.5(11), 2.5(14)
Halothane	dog	1.7(3), 3.5(38), 3.8(2)
1,1,2,2-Tetrachloroethane	man	121(10), 73(9), 141(6)
* PERC	man	13.1(10), 9.1(9), 18.9(6)
Toluene	man	15.6(1), 15.6(5), 14.7(8), 16.3(6)
Styrene	man	32(30), 52(1), 64(6)
Trichloroethylene	man	9(5), 9.5(9), 9.9(6), 9.9(15)

[a](1) Sato and Nakajima (1979); (2) Wagner *et al.*. (1974); (3) Young and Wagner (1979); (4) Dueck *et al.*. (1978); (5) Sherwood (1976); (6) Bocek (personal communication to Fiserova-Bergerova, ibid.); (7) Teisinger and Skramovsky (1947); (8) Sato *et al.*. (1972); (9) Morgan *et al.*. (1970); (10) Sato and Nakajima (1979); (11) Gibbs *et al.*. (1975); (12) Ellis and Stoelting (1975); (13) Saraiva *et al.*. (1977); (14) Eger *et al.* (1962); (15) Fink and Morikawa (1970).

Table VI-1-19

Analysis of Inter-Study Variation in Estimates of Blood/Air Partition Coefficients: Summary of Data in Table VI-1-18 (From Data Compiled by Fiserova-Bergerova (1983))

	Chemical	(Species)	GM[a]	SSDL[b]	S2[c]	#Studies
	Ethane	(man)	0.074	0.35	2.0	3
	Ethane	(dog)	0.080	0.62	3.4	3
	Cyclopropane	(man)	0.46	0.22	1.5	4
	Fluroxene	(man)	1.4	0.072	1.2	3
	Carbon Tetrachloride	(man)	2.0	1.1	9.8	3
	Halothane	(man)	2.6	0.044	1.1	6
	Halothane	(dog)	2.8	0.44	2.4	3
	1,1-Dichloroethane	(man)	5.0	0.16	1.4	3
	Benzene	(man)	7.7	0.12	1.3	5
	Methylene Chloride	(man)	8.6	0.18	1.4	3
	Chloroform	(man)	9.6	0.13	1.3	4
	Trichloroethylene	(man)	9.6	0.045	1.1	4
	Diethylether	(dog)	12	0.086	1.2	3
	Diethylether	(man)	12	0.039	1.1	5
*	PERC	(man)[d]	12	0.32	1.9	4
	Toluene	(man)	16	0.042	1.1	4
	1,2-Dichloroethane	(man)	20	0.013	1.0	3
	Styrene	(man)	47	0.36	2.0	3
	1,1,2,2-Tetrachloroethane	(man)	108	0.34	2.0	3
	Acetone	(man)	298	0.14	1.3	4
	Acetone	(dog)	341	0.14	1.3	3

[a]Geometric mean of available estimates. The table is sorted on this column, to demonstrate any relationship between GM and SSDL.

[b]Standard deviation (in ln scale) of available estimates.

[c]S2 = exp(2*SSDL).

[d]In addition to the values reported in Table IV-1-18, a value of 10.3 has been included based on communication of M. Andersen to Reitz and Nolan (1986).

Table VI-1-20

Estimates of the Log-Scale Standard Deviation (SDLE) Representing Variation Among Individual Humans and Animals in Tissue/Air Partition Coefficients, for Trichloroethylene, Haloethane, Cyclopropane and NO_2

	Trichloroethylene[a] 5 Humans		Halothane[a] 5 humans		Halothane[b] 18-38 horses	
	Mean	SDLE	Mean	SDLE	Mean	SDLE
Blood	8	0.3	2	0.2	2	0.2
Brain	21	0.2	6	0.5	5	0.1
Kidney	15	0.3	6	0.3	3	0.4
Liver	29	0.4	9	0.5	9	1.0
Lung	14	1.0	4	0.7	3	0.2
Muscle	19	0.3	8	0.2	4	1.0
Fat	569	0.03	222	0.1	--	--

	Cyclopropane[c] 5-9 Rabbits		NO_2[c] 5-6 Rabbits		Max SDLE[d]
	Mean	SDLE	Mean	SDLE	
Blood	0.7	0.04	0.5	0.01	0.3
Brain	0.8	0.1	0.4	0.02	0.5
Kidney	0.9	0.1	0.4	0.04	0.4
Liver	0.7	0.1	0.4	0.03	1.0
Lung	--	--	--	--	1.0
Muscle	0.51	0.1	0.4	0.07	1.0
Fat	--	--	--	--	0.1

[a]SDLE = CV, computed from standard deviations and means reported by Fiserova-Bergerova *et al.* (1984), each based on five subject-specific estimates (the same five subjects for each chemical). Tissues were from autopsied individuals.

[b]SDLE from Webb and Weaver (1981), each the square-root of a variance component computed in the log scale.

[c]SDLE based on variance components reported by Mapleson *et al.* (1970). Since computations were carried out in the log scale, square roots of reported variance components are estimates of the log-scale standard deviations. For NO_2, we use the reported between-animals component; for cyclopropane a separate between-animals component was not reported, and so the estimate used incorporates relatively more measurement error. For cyclopropane, our CV is the square root of the average of the "*in vivo*" and "*in vitro*" variance component estimates. Multiple strains of rabbits were used in the experiment.

[d]SDLE for a tissue is the maximum of the SDLE tabulated, over species and studies.

Table VI-1-21

Species-to-Species Variation in Measurements of Tissue/Air Partition Coefficients (Fiserova-Bergerova, 1983).

ISOFLURANE[a]

Tissue	Man	Monkey	Dog	Rat	GM[b]
Brain	1.9	2.0	2.5	2.2	2.1
Heart	1.8	1.6	2.7	2.8	2.2
Kidney	2.1	1.9	3.6	2.5	2.4
Liver	4.1	4.6	4.2	3.2	4.0
Lung	1.6	1.5	2.6	2.4	2.0
Muscle	2.4	1.4	3.4	1.6	2.1
Fat	69	66	75	63	68

METHYLENE CHLORIDE[c]

Tissue	Man	Monkey	Dog	Rat	GM[c]
Brain	7.1	6.8	7.9	7.4	7.3
Heart	7.1	6.3	7.4	9.2	7.4
Kidney	5.8	6.3	9.5	7.4	7.1
Liver	7.1	11	11	8.9	9.4
Lung	5.8	5.5	7.6	7.9	6.6
Muscle	4.7	3.7	7.9	4.7	5.0
Fat	84	86	97	91	89

[a]Data from Table 3 of Fiserova-Bergerova, 1983.
[b]Geometric mean of four species-specific estimates.
[c]Estimated from Figure 5 of Fiserova-Bergerova, 1983.

Table VI-1-22

Analysis of Species-to-Species Variation in Tissue/Gas Partition Coefficients, for Isofluorane and Methylene Chloride (Data Compiled by Fiserova-Bergerova, 1983; Species are Man, Monkey, Dog, and Rat.)

	ISOFLURANE			METHYLENE CHLORIDE		
Tissue	GM	Human/Rat Error[a]	S2	GM	Human/Rat Error[a]	S2
Brain	2.1	1.2	1.3	7.3	1.0	1.1
Heart	2.2	1.6	1.7	7.4	1.3	1.4
Kidney	2.4	1.2	1.8	7.1	1.3	1.6
Liver	4.0	1.3	1.3	9.4	1.3	1.5
Lung	2.0	1.5	1.7	6.6	1.4	1.4
Muscle	2.1	1.5	2.3	5.0	1.0	2.0
Fat	68	1.1	1.2	89	1.1	1.1

[a]Let R be the ratio of the human value to the rat value; the value reported is the larger of R and 1/R. (This corresponds to taking the absolute difference in the log scale, then transforming back to the original scale.)

Table VI-1-23

Summary of PERC Partition Coefficient Uncertainty Estimation

Source M:

1. Estimate from data SDLE's for directly measured tissue/air PC's:
   a. $SM1(i,b)^a$ $i = r,m,h.$
   b. $SM1(r,l)$
   c. $SM1(r,m)$
   d. $SM1(r,f)$
2. Estimate from data SDLE's corresponding to variations across species of tissue/air PC's: $SM2(j)^b$, $j = b,l,r,m,f.$
3. Estimate SDLE corresponding to difference between log of liver/air PC and log of rapid/air PC: $SM3(l,r)^c$.
4. Compute SDLE's for human or mouse tissue/air PC's (tissues other than blood or rapidly perfused organs) when these are based on measurements in rats:
   $$SM4(i,j) = [SM1(r,j)^2 + 2*SM2(j)^2]^{0.5},\ i = m,h;\ j = l,m,f.$$
5. Compute rapid/air PC's when these are based on rat liver/air measurements:
   $$SM4(i,r) = [SM1(r,l)^2 + SM2(l)^2 + SM2(r)^2 + SM3(l,r)^2]^{0.5},$$
   $$i = r,m,h.$$
6. Compute SDLE's and UF_M's for blood/air and tissue/blood PC's:
   a. Blood/air, for species i (i = r,m,h) -
      $SDLE(i,b) = SM1(i,b)$.
   b. Tissue/blood, for rats and tissue j other than rapidly perfused (j = l,m,f) -
      $SDLE(r,j) = [SM1(r,j)^2 + SM1(r,b)]^{0.5}$.
   c. Tissue/blood, for species i other than rats (i = m,h) and tissue j (j = l,r,m,f) or for rats and rapidly perfused tissue (i = r, j = r) -
      $SDLE(i,j) = [SM4(i,j)^2 + SM1(i,b)^2]^{0.5}$.

In each case, $UF_M(i,j) = \exp(2*SDLE(i,j))$.

Table VI-1-23 (continued)

Summary of PERC Partition Coefficient Uncertainty Estimation

Source I:

1. Estimate from data SDLE's representing individual variation in tissue/air PC's. Denote these by
   $SI1(i,j)^d$ $i = r,m,h$; $j = b,l,r,m,f$.
2. Compute SDLE's and UF_I's for blood/air and tissue/blood PC's:
   a. Blood/air, for species i ($i = r,m,h$) -
      $SDLE(i,b) = SI1(i,b)$.
   b. Tissue/blood, for species i ($i = r,m,h$) and tissue j ($j = l,r,m,f$) -
      $SDLE(i,j) = [SI1(i,j)^2 + SI1(i,b)^2]^{0.5}$.

   In each case, $UF_I(i,j) = \exp(2*SDLE(i,j))$.

Total Uncertainty:

1. Combine source M and source I uncertainty factors for species i and tissue j ($i = r,m,h$; $j = b,l,r,m,f$):
   $UF_T(i,j) = \exp[(\ln(UF_M(i,j))^2 + \ln(UF_I(i,j))^2)^{0.5}]$.

---

[a]The first variable in parentheses is a species code and the second is a tissue code. This notation corresponds to that in Figure VI-1-2.

[b]The variable in parentheses is a tissue code. This notation corresponds to that in Figure VI-1-2.

[c]The two variables in parentheses are tissue codes. This notation corresponds to that in Figure VI-1-2.

[d]The variables in parentheses are like those for the analogous SM1 standard deviations, i.e., a species code followed by a tissue code.

Table VI-1-24

Source I, Source M, and Total Uncertainty Factors
for PERC Partition Coefficient Estimates

PBPK Parameter	Preferred Parameter Value	UF_I	UF_M	UF_T
		RATS		
Blood/air	18.8	1.8	2.0	2.5
Liver/blood	3.74	8.1	2.7	10
Rapid/blood	3.74	3.2	4.3	6.5
Muscle/blood	1.06	8.1	2.7	10
Fat/blood	87.1	1.9	2.7	3.2
		MICE		
Blood/air	16.9	1.8	2.0	2.5
Liver/blood	3.01	8.1	2.7	10
Rapid/blood	3.01	3.2	4.3	6.5
Muscle/blood	2.59	8.1	2.7	10
Fat/blood	48.3	1.9	2.7	3.2
		HUMANS		
Blood/air	10.3	1.8	1.4	2.0
Liver/blood	5.88	8.1	3.1	11
Rapid/blood	5.88	3.2	3.8	5.8
Muscle/blood	3.10	8.1	4.1	12
Fat/blood	119.1	1.9	3.1	3.6

Table VI-1-25

Variation in Vmaxc Estimates

Source	Estimates ($mg/hr/kg^{0.74}$)	GM	Reitz & Nolan[a]	SSDL[b]
	Mice			
Hattis *et al.* "Schumann"	1.53[c]	3.96	8.34	0.721
Hattis *et al.* "Mitoma"	5.34			
Reitz & Nolan "Mouse 1"	8.34			
Reitz & Nolan "Mouse 2"	3.60			
	Rats			
Hattis *et al.* "Pegg"	2.39	2.03	1.27	0.414
Hattis *et al.* "Mitoma"	2.78			
Reitz & Nolan, F344	1.27			
	Humans			
Hattis *et al.* "Ohitsu(+)"	0.389	0.330	0.346	0.195
Hattis *et al.* "Ikeda(+)"	0.266			
Reitz & Nolan	0.346[d]			
	Weighted Average SSDL[e]			0.532

[a]Preferred values from Reitz & Nolan, using the "Mouse 1" estimates for mice.

[b]SSDL = standard deviation of ln estimates.

[c]From the range of values 1.53-1.72 suggested by Hattis *et al.*, the value 1.53 yields the largest value of SSDL.

[d]A value of 0.256 is given in Table 1 of our draft of Reitz and Nolan. We have used the value of 0.346 from the text of the draft, which we believe to be the correct value.

[e]Weighted average = $[\text{sum}(df*SSDL^2)/\text{sum}(df)]^{0.5}$ where summation is over mice, rats, and humans, and df is the number of measurements available for a species, minus one (*e.g.* df = 3 for mice).

Table VI-1-26

Variation in Km Estimates

Source	Estimates (mg/liter)	GM	Reitz & Nolan[a]	SSDL[b]
	Mice			
Hattis *et al.* "Schumann"	0.268[c]	1.47	4.56	1.54
Hattis *et al.* "Mitoma"	5.35			
Reitz & Nolan "Mouse 2"	2.22			
	Rats			
Hattis *et al.* "Pegg"	6.78	8.19	4.56	0.700
Hattis *et al.* "Mitoma"	17.8			
Reitz & Nolan, F344	4.56			
	Humans			
Hattis *et al.* "Ohitsu(+)"	5.69	1.86	4.56	1.58
Hattis *et al.* "Ikeda(+)"	0.609			
	Weighted Average SSDL[d]			1.28

[a]Preferred values from Reitz & Nolan, equal to the rat estimate for all three species.

[b]SSDL = standard deviation of ln estimates.

[c]From the range of values 0.268-0.669 suggested by Hattis *et al.*, the value 0.268 yields the largest value of SSDL.

[d]Weighted average = $[\text{sum}(df*SSDL^2)/\text{sum}(df)]^{0.5}$ where summation is over mice, rats, and humans, and df is the number of measurements available for a species, minus one (*e.g.* df = 3 for mice).

Table VI-1-27

Hour Volumes (in liters) for Ventilation (qp), Cardiac Output (qc), and Muscle Blood Supply (qm), as a Function of Activity Level in Humans (from Hattis *et al.*, 1986)

Activity Level	qp	(incr.)[a]	qc	(incr.)[a]	qm	(incr.)[a]
Sitting	420		400		66	
"Shoeworker"[b]	683	(263)	489	(89)		
Light exercise	960	(540)	582	(182)	324	(258)
Heavy exercise	2040	(1620)	840	(440)	540	(474)

[a]Increment from sitting value = value at specified activity level minus corresponding sitting value.

[b]qp from Brugnone *et al.* (1980), assumed typical for occupational settings; qc estimated by linear interpolation between adjacent values of qp and qc.

Figure VI-1-1

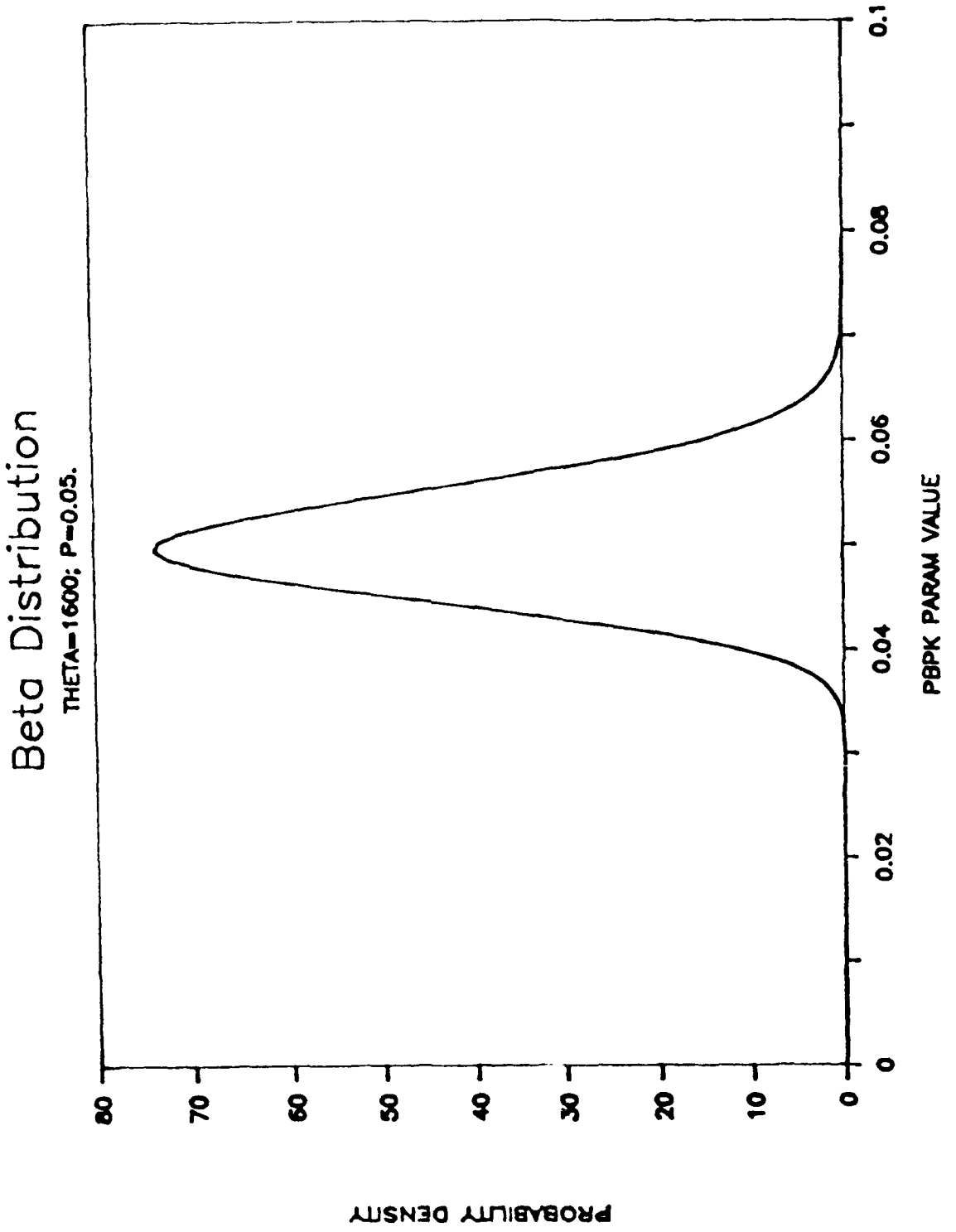
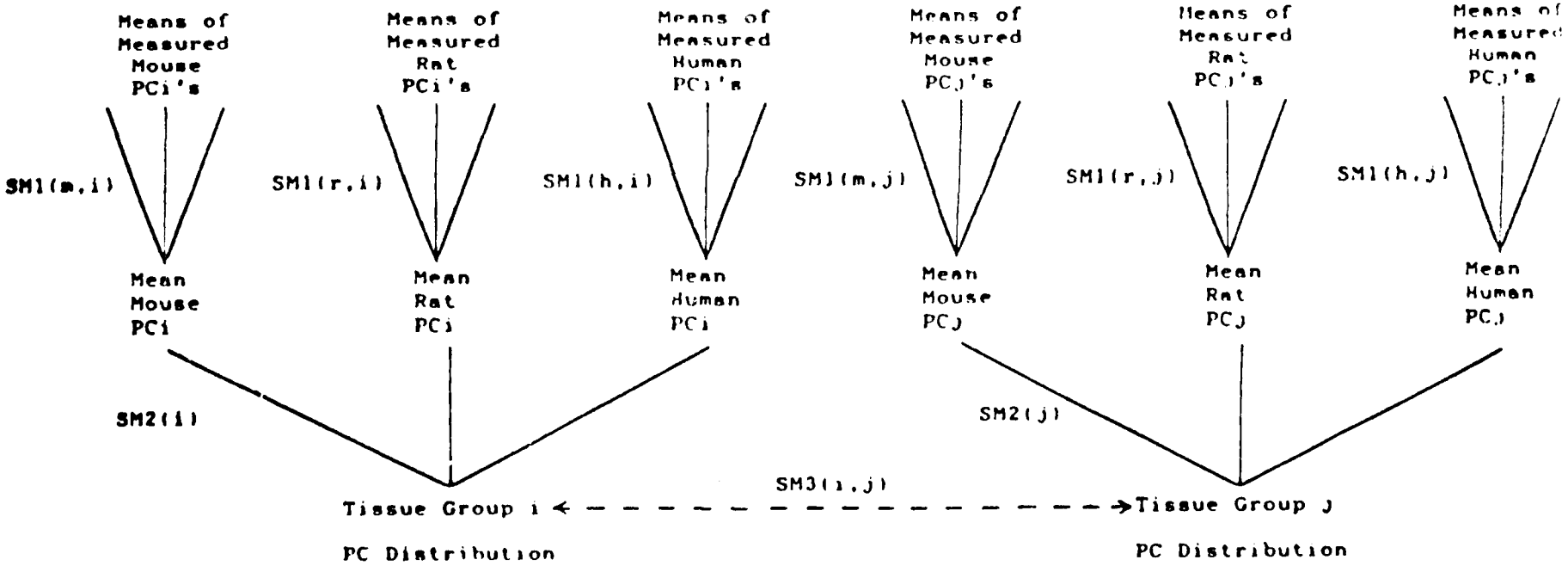

Beta Distribution
THETA=1600; P=0.05.
PROBABILITY DENSITY
80
70
60
50
40
30
20
10
0
0
0.02
0.04
0.06
0.08
0.1
PBPK PARAM VALUE

Figure VI-1-2

Representation of Source M Uncertainty Estimation
for Partition Coefficients



APPENDIX VI-1-A

EVALUATION OF UNCERTAINTY IN INPUT PARAMETERS TO PHARMACOKINETIC MODELS AND THE RESULTING UNCERTAINTY IN OUTPUT

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Evaluation of uncertainty in input parameters to pharmacokinetic models and the resulting uncertainty in output

David Farrar, Bruce Allen, Kenny Crump and Annette Shipp

Clement Associates, Inc., Ruston, LA (U.S.A.)

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SUMMARY

Physiologically-based pharmacokinetic (PBPK) models may be used to predict the concentrations of parent chemical or metabolites in tissues, resulting from specified chemical exposures. An important application of PBPK modeling is in assessment of carcinogenic risks to humans, based on animal data. The parameters of a PBPK model may include metabolic parameters, blood air and tissue:blood partition coefficients, and physiological parameters, such as organ weights and blood flow rates. Uncertainty in estimates of these parameters results in uncertainty regarding tissue concentrations and resulting risks. Data are reviewed relevant to the quantification of these uncertainties, for a PBPK model-based risk assessment for tetrachloroethylene. Probability distributions are developed to express uncertainty in model parameters, and uncertainties are propagated by a sequence of operations that simulates processes recognized as contributing to estimates of human risk. Distributions of PBPK model output and human risk estimates are used to characterize uncertainty resulting from uncertainty in model parameters.

INTRODUCTION

Physiologically-based pharmacokinetic (PBPK) models can be used in the animal-based assessment of human cancer risks. A PBPK model is assumed for rodents and humans (with parameter values that are possibly species-specific) and dose surrogates are calculated on the basis of that model. A dose surrogate is a particular measure of chemical delivered to a putative target tissue. The dose surrogate values corre-

Address for correspondence: B. Allen, Clement Associates, Inc., 1201 Gaines Street, Ruston, LA 71270, U.S.A.

sponding to the dose levels and dose regimen followed in a chosen carcinogenicity bioassay and the observed tumor count data are the input to dose-response model (e.g., the multistage or Weibull models [1]). Values of the chosen surrogate dose are estimated for humans, corresponding to specified external exposures, and human risks are estimated based on the fitted dose-response curve.

This report describes the development of methods for analyzing the effect of uncertainties associated with PBPK model parameters. The methods are illustrated in the context of a risk assessment for tetrachloroethylene that employs a multicompartment PBPK model and a carcinogenic end-point observed in female mice [2].

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR TETRACHLOROETHYLENE

The Environmental Protection Agency (EPA) [3] and Hattis et al. [4] have developed two slightly different PBPK models for tetrachloroethylene. Reitz and Nolan [5] have reviewed the parameter values adopted by the EPA and have recommended some revisions. In order to evaluate uncertainties within the framework of a given PBPK model, analyses presented here are based on the EPA model (Fig. 1). Flow-

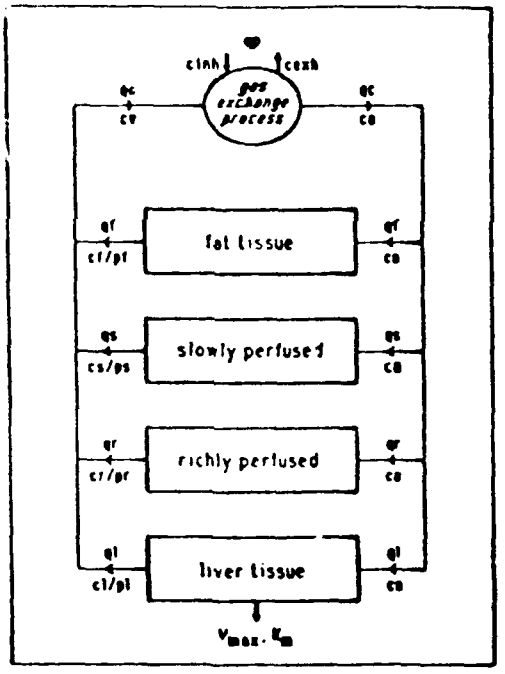

Fig. 1. Tetrachloroethylene PBPK model. Notation: c_{inh}, c_{exh}, c_a and c_v are concentration of parent in inhaled air, exhaled air, arterial blood, and venous blood; q_i, c_i and p_i are the perfusion rate, parent concentration, and tissue/blood partition coefficient for compartment i; and V_{max} and K_m are constants determining the rate of metabolism in liver.

limited compartments and Michaelis- Menten metabolism (occurring in the liver) are assumed.

Notation and preferred values for model parameters are listed in Table I. In gener-

TABLE I

PARAMETERS OF THE TETRACHLOROETHYLENE PBPK MODEL, WITH PREFERRED VALUES AND UNCERTAINTY FACTORS (UF[a])

Parameter	Preferred values (UFs)			
	Mice		Humans	
Body weight (bw; kg)	0.028		70	
Compartment proportions (range 0–1)				
Liver (v_l)	0.056	(1.24)	0.026	(1.35)
Rapidly perf (v_r)	0.049	(1.24)	0.050	(1.25)
Slowly perf (v_s)	0.767	(1.03)	0.620	(1.04)
Fat (v_f)	0.049	(1.25)	0.230	(1.09)
Cardiac output (l/h) (q_c)	1.13[b]	(1.08)	348[c]	(1.12)
Waking value ($q_{c,w}$)	-		486[d]	(1.12)
Alveolar ventilation rate (l/h)				
(q_p)	1.64	(1.11)	288[c]	(1.50)
Waking value($q_{p,w}$)	-		683[d]	(1.26)
Compartment perfusions (l/h)				
Liver (q_l)	0.282	(1.24)	90.6	(1.35)
Rapidly perf (q_r)	0.576	(1.24)	153	(1.25)
Slowly perf (q_s)	0.170	(1.01)	87.0	(1.04)
Waking value ($q_{s,w}$)	-		225	(1.17)
Fat (q_f)	0.102	(1.25)	17.4	(1.09)
Partition coefficients (unitless)				
Blood gas (p_b)	16.9	(1.97)	12.0	(1.97)
Liver/blood (p_l)	3.01	(2.69)	5.05	(9.37)
Liver/gas (p_{lg})	50.9	(1.97)	60.6	(8.36)
Liver gas (p_{lg})	50.9	(1.97)	60.6	(8.36)
Rapid/blood (p_r)	3.01	(4.14)	5.05	(5.69)
Rapid gas (p_{rg})	50.9	(3.51)	60.6	(4.92)
Slow/blood (p_s)	2.59	(2.54)	2.66	(11.0)
Slow/gas (p_{sg})	43.8	(1.97)	31.9	(10.1)
Fat/blood (p_f)	48.3	(2.56)	102	(2.15)
Fat/gas (p_{fg})	816	(1.93)	1230	(2.15)
Metabolic constants				
$V_{max,c}$ (mg/h)	3.96	(2.83)	0.33	(2.84)
K_m (mg/l)	1.47	(12.4)	1.86	(12.3)

[a]The uncertainty factors are estimated from 1000 Latin-hypercube samples such that for preferred value A_p, the interval from A_p/UF to A_p × UF contains 95% of the values.

[b]Preferred values for mice, 24 h/d.

[c]Preferred values for sleeping humans, 8 h/d.

[d]Preferred values for waking humans, 16 h/d.

al the definition of model parameters and the units adopted follow Reitz and Nolan [5]. Absolute compartment volumes (v_i) (in liters) are related to compartment proportions (v_{ic}, Table I) by:

$$v_i = v_{ic} \times bw \quad (i = l,r,s,f)$$

were bw is body weight in kilograms. The maximal metabolic rate (V_{max}) is scaled according to $V_{max} = V_{maxc} \times bw^{0.74}$ [5]. The v_{ic} and V_{maxc} parameters are those for which uncertainties are estimated.

The partition coefficients that are measured directly in vitro are limited to some of the tissue/gas coefficients (p_b and p_{gi}, $i = l,r,s,f$). Tissue/blood partition coefficients, which are those actually used in the PBPK model, are estimated by dividing the tissue/gas coefficients by the blood/gas coefficient ($p_i = p_{gi}/p_b$, $i = l,r,s,f$).

The dose surrogates that are estimated herein, and for which uncertainties are estimated, are (1) the average daily area under the liver concentration–time curve for the parent (AUCL), (2) the average daily area under the arterial blood concentration–time curve for the parent (AUCA), and (3) the average daily amount of parent metabolized per volume of liver tissue (CML).

PARAMETER UNCERTAINTY EVALUATION FOR THE TETRACHLOROETHYLENE MODEL

For each parameter we have identified a preferred value (Table I), and have specified a probability distribution to represent uncertainty. The preferred value represents some summary of information available from the literature (see below). The probability distribution is selected in such a way as to assign relatively high probability to values that are close to the preferred value. A useful device for communicating uncertainties is to define an 'uncertainty factor' (UF ≥ 1) such that for a preferred parameter value (h_P) the range of values h_P / UF to $h_P \times$ UF is considered highly probable (we assume probability 95%). Table I gives uncertainty factors estimated from the observed distributions of the values generated for the parameters, which are based on the probability distributions described below.

Specification of probability distributions to express uncertainty in parameter values involves, first of all, selection of appropriate families of distributions for individual parameters, or in some cases for groups of interrelated parameters. The families selected for expressing uncertainty are the log-normal or truncated log-normal family (for partition coefficients and metabolic constants) and the Dirichlet family (for compartment volumes as proportions of total body volume). Other PBPK model parameters are functionally related to the compartment volumes and their empirical distributions depend on the distribution of the volumes.

Uncertainty factors were derived largely on the basis of analyses of variation in reported measurements or estimates. That being the case, different sources of uncertainty and variation must be recognized. Variation among average measurements re-

ported in different experiments represents a different source or sources of uncertainty than variation from measurements taken on different individuals in the same experiment. Furthermore, when a parameter value is not measured directly so that a value of another variable is attributed to that parameter, additional uncertainty is introduced. Consequently, the evaluation of total uncertainty theoretically requires combining distributions representing multiple levels of uncertainty.

Of interest for test species PBPK parameters is uncertainty with respect to an average parameter value. This is the case because an aggregate response variable (proportion of animals with tumor) is to be related to an aggregate predictor (the single dose surrogate value assumed for a treatment group). For humans, in contrast, interest is in individual variation of dose surrogate levels due to variation in PBPK model parameter values. Therefore, for humans, it is desirable to incorporate into an estimate of relevant total uncertainty a component representing inter-individual variation specifically. Consequently the probability distributions derived below for humans apply to individuals selected at random, but distributions derived for rodents apply to mean values for groups of animals in the bioassay considered.

Presented here are uncertainty derivations for mice and humans. The concepts exemplified by the analysis for mice generalize to other test species.

Partition coefficients

Preferred values for tissue/gas partition coefficients (equated with the medians of the log-normal distributions characterizing uncertainties) are taken from Reitz and Nolan [5]. The blood/gas preferred value (12) is the geometric mean of the value from Reitz and Nolan [5] (10.3) and 3 values (9.1, 13.1, 18.9) reported by Fiserova-Bergerova [6].

Three sources of uncertainty are recognized, one or more of which are relevant to the estimation of total uncertainty for a given partition coefficient. For parameters measured directly by vial equilibration techniques, uncertainty is due to differences in values estimated in different experiments (source 1). These parameters are the rodent blood/gas, liver/gas, fat/gas, and slow/gas (actually muscle/gas) and the human blood/gas coefficients. The unmeasured parameters - the rapid/gas coefficient in rodents (equated to the rodent liver/gas coefficient) and all tissue/gas coefficients other than blood/gas in humans (equated to the average of the corresponding coefficients estimated in rats and mice) - have additional uncertainty due to the attribution of the measured values to those parameter values (source 2). For all human coefficients, individual variation is also relevant (source 3).

Each source of uncertainty is represented by a separate log-normal distribution with specified geometric standard deviation (GSD). Therefore, the distribution describing total uncertainty is also log-normal, with geometric variance (squared geometric standard deviation) given by the sum of geometric variances expressing specific contributing uncertainties. Details of the derivation of source-specific uncertainty distributions are available from the authors.

It is common practice in uncertainty analyses to derive distribution parameters by directly assigning subjective probabilities to ranges of parameter values [7]. An analogous procedure has been used here to modify the uncertainty estimates for the partition coefficients.

Plausible constraints on values of partition coefficients are $p_{g*} < p_{gl} < p_{gf} < p_{go} (i = l,r,s,b)$ where p_{g*} and p_{go} are the water/gas and oil/gas partition coefficients, respectively. Using the preferred values (Table I) and source-specific uncertainty factors as discussed above to define the log-normal distributions, the value of p_{gf} will be larger than the value of 1917 estimated for p_{go} by Sato and Nakajima [8], with probably about 20%. Similarly, in sampling from log-normal distributions for the partition coefficients, values of other tissue/gas partition coefficients were occasionally larger than values of the fat/gas coefficient. (Other suggested constraints were violated with very low probability.) Consequently, the distribution of the fat/gas partition coefficient was truncated at 1917, and other tissue/gas coefficients were truncated at the value of the fat/gas coefficient. These adjustments represent a significant alteration of both the shape and the variance of the distribution representing uncertainty of the fat/gas partition coefficient, and relatively slight alteration of the distributions for other tissue/gas partition coefficients.

Compartment volumes

For each species, uncertainty regarding compartment proportions of total body volume is expressed using the Dirichlet distribution. For a set of random complementary proportions, the Dirichlet distribution function can be expressed in terms of the expected proportions and a parameter θ which determines the variances and co-variances of the random proportions [9]. Consequently, it is convenient to equate the preferred proportions $v_{\kappa P}$ $(i = l,r,s,f)$ given in Table I to the expectations of the corresponding random proportions v_{κ} $(i = l,r,s,f)$. The value chosen for θ expresses the uncertainty regarding the joint distribution of the proportions; more specifically variance of v_{κ} is given by:

$$\mathrm{Var}[v_{\kappa}] = v_{\kappa P} \times (1 - v_{\kappa P})/(\theta + 1),\ i = l,r,s,f. \qquad (1)$$

Fixing the variance of one compartment proportion determines θ. Variation in liver volume had been used because preliminary analysis revealed that, for the 3 dose surrogates studied and for inhalation exposure, the model is roughly equally sensitive to all compartment volumes (for the AUCL and AUCA surrogates) or more sensitive to the liver volume than to other volumes (for the CML surrogate). Also, published information is easily incorporated since the compartment is identified unambiguously with a specific organ.

On the basis of liver volume measurements for mice reported in Arms and Travis [10], the preferred value of v_{l*} and θ for mice are estimated to be 0.056 and 1456, respectively. Volume proportions for other compartments are taken from Reitz and Nolan [5], adjusted for the change in v_{l*}.

For humans, the parameter value of v_k (0.026) is also taken from Arms and Trav [10] and other volume proportions from Reitz and Nolan [5], adjusted, again, for t difference between v_k values from the two sources. For θ, individual variation shou be considered (as discussed above). Data on individual variation in liver volume humans (as a proportion of body weight) is not available. Caster et al. [11] repc variances of v_k estimates for individual rats, which we assume to express plausit levels of individual variation for humans. Using value from Caster et al. [11] in Equ tion (1), the solution for θ is 1621. We tentatively adopt this value to represent unce tainty for humans, noting, however, that individual differences do not account f total uncertainty regarding compartment volumes, so that some under-estimate uncertainty is possible.

Circulatory and ventilatory parameters

The parameters defining the compartment proportions of body volume, cardi output (q_c), ventilatory output (q_p), and perfusion rates of specific compartments ($i = l,r,s,f$) are functionally interrelated. An account presented here demonstrates t sort of physiological considerations that are relevant, along with possibilities f quantifying the way that these considerations may determine uncertainty. Data r garding circulatory and ventilatory parameters for rodents and humans have be summarized by Arms and Travis [10].

Distributions for q_c, q_p, and the q_i's are derived as follows:

(1) Given a v_k value sampled from the Dirichlet distribution and the correspondi absolute compartment volume (v_i), q_i (the absolute compartment perfusion rate) given by $q_i = (v_i \times q_{cP} \times q_{cP})/v_{iP}$ where q_{cP} is the preferred proportion of total cardi output directed to compartment i [10] and q_{cP} is the preferred total cardiac outpu The total cardiac output is then given by $q_c = q_l + q_r + q_s + q_f$. These values of tot and compartment-specific perfusion are assumed to hold for sleeping humans ar for rodents during all hours.

(2) For humans, sleeping and waking values of cardiac output and alveolar ventil tion rate are desired. The 'waking' value (q_{cw}) is computed by $q_{cw} = q_c + (q_{cwP} - q_{cP}) \times edq_c$, where q_c is the 'sleeping' value computed in step 1, q_{cP} and q_{cwP} are preferred values for sleeping and waking individuals, and edq_c is distributed log-normally with preferred value 1 and uncertainty factor to be specified. The increment to total cardiac volume is assumed to be directed entirely to the slowly perfused compartment. Preferred values of waking and sleeping cardiac output and alveolar ventilation rate are derived from Hattis et al. [4].

An uncertainty factor for edq_c is also based on the data presented in Hattis et al. [4]. An arbitrarily low probability (0.001) is assigned to an increment in q_c values ($q_{cw} - q_c$) as large as the difference between values for sleeping and light exercise (234 l/h). Using this difference, the assumed difference in preferred values ($q_{cwP} - q_{cP} = 138$), the log-normal distribution assumed for edq_c, and the relationship be-

tween the GSD of the distribution and the associated UF, the uncertainty factor of 1.3 is obtained.

(3) Ventilatory volume (q_p) is assumed to be related linearly to the cardiac volume (q_c). For humans, $q_p - q_{p,P} = [(q_{p,a} - q_{p,P})/(q_{c,a} - q_{c,P})] \times (q_c - q_{c,P})$. The same relationship holds for waking values, $q_{p,a}$ and $q_{c,a}$. For mice, an analogous treatment requires specification of a second q_c–q_p coordinate (to replace $q_{c,P}$ and $q_{p,P}$), in addition to the values representing ordinary activity levels. Data from anesthetized mice have been used to determine the second coordinate [10].

Metabolic constants

After scaling V_{max} estimates (to get $V_{max,c}$ values) and expressing K_m in comparable units, geometric means of the values reported in Hattis et al. [4] and Reitz and Nolan [5] are equated with the preferred values.

It is assumed here that the uncertainty regarding the metabolic parameters arises chiefly from different experimental approaches and assumptions applied in deriving estimates. It may be argued that an uncertainty factor based on a geometric standard error is an appropriate measure of uncertainty; the geometric standard error is related inversely to the sample size, representing formally the inverse relationship between uncertainty and additional information. However, such a formalization is based on the assumption that the available measurements are independent and identically distributed (i.i.d.). The estimates of metabolic constants are suspected to deviate strongly from these assumptions. A likely pattern of deviation from the i.i.d. case is for clusters of estimates based on similar procedures to assume similar values. Such a pattern can lead to too-small estimates of uncertainty. Here, some conservatism (implying greater uncertainty) is introduced by basing an uncertainty factor on the geometric standard deviation rather than the geometric standard error. In other words, uncertainty regarding the metabolic constants is represented by a $\log_e$-normal distribution having the same geometric standard deviation as the sample geometric standard deviation computed from the available estimates [4, 5].

A tentative uncertainty factor for $V_{max,c}$ is given by $\exp(2 \times 0.532) = 2.9$, for mice and humans, where 0.532 is a GSD derived as a weighted average of species-specific GSD values. Uncertainty for K_m is evaluated in a similar manner, resulting in an uncertainty factor of 13 for mice and humans. The representation of uncertainty with respect to these metabolic constants is considered problematic, especially because their estimation via optimization of the model fit to in vivo data is dependent on estimates for the other model parameters. The 'Discussion' (see below) elaborates on this point.

PROPAGATION OF PARAMETER UNCERTAINTIES

Procedure

Parameter distributions defined in the section on 'Parameter Uncertainty Evalua-

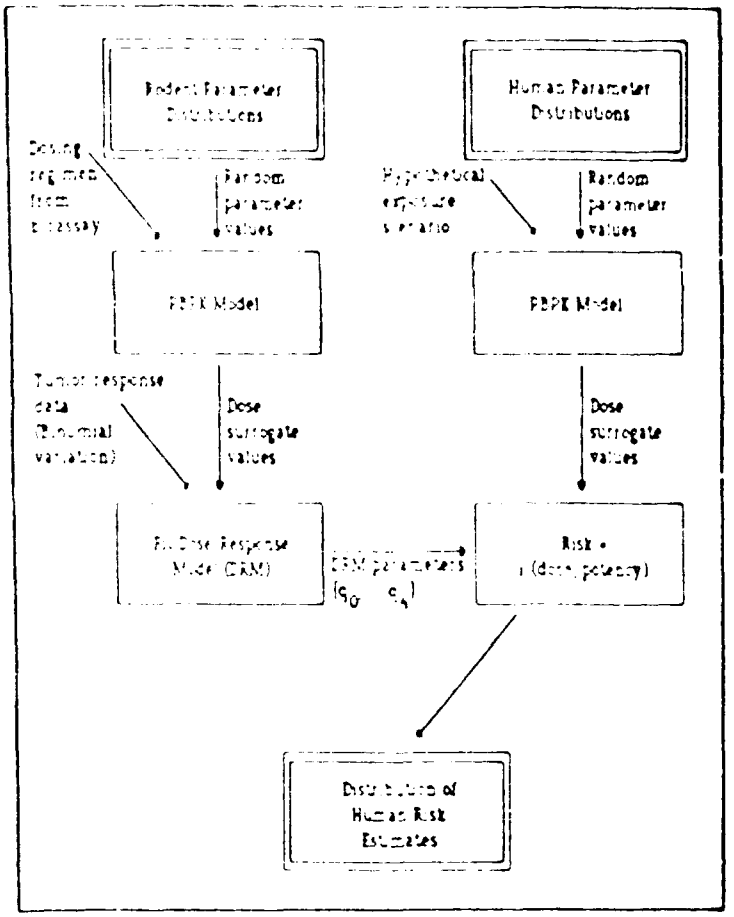

Fig. 2. Propagation of parameter uncertainties for PBPK model-based risk assessment.

tion' for mice and humans were sampled using the Latin-hypercube method [7, 12]. For each random selection of parameter values, the operations depicted in Figure 2 were performed to generate distributions of dose surrogate values and risk estimates.

In order to characterize uncertainty in the 3 dose surrogates (AUCL, AUCA, CML), 100 sets of parameters were generated and the PBPK model was run for each set. Mice were exposed as in the carcinogenicity bioassay via inhalation at 0, 100 and 200 ppm, for 6 hours per day in 5 consecutive days per week [2]. Humans were assumed to be exposed to 50 ppm (the current OSHA standard) for 8 hours per day in 5 consecutive days per week.

Uncertainty in dose surrogate values was propagated further to evaluate uncertainty in human risk estimates. A single tumor response, hepatocellular carcinoma, is considered here for illustrative purposes.

Surrogate dose values were related to the tumor response rates using a version of GLOBAL82 [13] to implement the multistage model. For the estimation of risk uncertainty, tumor response rates were also considered uncertain. For each of 100 randomly generated sets of dose surrogate values (one dose surrogate value per treatment group) the multistage model with a fixed number of stages was refitted, based on those dose surrogate values and random tumor counts for the treatment groups generated from a binomial distribution with parameters N_i and p_i. Here N_i is the number of animals in treatment group i (48 at 0 ppm, 50 at 100 and 200 ppm), while p_i is the estimated response proportion for dose group i obtained by fitting the multistage model to the observed response rates (1 at 0 ppm, 13 at 100 ppm, and 36 at 200 ppm) and the dose surrogate values corresponding to the preferred mouse parameter values.

Finally, a distribution of human maximum-likelihood and upper bound estimates of risk were generated by pairing each of the 100 sets of multistage model parameters with a randomly selected human dose surrogate value corresponding to the human exposure scenario of interest. Extra risk is defined by $R = (P(d) - P(0))/(1 - P(0))$, when $P(d)$ is the lifetime probability of observing a tumor given exposure that results in a surrogate dose value of d. No adjustments were made to the risk estimates obtained in this manner. Thus, for example, no adjustment was made to account for the somewhat different proportions of the human and mouse lifespans lived prior to first exposure.

RESULTS

Table II gives selected percentiles for the distributions of human dose surrogate values and extra risks. Median surrogate values are approximately equal to the dose surrogate values computed using preferred parameter values, which are 23.9 for AUCA, 118 for AUCL, and 27.8 for CML.

Median risk estimates vary substantially among the 3 surrogate doses. For comparison, consider the risks estimated in the 'traditional' manner, using applied doses with no pharmacokinetic transformations (Table III). Maximum-likelihood risk estimates (MLE) obtained without using a PBPK model (assuming mice and humans are equally susceptible to doses expressed in mg/kg body wt. per day) are higher than maximum likelihood estimates based on metabolite in liver, but lower than MLEs based on parent concentrations. For tetrachloroethylene, it appears that the structural uncertainty associated with the selection of an appropriate dose metric for cross-species extrapolation is of relatively greater importance than is the uncertainty associated with the values of the PBPK model parameters.

Also shown in Table III are the risk estimates obtained by using the preferred mouse and human PBPK parameters to estimate risk (i.e., the risk estimates that would be obtained if the parameter values were known without uncertainty to be equal to the preferred values). Except for the estimates based on CML, the median

TABLE II

SELECTED PERCENTILES FROM THE SIMULATED DISTRIBUTIONS OF HUMAN DOSE SURROGATE AND RISK VALUES (BASED ON 100 LATIN-HYPERCUBE SAMPLES)

Surrogate	Percentiles				
	2.5%	25%	50%	75%	97.5%
Dose surrogates					
AUCA (average daily area under the arterial concentration curve, mg h^{-1} l^{-1})	12.1	18.9	24.4	30.3	43.8
AUCL (average daily area under the liver concentration curve, mg h^{-1} l^{-1})	13.1	54.9	116	243	1060
CML (average daily amount metabolized per liver volume, mg/l)	2.24	13.9	27.8	51.8	117
Maximum likelihood estimates (MLEs) for extra risk[a]					
AUCA	0.0275	0.0910	0.164	0.243	0.494
AUCL	0.0391	0.244	0.480	0.700	0.982
CML	3.65E-9	3.73E-5	8.50E-4	7.06E-3	0.0495

[a]Extra risk is $(P(d) - P(0))/(1 - P(0))$, where $P(d)$ is the lifetime probability of tumor when exposed to dose d. Here d corresponds to an 8 h/d, 5 d/w exposure to 50 ppm.

TABLE III

RISK ESTIMATES[a] OBTAINED WITHOUT USE OF A PBPK MODEL, AND USING THE PBPK MODEL WITH NO UNCERTAINTY

Analysis	Risk estimates	
	MLE	Upper bound
No PBPK transformation[b]	5.57×10^{-3}	4.28×10^{-2}
PBPK transformation with no uncertainty		
AUCA	0.126	0.238
AUCL	0.425	0.506
CML	1.95×10^{-5}	7.00×10^{-3}

[a]Extra risks for 8 h/d, 5 d/w exposures to 50 ppm.

[b]Humans and mice are assumed to be equally susceptible to tetrachloroethylene when administered doses are expressed in mg kg^{-1}-d^{-1}.

of the simulated risk estimates is close to the risk estimate obtained with no uncertainty. In the case of CML, risks tend to be skewed to the right in comparison with the estimate corresponding to the preferred parameter values. This is a result of the

fact that the linear term of the multistage model fit to the preferred-value dose and response data is zero. Alteration of CML doses and response rates introduced in the course of the uncertainty analysis cannot decrease the linear term and this would tend to increase the risk estimates at the relatively low dose corresponding to the human exposure scenario. With the AUCL and AUCA surrogates, the linear terms are positive when the multistage model is fit to the observed response rates and doses corresponding to the preferred parameter values.

DISCUSSION

Evaluations of the carcinogenicity of tetrachloroethylene have implicated a metabolite as the moiety responsible for the tumorigenic responses following exposure [14]. The high degree of non-linearity between the CML dose surrogate and the hepatocellular carcinoma response rates in female mice (as discussed in the preceding section) is interesting in that light. Either there are non-linear steps, in addition to the saturable metabolic transformation, leading to the interaction that initiates the carcinomas (i.e., the surrogate represented by CML is not close enough to the events causing cancer to yield linearity between that measure of dose and the response), or the PBPK model is not adequate for describing tetrachloroethylene pharmacokinetics. We are continuing investigation of tetrachloroethylene carcinogenicity in hopes of elucidating the substance and the mechanisms responsible. This may entail the development of alternative means of characterizing and calculating the risks.

The approach to uncertainty analysis that has been illustrated in the context of tetrachloroethylene risk assessment has several features that are essential for an appropriate uncertainty assessment. First, the uncertainties related to the PBPK model parameters are formally expressed in terms of probability distributions that can incorporate multiple levels (or sources) of uncertainty. The parameter uncertainty distributions are based on the inspection and analysis of relevant data, sometimes in combination with the application of more subjective evaluations (or those based on expert opinion) of reasonable bounds for particular parameter values. Second, the parameters are not, in general, regarded as being independent of one another. The implied correlation structures (as, for example, among the volumes of the compartments) are modeled using multivariate distributions. Moreover, certain assumed functional relationships between the parameters have been maintained, even though the magnitudes of those relationships (the ratio of cardiac and pulmonary rates, for example) are subject to uncertainty. Finally, the approach employs an efficient technique (Monte Carlo stimulation using the Latin-hypercube procedure) to make explicit the relationships between parameter uncertainties and uncertainties in dose surrogate values and risk estimates.

By far the most difficult aspect of this approach is the characterization of the uncertainties in the parameters. This requires extensive review of the literature and attention to the different sources of uncertainty that are inherent in the observed varia-

tions in measurements. It is appropriate to stress that the estimates of uncertainty derived here are tentative. First of all, as illustrated by the measurement of individual variation in liver volume as a proportion of body weight, various of the GSD estimates are not based on data that are directly relevant, but on data from contexts that are considered more or less analogous to the context of immediate interest. Moreover, the variations identified with specific sources of uncertainty may not reflect only those sources of uncertainty. For example, individual variation will contribute to observed variation between mean measurements reported by different laboratories, particularly if the number of animals per mean is small.

Certain parameters are difficult to measure and equally difficult to characterize with respect to their uncertainty. When parameter estimates are based upon data collected in vivo, as is the case with metabolic constants, the values obtained are determined in part by the values assumed for other physiological parameters, as well as by the structural assumptions underlying the PBPK model. Also, uncertainties in estimates of parameters fitted simultaneously by an optimization program are not independent, because the fit of the model to the data is determined jointly by all of the fitted parameters. Information on the correlation among estimates of such parameters is necessary for a complete treatment of their uncertainty. This information is not currently available, and so, for example, V_{max} and K_m are treated as though they were independently determined. Because of these difficulties, the evaluation of uncertainty for metabolic constants could be improved by techniques that account for the uncertainty in the other parameters and that simultaneously estimated uncertainty for V_{max} and K_m.

Other refinements to the characterization of uncertainties in PBPK model parameters are possible. *In particular, the use of variance components analysis to derive* more pure estimates of source-specific uncertainties is of interest.

The results of the analysis, the distribution of surrogate doses and of risk estimates, have several potential uses. The distribution of simulated risk estimates can be interpreted as follows, keeping in mind the fact that, precisely because of uncertainty with respect to the parameter values and individual variation, consideration of a single risk estimate is not adequate. The proportion (P) of the simulated estimates that fall in an interval (I) may be interpreted as the probability that the true risk for a randomly selected individual is in I, when the uncertainties are taken into account. This interpretation should be useful in risk management decision-making contexts. One major advantage of this type of analysis (i.e., risk assessment that uses PBPK modeling and considers input uncertainties) is that reasonable variations in risk estimates become explicit. Traditionally, using administered doses, the uncertainties associated with the point estimates of risk have not been emphasized.

The distribution of the dose surrogates and the risk estimates result from uncertainties concerning true (average) values as well as variation around those averages. The uncertainty regarding the true values is theoretically reducible via further experimentation. The approach illustrated here can assist in directing the efforts to reduce

uncertainty. If, for example, a set of parameters is fixed at an assumed average value, the reduction in the spread of the risk estimates, below that obtained when all parameters are allowed to vary, provides an indication of the potential value of additional information with respect to that set of parameters. Such analyses take into account both sensitivity of model output to the values assumed for the parameters, and the current level of uncertainty regarding the parameters.

The approach to uncertainty analysis presented here has been illustrated for a simple PBPK model for tetrachloroethylene. It is applicable to PBPK models of greater complexity as well. Some of the parameters of those models – such as partition coefficients, compartment volumes, and flow rates – are identical to those in the tetrachloroethylene model used herein. Thus, for those parameters, the ground work has been laid here for their analysis. In general, the considerations described here are meaningful and useful for any analysis of PBPK model-based risk assessment.

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VOLUME VI

SENSITIVITY/UNCERTAINTY ANALYSIS

PART 2 OF 2 PARTS

CONTRIBUTIONS OF INDIVIDUAL PARAMETERS TO OVERALL UNCERTAINTY

A. INTRODUCTION

In Part 1 of this volume, a sensitivity analysis for individual parameters of a simple PERC PBPK model was completed. Appendix VI-1-A presented an uncertainty analysis for that same model. The uncertainty analysis built upon the discussion in Part 1 concerning parameter uncertainties. The uncertainty analysis also presented results in terms of distributions of output variables, in particular distributions of risk estimates.

In this part of the volume, one other set of results is presented. Those results also apply to the uncertainty analysis and again focus primarily on the distribution of output variables. In this case, however, the analysis highlights the contributions of individual parameters or sets of interrelated parameters.

The goal of the analysis reported here was to determine which parameters contributed most significantly to the variability in the dose surrogate or risk estimates. A variable can contribute to output uncertainty through a combination of 1) the sensitivity of the models to the output, and 2) the degree of uncertainty (variability) associated with the parameter estimate. A model is said to be sensitive to a parameter if relatively small changes in the parameter values result in relatively large changes in model output values. As stated in Section 1 of Part 1 of this volume, a parameter with a high degree of uncertainty associated with it does not contribute greatly to output uncertainty if the model is relatively insensitive to that parameter. Conversely, a parameter to which the model is highly sensitive may contribute

substantially to the output uncertainty even if the uncertainty associated with the parameter is relatively minor.

B. METHODS

The technique that was used to measure the contribution of individual parameters involved estimation of the variance of the output (dose surrogate or risk estimates). The variance for a sample of N values (x_1, x_2, ..., x_N) is defined as

$$(1) \qquad V = \sum(x_i - \mu)/(N-1)$$

$$(2) \qquad \mu = \sum x_i/N$$

where the sums in each equation are over all values of i (= 1, 2, ..., N).

Let V_T denote the variance associated with the output variable values when all parameters were allowed to vary according to their uncertainty distributions. Similarly, let V_i denote the variance associated with the output variable values when parameter i (or parameter set i) was allowed to vary, but all other parameters were fixed. Then

$$(3) \qquad R_i = V_i/V_T$$

is a representation of the contribution of parameter i (or parameter set i) to the overall uncertainty associated with the output variable values. When R_i is small, parameter i has a small impact on uncertainty in the output

variable. Larger values of R_i indicate greater impact on the variability of the output variable.

The representation characterized by the R_i values combined two factors. One was the amount of variation allowed for a parameter, as represented by its uncertainty distribution. Clearly, if a parameter was assumed to be known with certainty (i.e., the uncertainty distribution allowed for no variability), then R_i for that parameter would be zero (since V_i would be zero). The more uncertain a parameter value is (i.e., the greater the variation allowed by its associated uncertainty distribution) the greater the chance that the R_i for that parameter will be larger.

However, the other factor that affected the values of R_i influenced the degree to which increasing parameter uncertainty is manifested in increased output variable uncertainty. The second factor was the sensitivity of the model to the parameter under consideration. Thus, a parameter to which the PBPK model is relatively insensitive will probably have a small R_i value, even if the uncertainty associated with that parameter is very large. Sensitivity for the perchloroethylene PBPK model was addressed in detail in Part 1.

C. RESULTS

Tables VI-2-1 and VI-2-2 display results that extend the analysis of the perchloroethylene example presented in Appendix VI-1-A. Those tables show the R_i values for each parameter or set of parameters when the output variable was either a dose surrogate estimate or a risk estimate. The dose surrogates examined include those discussed in Part 1 of this volume and Appendix VI-1-A.

In one instance (the contribution of uncertainty in pla to uncertainty in the estimation of human AUCL; Table VI-2-1), the variability in the output found when a single parameter was varied exceeded that observed when all parameters varied. This result may have arisen as follows. When all parameters were allowed to vary, the random matching of sampled parameter values may have moderated some of the extreme AUCL values produced when pla alone varied. That would tend to decrease the variability; i.e., AUCL variance associated with uncertainty in the pla parameter would be smaller than that associated with uncertainty in all the parameters, as observed. This result will be examined further.

The contributions of all of the individual parameters or sets of parameters to risk uncertainty were small when virtual concentration of metabolite was used as the dose surrogate (Table VI-2-2). This can be seen by comparing values in the three columns of Table VI-2-2. This result was accompanied by a shift in the median risk estimates; i.e., the median risk estimate when all parameters were allowed to vary was higher than the risk estimate medians observed when individual parameters were allowed to vary.

This result could have arisen in the following way. The original fit of the multistage model (using the observed response rates and the preferred PBPK parameter values) estimated that the linear term of the model was zero. Changes in parameter values affect risk estimates by altering multistage model parameter estimates, but such changes could never decrease the linear term. Consequently, risk estimates would tend to be increased, especially at low doses where the linear term dominates. Thus, when all parameters were allowed to vary, the linear term tended to be increased more often than not because of the overall variability. However, when individual parameters were varied, the

variability of the dose surrogate estimates was not so great, inducing a smaller change in risk estimates (i.e., tending not to increase the linear term more often than not). This would cause a shift in the median risk estimates (smaller median values, more similar to that obtained with the original fit of the multistage model) when single parameters varied, and may also have resulted in less variability associated with the risk estimates.

A similar phenomenon was not observed and would not be expected for risk estimates based on the other two dose surrogates. This is because, in those cases, the linear term was positive when the multistage model was fit to the observed responses using the preferred PBPK model parameters. Thus, changes in the parameters (either one at a time or all together) could induce both increases or decreases in the linear term, tending to keep the median close to that observed with the original fit.

Despite the fact that the above arguments can explain the pattern of risk estimates observed for risks based on the metabolite virtual concentration, further investigation of this issue will be carried out.

D. DISCUSSION

The contributions of the individual parameters to the output uncertainties matched fairly closely the pattern observed when the model was examined with respect to sensitivity (Part 1 of this volume). Contribution to dose surrogate uncertainty corresponded extremely well with contribution to risk estimate uncertainty.

For the AUCL dose surrogate, the model was particularly sensitive to pla and pb. Those two parameters contributed most highly to the AUCL dose

surrogate uncertainty (Table VI-2-1). This was true for the human pb and pla values as well as for the mouse pb and pla values.

For the dose surrogate based on virtual concentration of metabolite, the response rates contributed most highly to the risk uncertainty. The metabolic constants were the most significant contributors to risk uncertainty of all the PBPK model parameters. Because of the relatively low atmospheric concentrations to which the mice were exposed (100 and 200 ppm), the mouse km parameter contributed more to risk uncertainty than did the mouse vmaxc parameter. Model predictions of virtual concentration of metabolite were found to be very sensitive to the value of the liver volume parameter (Part 1 of this volume). That sensitivity is reflected in the relatively large contribution of uncertainty in all volumes (varied together because of their interrelationships) to dose surrogate and risk uncertainty, when based on metabolite virtual concentration.

The dose surrogate AUCA was most sensitive to the blood/air partition coefficient. This parameter also contributed very significantly to uncertainty in the risk estimates. The contributions of uncertainties with respect to response rates, mouse vmaxc, and mouse km were also substantial for the risks based on the AUCA dose surrogate. The contributions of all other parameters were considerably less.

Some parameters were consistently unimportant in determining output uncertainties. Those parameters included the partition coefficients for the fat, slowly perfused, and richly perfused compartments. Of those partition coefficients, the one for the slowly perfused compartment contributed to output uncertainty more than the other two. The parameter describing the

relationship between the waking and sleeping cardiac output rates in humans (dqc) was also consistently unimportant for determining output uncertainty.

Table VI-2-1

Contributions of Individual Parameters
to Uncertainty in Dose Surrogate Estimates[a]

Parameter	Surrogate[b] AUCL	Virtual Concentration of Metabolite	AUCA
Vmaxc	4.6E-2 (4)	19 (2)	4.7E-1 (3)
Km	6.0E-2 (3)	22 (1)	7.0E-1 (2)
pb	3.8 (2)	4.1 (3)	97 (1)
pla	122 (1)	5.0E-2 (7)	7.0E-2 (6)
pra	1.8E-3 (7)	5.1E-2 (6)	6.8E-2 (8)
psa	1.1E-2 (5)	4.8E-1 (5)	3.4E-1 (4)
pfa	1.7E-3 (8)	4.0E-2 (9)	6.9E-2 (2)
Volumes[c]	1.5E-3 (9)	1.7 (4)	6.1E-2 (9)
dqc[d]	2.5E-3 (6)	4.6E-2 (8)	8.1E-2 (5)

[a] Presented are values of Ri*100 (see text for definition) and, in parentheses, a rank for the parameter. A rank of 1 indicates largest Ri. These are for the human PERC model with a 50 ppm, 8 hour/day, 5 day/week exposure scenario.

[b] The dose surrogates are average daily area under the liver concentration curve (AUCL), average daily amount metabolized per liver volume (virtual concentration of metabolite), and average daily area under the arterial blood concentration curve (AUCA).

[c] The volumes are correlated (they vary together according to a Dirichlet distribution) and so their contribution to output uncertainty constitutes one entry.

[d] The parameter defining the relationship between resting and active cardiac output rates. See Part 1 of this volume.

Table VI-2-2

Contributions of Individual Parameters
to Uncertainty in Risk Estimates[a]

Parameter	Surrogate[b] AUCL	Virtual Concentration of Metabolite	AUCA
Human Vmaxc	2.0E-1 (10)	1.3E-3 (3)	1.3E-1 (7)
Human Km	2.6E-1 (9)	6.0E-4 (5)	1.9E-1 (6)
Human pb	13 (2)	4.6E-5 (6)	32 (1)
Human pla	88 (1)	3.3E-7 (12)	2.0E-2 (15)
Human pra	7.5E-3 (16)	3.2E-7 (13)	1.9E-2 (17)
Human psa	4.6E-2 (14)	3.8E-6 (10)	9.7E-2 (13)
Human pfa	7.4E-3 (17)	2.5E-7 (16)	1.9E-2 (16)
Human volume[c]	6.1E-3 (18)	1.5E-5 (7)	1.7E-2 (18)
Human dqc[d]	1.0E-2 (15)	2.9E-7 (14)	2.3E-2 (14)
Mouse Vmaxc	8.8 (5)	7.7E-4 (4)	9.1 (4)
Mouse Km	1.0 (7)	1.7E-2 (2)	2.3 (5)
Mouse pb	13 (3)	5.2E-6 (8)	26 (2)
Mouse pla	13 (4)	1.4E-8 (18)	1.0E-1 (10)
Mouse pra	7.6E-2 (13)	1.9E-8 (17)	1.0E-1 (10)
Mouse psa	9.9E-1 (8)	2.8E-7 (15)	1.2E-1 (8)
Mouse pfa	1.0E-1 (11)	6.9E-7 (11)	1.1E-1 (9)
Mouse volumes[c]	8.0E-2 (12)	3.9E-6 (9)	1.0E-1 (10)
Response rates[e]	3.0 (6)	5.0 (1)	11 (3)

[a] Presented are values of R_1*100 (see text for definition) and, in parentheses, a rank for the parameter. A rank of 1 indicates the largest R_1. Risks were derived for a 50 ppm, 8 hour/day, 5 day/week human exposure scenario using the female mouse results discussed in Appendix VI-1-A.

[b] The dose surrogates are average daily area under the liver concentration curve (AUCL), average daily amount metabolized per liver volume (virtual concentration of metabolite), and average daily area under the arterial blood concentration curve (AUCA).

[c] The volumes are correlated (they vary together according to a Dirichlet distribution) and so their contribution to output uncertainty constitutes one entry.

[d] The parameter defining the relationship between resting and active cardiac output rates. See Part 1 of this volume.

[e] The bioassay response rates were allowed to vary according to a binomial distribution with probability of response defined by the multistage model fit to observed response rates with doses defined by preferred values of PBPK model parameters.

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