APPENDIX A
LIST OF REVIEWERS AND OBSERVERS

Panel Peer Review of the Draft NCEA Document "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

Arlington, VA
November 10-11, 2005

List of Participants

Gregory M. Blumenthal, Ph.D.
GMB Consulting
Chapel Hill, NC

James V. Bruckner, Ph.D.
University of Georgia
Athens, GA

Janusz Z. Byczkowski, Ph.D., D.Sc., D.A.B.T.
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CIIT Centers for Health Research
RTP, NC

Gary L. Ginsberg, Ph.D.
Connecticut Department of Public Health
Hartford CT, 06134

Panel Peer Review of the Draft NCEA Document "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

November 10-11, 2005

Observer Registration		
Name	**Organization**	**Nov. 10**
Tammie Covington	ENVIRON International Corp	Yes
George Cruzan	ToxWorks	Yes
Rob DeWoskin	US EPA/NCEA	Yes
Paul Dugard	HSIA	Yes
Jane Eickhoff	TOXCEL LLC	Yes
Kate Z. Guyton	US EPA	Yes
C. Eric Hack	TERA	Yes
Douglas Johns	US EPA/NCEA	Yes
Russ Keenan	AMEC Earth & Environmental, Inc.	Yes
Trevor Knowblich	Inside EPA News	Yes
Thomas McDonald	Arysta LifeScience North America Corporation	Yes
Robert McGaughy	US EPA/NCEA	Yes
Gary Mihlan	Bayer CropScience	Yes
Beth E. Mileson	Technology Sciences Group, Inc.	Yes
Pat Phibbs	BNA, Inc.	Yes
Micah Reynolds	TOXCEL LLC	Yes
Bob Sonawane	US EPA/NCEA	Yes
Chadwick Thompson	US EPA/NCEA	Yes
David Bottimore	Versar, Inc.	Yes
Amanda Jacob	Versar, Inc.	Yes

APPENDIX B
AGENDA

United States
Environmental Protection Agency

Panel Peer Review of the Draft NCEA Document "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

Hyatt Arlington
1325 Wilson Boulevard
Arlington, VA 22209

Agenda

THURSDAY, NOVEMBER 10, 2005

8:30AM	**Registration Begins**
9:00AM	**Welcome, Goals of Meeting, and Introductions** David Bottimore, Versar, Inc.
9:10AM	**Welcome** Bob Sonawane, EPA/NCEA
9:15AM	**Background on "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"** Chad Thompson, EPA/NCEA
9:30AM	**Chair's Introduction and Review of Charge** Gary Ginsberg, Chair
9:40AM	**Reviewer Roundtable of Overview Comments** Gary Ginsberg, Chair
10:00AM	**Observer Comment Period**
10:20AM	**Discussion Session and Responses to Charge Questions (with break as appropriate)**
12:00PM	Lunch
1:00PM	**Discussion Session and Responses to Charge Questions (continues, with break as appropriate)**
4:15PM	**Discussion of Public Comments**
4:30PM	**Recap of Comments/Recommendations and Plans for Writing**
5:00 PM	**Adjourn**

United States
Environmental Protection Agency

Panel Peer Review of the Draft NCEA Document "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

Hyatt Arlington
1325 Wilson Boulevard
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Agenda

FRIDAY, NOVEMBER 11, 2005

9:00AM	**Welcome and Goals of Panel Writing Session** David Bottimore, Versar, Inc.
9:15AM	**Panel Writing Session** Gary Ginsberg, Chair
11:45AM	**Closing Remarks and Next Steps**
12:00PM	**Adjourn**

APPENDIX C
CHARGE QUESTIONS

Panel Peer Review of the Draft NCEA Document "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

November 10-11, 2005

External Peer Review Panel Workshop Charge Questions

Purpose of the Document:
The purpose of the draft document is to describe some approaches for the use of physiologically-based pharmacokinetic (PBPK) models in risk assessment. PBPK models represent an important class of dosimetry models that are useful for predicting internal dose at target organs for risk assessment applications. Dose-response relationships that appear unclear or confusing at the administered dose level can become more understandable when expressed on the basis of internal dose of the chemical. To predict internal dose level, PBPK models use pharmacokinetic data to construct mathematical representations of biological processes associated with the absorption, distribution, metabolism, and elimination of compounds. With the appropriate data, these models can be used to extrapolate across species and exposure scenarios, and address various sources of uncertainty in risk assessments. This report addresses the following questions: (1) Why do risk assessors need PBPK models; (2) How can these models be used in risk assessments; and (3) What are the characteristics of acceptable PBPK models for use in risk assessment?

Workshop Purpose:
The purpose of the workshop is to carry out an independent external peer review of the draft framework document entitled, "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment." Independent external experts in PBPK modeling and risk assessment have been invited as panelists to provide review and comment on the document, as well as address the particular charge questions (see below). Further, we ask that you consider and provide remarks on the public comments. We hope your feedback will improve the usefulness and clarity of the final document.

Questions:
1. What is the panel's overall view of the thoroughness, clarity, and applicability of this report?

2. Are the graphical examples explaining various concepts clear and helpful? If not, do you have suggestions for improving clarity?

3. Does this document reasonably describe the major potential uses and advantages of PBPK modeling in risk assessment, are there risk assessment applications of PBPK modeling that have not been addressed?

4. Are there improvements to the document that would substantially help risk assessors who are less familiar with PBPK modeling better understand the potential strengths and limitations of PBPK modeling?

5. Do you think that PBPK modelers outside the EPA, and those less familiar with risk assessment practices, would find this document useful as far as fostering the kinds of research and model development useful for risk assessment?

6. Are there current research needs and data gaps not highlighted in this report that would improve the utilization of PBPK models in risk assessment?

7. Are there future reports that you could envision which would compliment or expand upon the topics covered in the current document? For example, would a report focused on dosimetry models for reactive gases be helpful? Or a report focused on extrapolation across life stages using PBPK modeling?

8. Do you have additional comments/suggestions that were not covered in the above questions?

APPENDIX D
POWERPOINT PRESENTATIONS

David Bottimore
Welcome, Goals of Meeting, and Introductions

Chad Thompson, EPA/NCEA
Background on "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

APPENDIX E
WRITTEN COMMENTS FROM REVIEWERS

PRE-MEETING COMMENTS SUMMARY REPORT

Panel Peer Review of the Draft NCEA Document "Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment"

Prepared for:
U.S. Environmental Protection Agency
National Center for Environmental Assessment
808 17th Street, N.W.
Washington, DC 20074

Prepared by:
Versar, Inc.
6850 Versar Center
Springfield, Virginia 22151

Contract No. C68-C02-061
Task Order 89

Reviewers:
Gregory M. Blumenthal, Ph.D.
James V. Bruckner, Ph.D.
Janusz Z. Byczkowski, Ph.D.,D.Sc.,D.A.B.T.
Harvey J. Clewell
Gary L. Ginsberg, Ph.D.

November 9, 2005

Specific Comments Section Revised with Additions on November 16, 2005

TABLE OF CONTENTS

I. INTRODUCTION

The objective of this Task Order is to perform a panel peer review of the draft EPA document, developed by the National Center for Environmental Assessment (NCEA), entitled "Approaches for the Application of Physiologically Based Pharmacokinetic Data and Models in Risk Assessment." Pharmacokinetics is the study of the biological processes that affect the absorption, distribution, metabolism and excretion of a substance, such as a drug or toxicant. Pharmacokinetic data and models have important applications in risk assessment. Given sufficient physiological and pharmacokinetic data, physiologically based pharmacokinetic (PBPK) models, which mathematically represent pharmacokinetic processes based on known biological properties, can be developed. Such models can then predict an internal dose (generally blood level or target tissue level) that would result from different exposure regimens or in different species. Pharmacokinetic data and PBPK models can also support quantitative estimates of intraspecies internal dose variability. Examples include the variability in physiological values (e.g., tissue volumes or fluid flows), or in metabolic capacity associated with the presence of enzyme polymorphisms. Predicted internal dosimetry from PBPK models can also be used with benchmark dose or other empirical approaches for dose-response analysis.

The objective of this document is to provide a description of approaches for using PBPK data and models in human health risk assessment. The document primarily focuses on the evaluation and use of these models in predicting internal doses at target organs for risk assessment applications, based on EPA guidelines. The document assumes that risk assessors are familiar with the basic concepts of PBPK modeling, and that model developers are familiar with basic concepts of risk assessment. Hence, exhaustive descriptions of PBPK modeling and/or risk assessment methods are not presented. However, brief descriptions of both disciplines, as well as appropriate references to secondary review articles and reports, are included in the document.

II. CHARGE TO THE PEER REVIEWERS

Purpose of the Document:
The purpose of the draft document is to describe some approaches for the use of physiologically-based pharmacokinetic (PBPK) models in risk assessment. PBPK models represent an important class of dosimetry models that are useful for predicting internal dose at target organs for risk assessment applications. Dose-response relationships that appear unclear or confusing at the administered dose level can become more understandable when expressed on the basis of internal dose of the chemical. To predict internal dose level, PBPK models use pharmacokinetic data to construct mathematical representations of biological processes associated with the absorption, distribution, metabolism, and elimination of compounds. With the appropriate data, these models can be used to extrapolate across species and exposure scenarios, and address various sources of uncertainty in risk assessments. This report addresses the following questions: (1) Why do risk assessors need PBPK models; (2) How can these models be used in risk assessments; and (3) What are the characteristics of acceptable PBPK models for use in risk assessment?

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6. Are there current research needs and data gaps not highlighted in this report that would improve the utilization of PBPK models in risk assessment?

7. Are there future reports that you could envision which would compliment or expand upon the topics covered in the current document? For example, would a report focused on dosimetry models for reactive gases be helpful? Or a report focused on extrapolation across life stages using PBPK modeling?

8. Do you have additional comments/suggestions that were not covered in the above questions?

III. GENERAL COMMENTS

Gregory M. Blumenthal
The report is relatively thorough and applicable, although it presupposes that the readers already have a good grasp of such pharmacokinetic concepts as “first-pass effect”; zero-order, first-order, and second-order metabolic processes; and metabolic saturation. The clarity of this report to EPA scientists and risk assessors without a thorough background in these pharmacokinetic concepts may be severely limited.

An additional minor improvement could be made by heavily emphasizing the term “dose metric”. This massively useful concept is the key to the applicability and utility of PBPK models in risk assessment and its importance cannot be overstated.

In general, the graphical examples are appropriate and useful. However, the authors should provide text for each figure, even if it repeats material in the main text.

Innovation in computational biology and its application to risk assessment is an ongoing process. This report should be envisioned as a living document, with room to expand as novel methods and applications arise. Key among the areas with explosive growth occurring are acute dosimetry, point-of-contact dosimetry, whole-life and developmental modeling, maternal-fetal dosimetry, and metabolic network modeling to identify sensitive subpopulations.

James V. Bruckner

Janusz Z. Byczkowski
The reviewed document “Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment” seems to be the first comprehensive publication that systematically reviews application of PBPK modeling in risk assessment. The authors of this document should be commanded for preparing thorough, clear and well organized report.

This document reads smoothly. It clearly states the scope and intent of the report in the introductory section. The PBPK model applications, issues and potential pitfalls are presented transparently and discussed appropriately. The “boxes”, tables and figures are easily readable and informative. The schematic diagrams are appropriate and well designed. The rest of the figures adequately illustrate the issues discussed in the text. However, because the mathematical equations in the text are kept to the absolute minimum, it would be informative if an example of the computer codes, for instance of the “classic” Ramsey and Andersen's PBPK model, was provided in an appendix to the document. The extent of literature review, supplemented by the list of all pertinent references in the Appendix 2, seems to be adequate and complete - up to the beginning of the year 2003. An exhaustive list of partition coefficients and metabolic constants for PBPK modeling of many classes of chemical compounds is provided in the Appendix 3.

While this document is addressed to risk assessors rather than pharmacokineticists, it contains many clarifications and suggestions that may be useful in developing both PBPK and

pharmacokinetic models in general. For example, the Appendices provide extremely useful for pharmacokineticist compilation of references and parameters.

A public discussion, initiated over the Internet, proved that there is a genuine interest in this report among the international experts from both fields - pharmacokinetics and risk analysis (involved were members from three mailing lists: PharmPK < http://www.boomer.org/pkin/ >, Risk Anal <http://lyris.pnl.gov/cgi-bin/lyris.pl?enter=riskanal&text_mode=0 >, and DRSG <http://www.sra.org/drsg/ >). The participants of this discussion emphasized that in addition to physiological modeling of pharmacokinetics, there is a need for "...pharmacodynamic and disease progress modelling to get the full picture..."

This is also this reviewer's belief, that although this document represents an opportunity for the Agency and a good beginning of the process to depart from defaults in order to base its chemical risk assessment paradigm on physiologically realistic and scientifically defensible methodology, much more work needs to be done. Particularly, linking the internal dosimetry with the effects of environmental contaminants represents a real challenge as it needs further research. Even though, the section 4.4 of this report attempts to address the issue of linkage of PBPK to pharmacodynamic models, it also demonstrates that still additional science is needed to be able to predict and quantify the health effects of many classes of chemical compounds.

If the U.S. EPA chose to continue exploring this trend, this reviewer would suggest that the future report by the Agency, perhaps entitled "Approaches for the Application of Physiologically Based Pharmacodynamic (PBPD) Models and Supporting Data in Risk Assessment", may be essential in order to stimulate research in the PBPD, to summarize the endeavor so far, and to support progress in this field.

Harvey J. Clewell

Gary L. Ginsberg

This is a well written document, primarily of use to those experienced in pharmacokinetics, PBPK modeling and risk assessment. It makes many cogent points, describing both general principles and special considerations needed to successfully develop, evaluate and apply PBPK models. It does not provide a great deal of detail in any area which makes the document easier to read cover to cover, and get an overview of the subject matter. However, the lack of details and specific case examples prevents this from being a PBPK "cookbook" which one could use as a stand alone resource to actually construct a PBPK analysis. One would already have to know about modeling software, model structure, mathematical relationships, optimization/backfitting techniques, evaluation techniques (e.g., residuals calculations, mass balance calculations), and probabilistic techniques (Monte Carlo, Bayesian) to really make use of this document. However, for those in the field or at EPA who are applying PBPK to risk assessment, this should be a helpful set of principles. To some extent, the document has the potential to standardize modeling practice, not in terms of which parameters to use, but in terms of basic approaches and applications.

The document focuses upon the use of PBPK modeling to address certain extrapolation issues in the setting of reference values and slope factors: cross-species, dose route, high dose to low dose,

temporal adjustments, and degree of variability. However, it doesn't provide anything of substance on several other uses of PBPK modeling: across age group extrapolations; in utero modeling; variability due to genetic polymorphisms, lactational models, etc. Additionally, the document does not put PBPK modeling into an overall context of pharmacokinetic analysis in which classical one and two compartment models have been used for many years to provide adequate description of chemical fate, and which have been applied to risk assessment (e.g., methyl mercury, dioxin).

The appendices are a useful compilation of equations that can be used to derive parameter inputs if empirical data are not available. It is also helpful to have parameter data sheets for a variety of xenobiotics. However, these parameter data sheets are not optimally useful because the model structure for which these values were derived is not specified. In some cases, references are provided so that the reader has a head start if he/she needs to develop a model for that analyte. However, in many cases references are not provided.

In spite of the aforementioned, the document does a good job of introducing and providing perspective on many important topics. In a number of cases, it doesn't go far enough. For example, it introduces the concept that PBPK modeling can be used to help interpret biomonitoring data. This is an emerging and essential application for PBPK modeling because it offers the opportunity to convert a urinary or blood biomarker level into an exposure dose from which key questions about population and individual risk may begin to be assessed. The document may be enhanced by a more complete description of this application including its methodological and interpretative issues.

IV. RESPONSE TO CHARGE QUESTIONS

1. What is the panel's overall view of the thoroughness, clarity, and applicability of this report?

Gregory M. Blumenthal
The report is relatively thorough and applicable, although it presupposes that the readers already have a good grasp of such pharmacokinetic concepts as "first-pass effect"; zero-order, first-order, and second-order metabolic processes; and metabolic saturation. The clarity of this report to EPA scientists and risk assessors without a thorough background in these pharmacokinetic concepts may be severely limited.

James V. Bruckner

Janusz Z. Byczkowski
From this reviewer's point of view, this is the first comprehensive publication that systematically reviews application of PBPK modeling in risk assessment. The authors of this document should be commanded for preparing thorough, clear and well organized report.

Harvey J. Clewell
In general, I was very impressed with the thoroughness and clarity of the report. It is well written, easy to read, and covers most of the important issues for applying PBPK modeling in risk assessment. There are, however, a few additional areas that should be discussed in the report:

- Sections 2.5 and 2.6 should include multiple references to the IPCS document on chemical specific adjustment factors (CSAFs) in order to clarify the relationship between the model applications discussed in these sections with the description of the CSAF approach in the IPCS document.

- Section 2.5.5 (p. 2-11) incorrectly states that "The IVF of 10 conventionally used in RfC dereivation implies that for the same level of response or nonresponse, the potential doses among individuals may differ by as much as – but not more than – an order of magnitude." This is a common misunderstanding. In fact, the IVF of 10 is associated with the potential ratio of the equitoxic doses for an average individual as compared to a sensitive individual. Therefore, an IVF of 10 is consistent of a range of equitoxic doses across the population of about two orders of magnitude.

- The first paragraph in Section 3.3 is incorrect: a mass-balance differential equation consists of a series of rate terms (not clearance terms), and the units are mass per time (not volume per time). These rate terms are often calculated as the product of a clearance (in units of volume per time) and a concentration (in units of mass per volume). Also, the uptake of a chemical in systemic circulation by a tissue is proportional to its activity gradient, where the tissue: blood relationship

of activities is related to the concentration difference by the partition coefficient. It just confuses things to mention Fick's law of diffusion since the transport is generally blood-flow limited, not diffusion limited.

- Section 3.5 should include a description of the process of internal verification of the model code. I disagree completely with the suggestion in the document that the risk assessor is not responsible for ensuring that the computer implementation of the model is free from error. Curiously, the summary (Section 3.9) actually includes considerations relevant to internal verification (in the second to last bullet) that are not mentioned at all in this section.

- Section 3.6 should include a discussion of the necessity of evaluating the model specifically for the dose metric that will be used in the risk assessment. For example, if the validation of the human model is based solely on parent chemical kinetic data, it may not be valid at all for a metabolism dose metric.

- Section 4.3 should include (perhaps between sub-sections 4.3.5 and 4.3.6, a much more detailed explanation of the alternative approaches for calculating a dose metric for a repeated exposure, as discussed in Clewell et al. (2002):

    - Calculation of total dose metric over entire study divided by length of study
    - Single dose estimate (total AUC for single dose adjusted for exposure frequency)
    - Steady-state estimate (subtraction of consecutive periods after steady state or periodicity is achieved)

Gary L. Ginsberg

For the most part, this is a clear presentation of how PBPK modeling can be used in cancer and non-cancer risk assessment, building a logical progression from basic uses and of PBPK modeling in risk assessment (Chapter 2) to the ingredients, design and implementation of PBPK models (Chapter 3), to evaluation of PBPK models once constructed (later in Chapter 3), and finally to brief case examples of PBPK model use in risk assessment (Chapter 4). The report provides especially useful sections on parameter estimation (Section 3.4), model evaluation (Section 3.6), constructing models in data-poor situations (Section 3.8). Any risk assessment document is likely to leave out some application or question. In the case of this document there are several important cases which are largely unexplored but which are topics of considerable interest to risk assessors: 1) Extrapolation across age groups and for in utero and lactational exposure; 2) Extrapolation to those with genetic polymorphisms; 3) Use of PBPK modeling for interpreting human biomonitoring data; 4) Use of simpler compartmental models to address risk assessment questions. Further, other topics are treated incompletely such as chemical mixtures (covered to some extent in Section 4.3.9) and how models differ for different types of xenobiotics (most of discussion is based upon non-reactive volatile organics; Figure 3-1 shows different models for different chemicals but doesn't explain whats going on).

While the document provides excellent background information and general principles, it is not detailed enough to constitute a cookbook or user's manual for PBPK modeling. As such, it is most useful for those already in the field to ensure consistency of approach. It is also perhaps a way for non-modeler risk assessors to become better able to understand and interpret the output

of PBPK models. On the last point, it may be very useful to include a section on "Interpreting the Output of PBPK Models." This could help the generalist and the modeler alike.

As described in the ES and Introduction, this document relies on experience with water-insoluble gases and some non-volatile organics. The explanation given is that PBPK modeling has thus far focused on these types of chemicals. There is a wide range of PBPK models from dioxins/PCBs to pesticides, to inorganics (metals, perchlorate) to VOCs. It would be good to start the document off with a broad rather than narrow description of PBPK uses thus far and provide descriptions of how PBPK modeling may differ for chemicals with very different properties (e.g., metals, bone or binding sites may be important to disposition; lipid binding and metabolism less important; lipophilic slowly metabolized organics (PCBs) – lipid partitioning important determinant of fate; perchlorate – transport via symporter, etc. etc.).

2. Are the graphical examples explaining various concepts clear and helpful? If not, do you have suggestions for improving clarity?

Gregory M. Blumenthal

In general, the graphical examples are appropriate and useful. However, several minor changes might be made to improve the clarity of some of the examples:

Figure 2-1 – Use bold type for the axis labels of the graphs in order to highlight the role of the dose metric (rate of amount metabolized, in this case) in linearizing the dose-response function.

Figure 2-2 – Shade the area under the curves to emphasize the AUC as dose metric.

Figure 3-1 – More closely associate each diagram (A, B, C, D) with its respective literature citation.

Figure 3-2 – Provide text for the figure, even if it repeats material in the main text.

Figure 3-3 – Provide text for the figure, even if it repeats material in the main text. Additionally, note that this is just an illustration and not real data from any particular model.

Figure 3-4 – Provide text for the figure, even if it repeats material in the main text. Additionally, some indication of how many Monte Carlo iterations are involved may be useful.

Figure 4-1 – If the parameters are not available, you want benchwork to precede model development, if at all possible.

Figure 4-2 – Use the phrase “dose metric” whenever possible.

Figure 4-4 – Shade the area under the curves to emphasize the AUC as dose metric.

Figure 4-5 – Shade the area under the curves to emphasize the AUC as dose metric.

Figure 4-6 – Replace the diagonally-oriented curly brackets for the interindividual variability factor with horizontally-oriented square brackets at the x-axis.

Figure 4-8 – Add a note that this is an illustration, not actual data from a specific chemical.

James V. Bruckner

Janusz Z. Byczkowski

The “boxes”, tables and figures are easily readable and informative. The schematic diagrams are appropriate and well designed. The rest of the figures adequately illustrate the issues discussed in the text.

Harvey J. Clewell

Figures 2-2 and 4-5 give a misleading illustration of a human equivalent exposure calculation or temporal adjustment. The comparison shown, which appears to accumulate the AUC over just 24 hours, would only be appropriate if the animals/subjects were exposed only for a single day and were sacrificed/tested at exactly 24 hours. The comparison that appears to be intended, i.e., adjustment to continuous exposure, should either continue to accumulate the AUC after the 24-hour exposure until the chemical is completely cleared, or should calculate an AUC for consecutive 24 hour time-points after steady-state is achieved.

Figure 3-2, panel B should show an inhalation profile of the correct duration rather than an oral profile. I would suggest a case where the end-exposure concentration is overpredicted by an order of magnitude and the predicted post-exposure concentrations decrease much too rapidly so they cross through the data.

Figure 3-3 should not include a parameter sensitivity as high as 2 without some additional discussion explaining that, in actual practice, it is unusual for a PBPK model to have a parameter sensitivity much greater than zero in absolute value, and that such a condition is a cause for concern since it indicates amplification of input error (Allen and Clewell 1996).

Figure 4-2 should be revised to eliminate any extensive variables (amounts/quantities). Only intensive variables (concentrations, AUCs, amounts produced per unit volume) should be considered for use as dose metrics.

On p. 4-12, there is a reference to Figure 2-2 indicating that the model accurately predicts the observed kinetics in the human, but there is no data shown in the Figure.

Figure 4-5 shows rat and human panels, but the caption and text refers to both panels as being for rats.

Gary L. Ginsberg

The figures have good content and purpose and are generally simple enough for the casual reader to learn from. The following are some points for improving the figures:

Figure 2-2 (pg 2-6)- For direct interspecies comparison of kinetics, it would be good to show the human 6 hr exposure curve at 50 ppm and then show in legend the concentration needed in humans for 6 hrs to match the rodent AUC from 50 ppm. Then the use of PBPK models to simulate different scenarios (continuous exposure) with the bottom chart, along with different species, would be clearer.

Section 2.5.1 – a graphic from the RfC document or elsewhere showing different modeling approaches for different types of gases/particles would be helpful.

Figure 3-1 – this is a potentially important figure and can set the stage for comparison of PBPK modeling approaches for different types of chemicals. However, the text and legend fall short of this treatment and it makes one wonder about the point of the figure. Also, the "D" part of the figure is cramped and difficult to read.

Section 3.4.2 – Partition coefficients – a graphic of vial equilibration technique and how one gets to tissue:blood from separate measurement of tissue:air and blood:air. In fact the text does not talk about this common approach for deriving tissue:blood.

Figure 3-2 – pg 3-18 – can the figures be represented on a linear scale to more clearly see goodness of fit or at least discuss the issue of log vs linear y axis. The text that goes with this figure (3-18 bottom) promises to show how adequacy of model structure can be judged from the graph. However, the text states that the poor fit in Fig 3-2B may be due to either inadequate structure or problems with model parameters. So this example falls short of teaching us how to judge structural issues from the data. On top of that, it appears that the data and models in A,B, and D are describing a steady state situation in which chemical is continuously taken up and eliminated, followed by a break point wherein chemical is no longer taken up but just eliminated. Figure B appears to show a much different uptake pattern (bolus) and may be off not because of model structure but because the exposure parameters are wrong.

Figure 3-4 – it's a good figure. An enhancement would be to show how an input distribution (e.g., bimodality in metabolic enzymes) can be transmitted thru to the distribution of AUC results.

Figure 4-2 – very busy, not easy to follow. Perhaps remove the Amount→quantity in tissue boxes under parent and metabolite, and the Average → mg formed boxes under rate of production. Also need a new box under Unknown→AUC-→ "Evaluate Correlations to Tox Response". Also the bottom right box should be in units of per tissue volume.

Figure 4-3 – should the toluene parent compound plot have more of an upward bend to it to match saturation above 500 ppm?

Page 4-12 – 2nd para – discussion of Figure 2-2 which allegedly demonstrates predictiveness of a toluene rat model for humans. However, Figure 2-2 has no data points, only simulated lines, so one cannot tell good the fit is or the predictiveness of the model.

Figure 4-5 – heading for lower figure should be rat and break point in upper figure when concentration starts decending should be 4 hr, not later. Also, the conversion from 4 hr to 24 hr dose for equivalent AUC is not quite linear – it would be helpful to point out why the lower dose over longer time is somewhat less efficient (is there saturation at the higher dose?).

Figure 4-6, Page 4-15 – nicely laid out figure. However, its important to indicate that 99th to 50th can also be used to indicate size of variability factor. The figure represents the degree of intraspecies variability as being determined on the basis of the 95th to 50% comparison. However, some have used 99th % as another upper bound for evaluating population variability. This may be important to ensure that low percentage subgroups which have distinct pharmacokinetics are not diluted out of the analysis. (Ginsberg, et al. 2001 – ALDH analysis which showed that a metabolic polymorphism in an ethnic minority (Asians) that leads to much less acetaldehyde detoxification and greater risk of ethanol toxicity could be overlooked when that population is averaged in the general pop. To see this subgroup in variability statistics, one needed to compare 99th to 50th, not 95th.)

3. Does this document reasonably describe the major potential uses and advantages of PBPK modeling in risk assessment, are there risk assessment applications of PBPK modeling that have not been addressed?

Gregory M. Blumenthal
The use of PBPK models in quantifying age-dependent pharmacokinetic adjustments, allowing departure from ADAFs for mutagenic carcinogens, should also be addressed.

James V. Bruckner

Janusz Z. Byczkowski
Yes. This document seems to be reasonable, thorough and complete.

Harvey J. Clewell
I think the document adequately describes the major potential uses and advantages of PBPK modeling in risk assessment.

Gary L. Ginsberg
Overall, the answer is yes. See answer to #1 for comments on what applications have not been fully covered.

4. Are there improvements to the document that would substantially help risk assessors who are less familiar with PBPK modeling better understand the potential strengths and limitations of PBPK modeling?

Gregory M. Blumenthal
A chapter with a basic introduction to critical pharmacokinetic concepts is necessary.

James V. Bruckner

Janusz Z. Byczkowski
Yes. This reviewer would suggest to include an example of source codes for a PBPK model, for instance, codes of the "classic" Ramsey and Andersen (1984) PBPK model for styrene.

Harvey J. Clewell
Perhaps a few case-studies where the strengths and limitations of a particular model used in a risk assessment were evaluated.

Gary L. Ginsberg
As mentioned above, a section on interpretation of model output could be helpful for non-modeling risk assessors in reviewing the results of a modeling exercise. Areas of interpretation can be confidence in the model (extent to which it is calibrated and tested against external datasets, goodness of fit, number of parameters needing back-fit), more on sensitivity analysis (what is level of confidence and key uncertainties in the most sensitive parameters; what are reasonable bounds for these parameters; how much might they influence the assessment), more on non-linearities and how they affect results, etc.

The document could do a better job of educating the reader on the source of toxicokinetic non-linearities. Michaelis-Menten kinetics are not described, perhaps because this is too technical a subject? There are numerous opportunities to provide background on non-linear kinetics, whether due to saturable absorption, binding, metabolism, or excretion (e.g., Section 3.4.3 – biochemical parameters).

The RfC (1994) methodology comes up in several locations. One clear presentation of the role of PBPK modeling within the RfC approach would be useful, especially in terms of simplications taken that are not normally used in full PBPK modeling assessments. Additionally, it would be good to explain some of the differences between reactive gas and non-reactive gas models (local vs. systemic effect).

Other improvements would be to consolidate and clarify some aspects of Section 2. Specifically: Page 2-12 to 2-15 – RfD Development Section – much of this material is redundant with RfC section. It would be more efficient to combine the bulk of the RfC and RfD sections into one general principles section and then have subsections that discuss any techniques specific to the inhalation or oral dose route.

Also: Page 2-15 – the box – has essentially the same rules as in the RfD box, but here they are worded differently – any reason for this? It can confuse the reader. Also, left out of Box 2-3 is PBPK use in duration adjustment. There are needs for duration adjustment in oral studies (e.g., 5 day a week gavage dosing to 7 day per week continuous exposure in drinking water). The RfD section should discuss the utility of PBPK models to extrapolate from one exposure scenario in animals (e.g., bolus dosing which may involve saturation of activation or detoxification systems) to a more continuous exposure scenario in humans to achieve the same AUC dose.

Also, Page 2-18 – Section 2.8 – This section should come before the RfD/RfC sections. PBPK models, at their most basic and primary level, are an advanced form of exposure assessment. The improved exposure metric is then more effectively used in non-cancer and cancer risk assessment. Therefore, the exposure assessment application should come before the risk assessment discussion. This section brings up an important and evolving use of PBPK modeling – conversion of biomonitoring data to exposure doses and application to risk assessment. This merits considerably more attention in this document. There are several case studies (dioxin, mercury, PFOA, phthalates) where this approach is used.

The differences between flow-limited and diffusion-limited kinetics as introduced on Page 3-3, bottom should be spelled out more fully.

Page 3-17, 2nd full para – near the end – "PBPK modeling is not a fitting exercise" – this is debatable since some parameters are fitted to an initial dataset. Better to state that since many parameters are not fitted, can't expect perfect match to the underlying PK dataset.

There can be more discussion of limitations of fitting procedures (e.g., metabolic constants) wherein one may have a fairly insensitive parameter and so the parameter estimate is still fairly uncertain. This may affect the predictiveness of the model since the uncertain parameter may not be very reliable for different conditions of exposure. It would be good to describe a bit more the iterative approach and use of multiple datasets to optimize the fit. Finally, the situation where there are multiple backfitted parameters (typically an absorption coefficient and metabolic constants) should be described as being particularly uncertain in terms of the optimal value of the individual parameters.

Same para on page 3-17 – where does the 2 SD rule for evaluating results come from?What other choices are there? An option is to use best model fit when considering multiple underlying datasets and iterative recalibration.

Issue of model calibration vs. validation (e.g., Page 3-19) - its confusing to say that a model which has been cross-checked against external datasets isn't really "validated" but is only "calibrated". Calibration signifies the adjustment of a model to match an underlying observation, not an independent test of the model predictiveness (without adjustment) against a new dataset. A different term is needed to described this activity and the confidence it provides. Perhaps "confirmation of predictive ability" for certain types of data.

More on model calibration - Page 3-21 – top section – should discuss case where underlying PK datasets disagree with one another. A single model will not be able to simulate both; must

decide which underlying dataset to trust more or consider 2 different versions of the model – one that fits one dataset optimally and the other fits the 2nd dataset.

Need for peer-reviewed model: Page 4-1 – 2nd bullet and page 4-3 – requirement that a PBPK model must be peer-reviewed before being used in risk assessment seems excessive, or at least should be caveated to state that the applications for which a model must be peer-reviewed to be considered for risk assessment. The point of this guidance is to describe the principles for developing and evaluating a model to enable it to be used in risk assessment. If one follows these principles the need for external peer review prior to the risk assessment is diminished. Perhaps it is USEPA policy that the model must first be peer reviewed to be used in deriving any value that goes onto IRIS. The best approach for this document may be to say that the policy at certain agencies or for certain risk applications may be to peer review a model prior to use.

Description of PBPK for Mixtures - Page 4-21 – this section would be helped by citing examples where mixtures were analyzed with PBPK models and how this was accomplished (cite work by Ray Yang), and by expanding the description of the HI approach. This appears to be a simple addition approach to His for multiple contaminants in a mixture in which the PBPK model is used to adjust the ingredient concentration as influenced by the other chemicals. The description should include how chemicals may interact (at metabolic enzymes or binding sites) and how this can be simulated in a model. Further, the description at the top of Page 4-22 is confusing due to the word "POD". I recommend replacing it with "environmental exposure concentration".

As described above, it would be good to relate PBPK to the classical PK frame of reference. This is only done in passing in this document. However, risk assessors will come across one compartment models from time to time (e.g., mercury, dioxin, chlorpyrifos) and will want to be able to distinguish these from pBPK and why is PBPK preferable (or are there cases where one compartment is just fine for RA?).

5. Do you think that PBPK modelers outside the EPA, and those less familiar with risk assessment practices, would find this document useful as far as fostering the kinds of research and model development useful for risk assessment?

Gregory M. Blumenthal
This document excels at describing the role that PBPK modeling plays in EPA risk assessment practices. This should greatly assist non-EPA PBPK modelers in developing and presenting models that serve EPA risk assessment purposes.

James V. Bruckner

Janusz Z. Byczkowski
Yes. While this document is addressed to risk assessors rather than pharmacokineticists, it contains many clarifications and suggestions that may be useful in developing PBPK and pharmacokinetic models in general. For example, the Appendices provide extremely useful for pharmacokineticists compilation of references and parameters. A public discussion, initiated over the Internet, proved that there is a genuine interest in this report among the international experts from both fields - pharmacokinetics and risk analysis.

Harvey J. Clewell
Yes, I think the document is particularly valuable for those who would like to develop a PBPK description for a chemical that would be of value to the agency for a risk assessment.

Gary L. Ginsberg
As stated above, the document is technical but fairly readable and gives risk assessors the whys and some of the hows for doing PBPK modeling. Without actually having an operative model in front of you to work with, one cannot fully appreciate what is involved. It is a very hands on activity. Perhaps a section that takes apart a case example could make this all more concrete. It could show the construction of a real world model from choice of structure, through parameterization (showing sources of parameter values), different simulations with different exposure inputs, matches to key datasets, and how the model is affected by sensitive parameters. The document may also describe how one learns how to do it (attending workshops; working with other modelers).

6. Are there current research needs and data gaps not highlighted in this report that would improve the utilization of PBPK models in risk assessment?

Gregory M. Blumenthal
Innovation in computational biology and its application to risk assessment is an ongoing process. This report should be envisioned as a living document, with room to expand as novel methods and applications arise. Key among the areas with explosive growth occurring are acute dosimetry, point-of-contact dosimetry, whole-life and developmental modeling, maternal-fetal dosimetry, and metabolic network modeling to identify sensitive subpopulations.

James V. Bruckner

Janusz Z. Byczkowski
No. This report represents the current state- of-the-art in the PBPK modeling.

Harvey J. Clewell
Not that I can think of.

Gary L. Ginsberg
This document doesn't really address research needs and data gaps, as it is more of an informational rather than an analytical document. Many of the research needs are chemical specific or host specific (age groups, polymorphisms, etc.), or have to do with chemical mixtures. I suppose a section that outlines these key areas of data needs would be helpful.

A related issue is where does the PK data come from to construct and calibrate models. The discussion in section 2.3 doesn't really cover this. There could be a discussion that this is not a regulatory-driven activity but the data are developed for specific risk assessment needs. It would be helpful to describe the PK data gathering that is required for FIFRA and TSCA and discuss the limitations for its use in PBPK modeling. Risk assessors familiar with these programs may wonder about the utility of these data for modeling.

7. Are there future reports that you could envision which would compliment or expand upon the topics covered in the current document? For example, would a report focused on dosimetry models for reactive gases be helpful? Or a report focused on extrapolation across life stages using PBPK modeling?

Gregory M. Blumenthal
See answer to #6.

James V. Bruckner

Janusz Z. Byczkowski
Yes. A report focused on PBPK modeling of gestational and lactational transfers of chemicals would be helpful in addressing unique problems of assessing risk from exposures of children to environmental pollutants.

Moreover, this reviewer can envision the future report by the Agency, perhaps entitled "Approaches for the Application of Physiologically Based Pharmacodynamic (PBPD) Models and Supporting Data in Risk Assessment".

Harvey J. Clewell
Both of the suggested reports would be of value.

Gary L. Ginsberg
As stated above, there are various areas which are not fully covered in this document. These could conceivably form the material of other in depth PBPK / risk reports. Even if this were the case, consideration should be given to providing more information on these areas in this report to make it more complete and representative of the field. In addition to the above, a document that is more of a "How To", providing a step-by-step methodology for conducting PBPK modeling and explains how some of the simulation software works might encourage more people to use the technology.

8. Do you have additional comments/suggestions that were not covered in the above questions?

Gregory M. Blumenthal
Appendix 1 requires significant explanation and discussion. Those formulas should not be published without appropriate descriptions and caveats.

James V. Bruckner

Janusz Z. Byczkowski
Yes. Because numerous typing errors were spotted in the Appendices (see below), this reviewer would suggest a thorough "quality control" of the Appendices (at least, a "quality reading" by the professional technical proofreader).

Harvey J. Clewell
Table 3-1: I would remove the subscripts for current and previous simulation time. These subscripts are only accurate for explicit integration algorithms such as the Euler method. They are not correct for the case of implicit algorithms such as the Gear (backward differentiation) method.

Similarly, specifying the "integration interval" is only relevant to constant step-size algorithms. For variable step-size algorithms, the appropriate element is the "error criteria". There are a number of places in the document where this oversight should be corrected.

P. 3-17: I would suggest also mentioning an alternative rule of thumb often used by modelers: that a model is successful if it is within a fator of two to three of the data more than half the time.

I don't understand, and probably don't agree with, the 6th bullet in section 3.9. I could agree with scaling metabolism by tissue weight, but not by body weight.

I have made a number of minor suggested edits to my copy of the document, which I will provide to the peer review organizers.

I have also suggested a number of additional references at various places in main document. They are listed here:

Abraham, MH; Weathersby, PK. *J Pharm Sci.* **1994**, *83*, 1450-1456.

Allen BC, Covington TR, Clewell HJ. 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. *Toxicology* 111:289-303.

Astrand, P., and Rodahl, K. Textbook of Work Physiology. McGraw-Hill, New York, 1970.

Beliveau, M; Lipscomb, J; Tardif, R; Krishnan, K. *Chem Res Toxicol.* **2005**, *18*, 475-485.

Clewell HJ, Andersen ME. 1985. Risk assessment extrapolations and physiological modeling. *Toxicol Ind Health* 1:111-131.

Clewell HJ, Andersen ME. 1996. Use of physiologically-based pharmacokinetic modeling to investigate individual versus population risk. *Toxicology* 111:315-329.

Clewell, H.J., Gentry, P.R., Covington, T.R., Sarangapani, R., and Teeguarden, J.G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol. Sci.* 79:381-393.

Gentry, P.R., Hack, C.E., Haber, L., Maier, A., and Clewell, III, H.J. 2002. An Approach for the Quantitative Consideration of Genetic Polymorphism Data in Chemical Risk Assessment: Examples with Warfarin and Parathion. *Toxicological Sciences* 70:120-139.

Lilly, P.D., Thornton-Manning, J.R., Gargas, M.L., Clewell, H.J., and Andersen, M.E. 1998. Kinetic characteristics of CYP2E1 inhibition in vivo and in vitro by the chloroethylenes. *Arch Toxicol* 72:609-621.

Sarangapani R, Teeguarden J, Andersen ME, Reitz RH, Plotzke KP. 2003a. Route-specific differences in distribution characteristics of octamethylcyclotetrasiloxane in rats: analysis using PBPK models. Toxicol Sci. 71(1):41-52.

Sarangapani, R., Gentry, P.R., Covington, T.R., Teeguarden, J.G., and Clewell, H.J. 2003b. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal Toxicol* 15(10):987-1016.

Gary L. Ginsberg
Yes, see below for a variety of broad and specific comments.

V. SPECIFIC OBSERVATIONS

Gregory M. Blumenthal

James V. Bruckner

p. xi, pgr. 3, line 7; p. xii, pgr. 1, line 7; and p. xiii, line 3: Replace the word "applied" with "administered".

p. xii, pgr. 4, lines 1 & 2: What is meant by the term "value" in the phrase "it is used to estimate the value of internal dose metrics"?

p. 1-1, pgr. 1, line 8: It should be noted here for less familiar readers that RfC methodology applies to inhalation exposures.

p. 2-1, pgrs. 1 & 2: It might be useful to include a short, transitional paragraph that clarifies the difference(s) between classical pharmacokinetic (PK) analysis (of data) and PK modeling.

p. 2-2, lines 25 – 28: I assume that Fig. 2-1 illustrates (is an example of) a chemical that is metabolically activated. If this is the case, it should be mentioned in the text and noted that the modeling provides evidence of this phenomenon.

p. 2-3, pgr. 1: It may be useful here to note the importance of being able to relate the mode of action to the biologically-active form of the chemical.

p. 2-5, pgr. 1: It would be worthwhile to point out that PK equivalence may not equate to toxicity equivalence, due to the potential existence of thresholds and toxicodynamic differences.

p. 2-5, line 21: The term "window of susceptibility" should be succinctly defined.

p. 2-7, line 11: Substitute "administered" for "applied" dose.

p. 2-9, pgr. 1: It should be recognized (and pointed out) here that estimation of equivalent absorbed doses (by inhalation and ingestion) does not take into account an important factor; namely the frequent differences in the duration of the oral (usually bolus) and inhalation (frequently hours) exposures. Bruckner and his colleagues have assessed the influence of first-pass effect with equivalent inhalation and oral exposures to VOCs by: (1) measuring the total absorbed dose for 2- to 3-hour inhalation exposures in rats; (2) administering this total dose by constant gastric infusion over the same time-frame; and (3) monitoring blood and tissue concentrations of the compound of interest during and post exposure [e.g., see Sanzgiri et al. *Toxicol. Appl. Pharmacol.* 134: 148-154 (1995)].

p. 2-9, lines 26 – 30: The magnitude of CNS depression is an example of an effect that is dependent upon the momentary concentration of a VOC in the blood/brain. PBPK models and objective measurements of trichloroethylene-induced CNS dysfunction in rats have been utilized to demonstrate that Haber's Rule and ten Berge et al's. *J. Hazard. Mater.* 13: 301-309 (1986) C^n

X t approaches to time scaling result in overestimation of risks when extrapolating from shorter to longer exposure times and underestimation when extrapolating from longer to shorter exposures [Boyes et al. *Environ. Health Perspec.* 108 (Suppl 2.): 317-322, 2000; Bruckner et al. *J. Toxicol. Environ. Health* *67* (Part A): 621-634, 2004].

p. 2-12, Box 2-2: Should "Interspecies uncertainty factor" be added?

p. 2-14, lines 26 – 29: This sentence is a bit unclear. I assume the authors intend to state that default uncertainty factors of 3 are used for potential interspecies and intraspecies pharmacodynamic differences in conjunction with PBPK modeling. See suggested editorial changes.

p. 2-15, line 26: Has the meaning of the abbreviation "IUR" been stated previously?

p. 2-17, lines 11 – 13: The paper of Lee et al. *Toxicol. Appl. Pharmacol.* 139: 262-271 (1996) might be cited here as an example of the influence of dose on the extent of first-pass hepatic and of first-pass pulmonary elimination of orally-administered trichloroethylene.

p. 2-18, line 1: Should drinking water "rate" be "volume"?

p. 2-18, lines 3 – 17: The authors seem unnecessarily tentative in stating that "PBPK models are potentially useful in evaluating the pharmacokinetic basis …". Furthermore, it has been clearly established a number of times in this document that potential pharmacokinetic and pharmacodynamic differences are separate/different entities.

p. 2-18, Box 2-4: Can't PBPK models be useful in certain intraspecies pharmacokinetic extrapolations (e.g., child to adult)?

p. 3-2, line 15: What is meant by the statement that there is no limitation to the size of a compartment? Doesn't a compartment's size have to be physiologically realistic?

p. 3-3, line 10: Is use of the term "reactors" necessary? It may not be familiar to the general reader.

p. 3-3, lines 25 & 26: Concentration gradient is, of course, just one factor in Fick's law of diffusion. See suggested editorial change in the text.

p. 3-8, lines 7 – 9: An ILSI committee is currently finalizing a series of manuscripts that include a compilation of some physiological values for immature rodents and humans.
p. 3-10, Table 3-5: The range of values given for pulmonary blood flow (as a % of cardiac output) is 11.1-17.8%. Doesn't 100% of the cardiac output pass through the pulmonary circulation?

p. 3-11, lines 14: The phrase "model validation" might be utilized rather than "external evaluation".

p. 3-13, lines 11 & 12: What do the authors mean when they state that *in vitro* to *in vivo* extrapolations are not clear?

p. 3-19, pgr. 2: It would be useful to briefly address the question of the quality of experimental PK data sets. Many factors can influence the accuracy of experimental measurements in animals and humans, from stress on the subjects to analytical chemistry problems. My primary role in PBPK model development has been to provide quality empirical time-course data, so I am aware of many of the potential problems.

p. 3-27, line 27: I assume that *in vivo* kinetic data may also be useful for model calibration.

p. 3-29, lines 1 – 3: Should the words "The model for" be inserted at the beginning of this sentence? It is problematic as presently written.

p. 3-29, lines 32: Some sort of disclaimer sentence should probably be added at the end of this section, emphasizing that a relatively high degree of uncertainty is usually inherent in the use of surrogate data.

p. 3-30, line 25: The term "steady-state" can be inserted between the words "*in vivo*" and "data".

p. 3-31, lines 3 – 6: Another bullet/point should be added addressing the use of kinetic data for model construction/calibration and the need for additional data sets for model validation.

p. 3-31, line 7: Still another bullet/point should be added at or near the beginning of this section. It should point that the model should include/focus on the proximate toxic moiety, if it and/or the MOA is/are understood.

p. 4-1, lines 6 & 7: Types of human studies, that may serve as the critical study, should be expanded to include clinical and experimental studies. See the recommended change in the text.

p. 4-3, Box 4-1, 2nd bullet: "Binding" should be included with storage.

p. 4-4, lines 24 – 26: Treatment with an inhibitor of the metabolism of a toxic parent compound may enhance its toxicity by decreasing its rate of metabolic clearance.

p. 4-5, pgr. 1: It would be worthwhile pointing out that the concentration of ligand at a particular point in time is often considered to be the most appropriate dose metric for adverse effects associated with receptor interactions.

p. 4-12, lines 26 & 27: The words "oral" and "gavage" are redundant.

p. 4-13, Fig. 4-4: Obviously, as shown here, exposure conditions that yield equivalent AUCs can be forecast, but the oral C_{max} is about 10-fold higher in the oral group. This relatively high concentration may exceed a toxicity threshold. Alternatively, the adverse effect may depend upon the momentary concentration.

p. 4-14, pgr. 1: It would be worthwhile to point out that PBPK models are now being used to make duration adjustments for derivation of acute exposure guideline levels (AEGLs) for a number of chemicals. AEGLs are established for 10- and 30-minute inhalation exposures, as well as 1-, 4- and 8-hour exposures. Typically, a critical study with a single duration of exposure serves as the basis for derivation of AEGLs for the other exposure durations. A PBPK model is used to forecast the inhaled concentration required for each time-period to produce the same dose metric as that associated with an adverse effect in the critical study. Bruckner et al. (2004) (referenced previously) described the AEGLs program and illustrated the use of PBPK for time scaling with trichloroethylene.

p. 4-20, Table 4-2: How is tumor prevalence expressed here? No units are given.

p. 4-23, line 11: Shouldn't the word "difference" be "differential"?

Janusz Z. Byczkowski

P. xii Line 29 - Quote: "*...which are then use to derive...*"
Change to: "...which are then used to derive..."

P. 2-2 Line 21 - please add a short paragraph on modeling of dermal exposures. Some of the following references, listed in the Appendix 2, may be used:

Auton, T. R., Ramsey, J. D., and Wollen, B. H. (1993). Modelling dermal pharmacokinetics using in vitro data. Part II. Fluazifop-butyl in man. Human & Experimental Toxicology 12, 207-213.

Auton, T. R., Ramsey, J. D., and Woollen, B. H. (1993). Modelling dermal pharmacokinetics using in vitro data. Part I. Fluazifop-butyl in the rat. Human & Experimental Toxicology 12, 199-206.

Blancato, J. N., and Bischoff, K. B. (1993). The application of pharmacokinetic models to predict target dose. In Dermal risk assessment. Dermal and inhalation exposure and absorption of toxicants. (R. G. M. Wang, J. B. Knaak, and H. I. Macbach, Eds.), pp. 31-46. CRC Press Inc.

Bookout, R. L. J., McDaniel, C. R., and Quinn, D. W. M. J. H. (1996). Multilayered dermal subcompartments for modeling chemical absorption. SAR QSAR Environ Res. 5, 133-150.

Corley, R. A., Markham, D. A., Banks, C., Delorme, P., Masterman, A., and Houle, J. M. (1997). Physiologically based pharmacokinetics and the dermal absorption of 2-butoxyethanol vapor by humans. Fundamental and Applied Toxicology 39, 120-130.

Corley, R. A., Gordon, S. M., and Wallace, L. A. (2000). Physiologically based pharmacokinetic modeling of the temperature-dependent dermal absorption of

chloroform by humans following bath water exposures. Toxicological Sciences 53, 13-23.

Jepson, G. W., and McDougal, J. N. (1999). Predicting vehicle effects on the dermal absorption of halogenated methanes using physiologically based modeling. Toxicological Sciences 48, 180-188.

Loizou, G. D., Jones, K., Akrill, P., Dyne, D., and Cocker, J. (1999). Estimation of the dermal absorption of m-xylene vapor in humans using breath sampling and physiologically based pharmacokinetic analysis. Toxicological Sciences 48, 170179.

McDougal, J. N., Jepson, G. W., Clewell, H. J., MacNaughton, M. G., and Andersen, M. E. (1986). A physiological pharmacokinetic model for dermal absorption of vapors in the rat. Toxicology and Applied Pharmacology 85, 286294.

McKone, T. E. (1993). Linking a PBPK model for chloroform with measured breath concentrations in showers: implications for dermal exposure models. Journal of Exposure Analysis and Environmental Epidemiology 3, 339-365.

Nichols, J. W., McKim, J. M., Lien, G. J., Hoffman, A. D., Bertelsen, S. L., and Elswick, B. A. (1996). A physiologically based toxicokinetic model for dermal absorption of organic chemicals by fish. Fundamental and Applied Toxicology 31, 229-242.

Roy, A., Weisel, C. P., Lioy, P. J., and Georgopoulos, P. G. (1996). A distributed parameter physiologically-based pharmacokinetic model for dermal and inhalation exposure to volatie organic compounds. Risk Analysis 16, 147-160.

Shatkin, J. A., and Brown, H. S. (1991). Pharmacokinetics of the dermal route of exposure to volatile organic chemicals in water: A computer simulation model. Environmental Research 56, 90-108.

Thrall, K. D., and Woodstock, A. D. (2002). Evaluation of the dermal absorption of aqueous toluene in F344 rats using real-time breath analysis and physiologically based pharmacokinetic modeling. Journal of Toxicology and Environmental Health 65, 2087-2100.

P. 2-14 Line 24 - Please add a sentence or two, explaining that Monte Carlo modeling coupled with PBPK represents a useful approach to quantify impact of parameter variability on simulated dose metrics (e.g., excerpt from page 3-24).

P. 2-15 Line 28 - Quote: "*...either a nonlinear (i.e., RfC or RfD) or linear...*"
Please delete "(i.e., RfC or RfD)"

P. 2-16 Line 1 - Quote: "*...the tumors observed in such studies are...*"

Please add "the number of tumors observed in such studies is"

P. 3-2 Line 24 - Quote: "*...biochemical parameters (i.e., partition coefficients)...*"
Please change to "physicochemical parameters (i.e., partition coefficients)"

P. 3-6 Line 12 - Please change in the equation for Tissue blood expression "(C_1+C_2)" to "$(C_1 - C_2/P_{t:b})$"
and in the equation for Cellular matrix "(C_1-C_2)" to "$(C_1 - C_2/P_{t:b})$"
Add below the table 3-2: "$P_{t:b}$ = partition coefficient tissue:blood"

P. 3-7 Line 18 - Quote: "...(*mg/L-hr*$^{-1}$)..."
This expression of unit, mathematically, does not make sense. Please change either to exponential "($mg \times L^{-1} \times hr^{-1}$)" or proportional "(mg/L/hr)" expression.

P. 3-17 Line 28 - Quote: "*...every single data...*"
Please change to "every single datum"

P. 3-29 Line 28 - Quote: "*... a web-based resource for PBPK modeling (http://www.capkr.man.ac.uk). The site provides instant access to resources such as data, methodology, and tools necessary to start PBPK modeling effort...*"
Unfortunately, the provided URL, points to the password-protected "members only" university web site, without public access to any resources.
Please either, provide URL to publicly accessible web site (for example, http://www.pbpk.org/) or delete this information.

P. 4-13 Line 4; 4-14 Lines 8 and 14 - Quote: "*...mg/L-hr...*"
This expression of unit, mathematically, does not make sense. Please change either to exponential "($mg \times L^{-1} \times hr^{-1}$)" or proportional "(mg/L/hr)" expression.

P. 4-16 Lines 23 - 26 - Quote: "*...Because the Agency has traditionally applied the uncertainty factors to the external dose and not to the internal dose, it may be useful to undertake a systematic evaluation of the outcome of applying the uncertainty factors to the external and internal doses for various chemicals and situations...*"
The Agency traditionally applied the uncertainty factors to the external dose because before accepting PBPK models it could not calculate realistically internal doses, delivered to the target. The very goal of applying PBPK models in risk assessment is to determine the internal dose metrics under relevant exposures, and thus, reverting to "external dose divided by uncertainty factor" as a basis for calculation of RfD and/or RfC would nix most of the advantage from using the PBPK model. Even

though, as already noted in the document, for the linear range of PK, the result is numerically the same, no matter whether uncertainty factors are applied to internal or external dose, the right method is to divide internal dose metrics by uncertainty factor(s), and then, to calculate human exposure (external) dose. Undertaking the "systematic evaluation", as suggested in the document, would just waste time and resources as its outcome can be easily predicted right now: it is always appropriate to divide internal dose metrics by uncertainty factor(s); while it is only sometimes appropriate to divide external dose by uncertainty factor(s) - only when dealing with linear pharmacokinetics.

This reviewer suggests to substitute the above quoted sentence with the recommendation to appropriately apply uncertainty factor(s) to internal dose metrics, before calculating human exposure (external) dose for RfD and/or RfC, applicable for both linear and nonlinear pharmacokinetics.

P. 4-17 Lines 7 and 15; 4-18 Line 15 - Quote: "*...mg/kg-d...*"
This expression of unit, mathematically, does not make sense. Please change either to exponential "($mg \times kg^{-1} \times d^{-1}$)" or proportional "(mg/kg/d)" expression.

P. 4-23 Line 11 - Quote: "*...difference equations...*"
Please change to: "differential equations"

P. G-1 Line 23 - Quote: "*...Cancer scope factor...*"
Please change to: "Cancer slope factor"

P. G-1 Line 34 - Quote: "*...Delivered dose: The amount of a substance available for interactions with biologically significant receptors in the target organ...*"
Although this definition is correct, risk assessors usually use the term "receptors" differently than pharmacologists. For the risk assessor, "receptor" often means the individual (human subject) exposed to a potentially harmful chemical compound.
To avoid confusion, this reviewer would suggests changing the definition of *Delivered dose*, to: "The amount of a substance available for interactions with biologically significant targets in the affected tissues".

P. G-2 Line 11 - Quote: "*...This adjustment may incorporate...*"
Please change to: "The adjustment of concentration from animal to human may incorporate".

P. G-2 Line 27 - Quote: "*...the relationship between external exposure level and the biologically effective dose at a target tissue...*"
Please add: "the relationship between external exposure level and the biologically effective dose at a target tissue over time".

P. G-2 Line 27 - Quote: "*...the relationship between a biologically effective dose and the occurrence of a tissue response...*"
Please add: "the relationship between a biologically effective dose and the occurrence of a tissue response over time".

P. G-2 Line 42 - "*Reference concentration (RfC)*":
Please change to revised IRIS definition (effective July 2005 < http://www.epa.gov/iris/gloss8.htm >: "An estimate of a continuous inhalation exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a BMCL, a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used."

P. G-3 Line 5 - "*Reference dose (RfD)*":
Please change to revised IRIS definition (effective July 2005 < http://www.epa.gov/iris/gloss8.htm >: "An estimate of a daily oral exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a BMDL, a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used."

P. G-3 Line 22 - Please explain the term "*window of susceptibility*".

P. R-12 ref. Price et al. (2003a) - Change "*Conelly*" to "Conolly"

P. R-12 ref. Price et al. (2003b) - Add in "J Toxicol Environ Health A66:"

Appendix 3, P. 6 Line 13 - Quote: "*...Oxydation...*"
Please correct to "Oxidation"

Appendix 3, P. 6 Line 16 and P. 7 Line 22 - Quote: "*...conjugaison...*"
Please change to "conjugation"

Appendix 3, P. 8 Lines 24, 25 - Quote: "*...Oxydation...*"
Please correct to "Oxidation"

Appendix 3, P. 19 Lines 33, 34, 35 37, 39, 40, 41, 43 - Quote: "*...Oxydation...*"
Please correct to "Oxidation"

Appendix 3, P. 20 Lines 18, 20, 22 - Quote: "*...Oxydation...*"
Please correct to "Oxidation"

Appendix 3, P. 22 - Please correct illegible fonts

Appendix 3, Pp. 25, 36 and 51 Line 5 - Quote: "*...coumpound...*"
Please change to "compound"

Appendix 3, P. 28 Line 17 - Quote: "*...conjugaison...*"
Please change to "conjugation"

Appendix 3, P. 46 Line 1 - Quote: "*...CHLOROFLUOROHYDROCARBONE...*"
Please change to "CHLOROFLUOROHYDROCARBON"

Appendix 3, P. 57 Line 14 - Quote: "...(*younth*)..."
Please change to "(youth)"

Appendix 3, P. 81 Line 15 - Quote: "*...disapearance ...*"
Please change to "disappearance"

Appendix 3, P. 83 Lines 36 and 39 - Quote: "*...conjugaison...*"
Please change to "conjugation"

Appendix 3, Pp. 100, 103 and 106 Line 19 - Quote: "*...caracteristics...*"
Please change to "characteristics"

Appendix 3, Pp. 119 and 120 Line 2 - Please translate into English.

Harvey J. Clewell
p. 1-2, lines 26-27: change "a compilation…environmental chemicals." to "and a case study based on the evaluation of a PBPK model for isopropanol (Clark et al. 2004)"

p. 2-2, line 31: Clewell and Andersen 1997 should be 1985

p. 2-4, lines 5 and 35; p. 2-14, line 29p. 2-16, line 7: Clewell et al., 2002 should be 2002a (Clewell Andersen and Barton)

p. 2-7, line 12: add "exposure" at beginning of line

p. 2-9, line 2: change was to could be

p. 2-13, line 8: change "overly" to "highly"

p. 2-18, line 4: add Clewell et al. 2004

p. 3-1, line 31: Clewell and Andersen 1997 should be 1987

p. 3-2, line 15: change “size” to “complexity”

p. 3-3, line 11: change “equal” to “related by the partition coefficient”

p. 3-3, line 22: delete “clearance” and change “volume” to “mass”

p. 3-3, line 23: change “liters” to “mg” and “milliliters” to “mg” and delete “clearance”

p. 3-3, line 25-26: delete “is described according to Fick’s law of simple diffusion, which states that the flux of a chemical” and change “concentration” to “activity (concentration adjusted by partition coefficient”

p. 3-3, line 27: delete “passive and”

p. 3-5, table: remove all subscripts “n” and “n-1” and related definitions in footmote

p. 3-6, table 3-1, footnote b: add “steady-state” before “arterial”

p. 3-6, table 3-2: correct equations (should be C1 – C2/P2)

p. 3-7, line 1: add “a” after “by”

p. 3-7, line 2: add “a” after “on”

p. 3-7, line 3: add “typically” before “been”

p. 3-7, line 4: delete “second order”

p. 3-7, lines 4-7: delete from “Conjugation” to “successfully”

p. 3-7, line 28: after “volumes”, add “absorption rate parameters (e.g., for dermal or oral uptake),”

p. 3-8, line 12: add Gentry et al. 2004; Gentry et al. 2006 (ILSI human perinatal parameters)

p. 3-8, line 23: delete “(e.g., Andersen et al., 1987).” and add “but decreases with activity (Astrand and Rodahl, 1970).”

p. 3-8, lines 14-16: change 91% to 85% and 9% to 15% and add reference to Brown et al. 1997.

p. 3-8, lines 18-19 change “which may seem questionable...in risk assessment.” to “is a reasonable approximation since tissue densities typically range from 0.9 kg/L for muscle to 1.06 kg/L for fat (Ross et al. 1991, Mendez and Keys, 1960)

p. 3-9, table 3-3 title: change “Reference” to “Typical”

p. 3-10, Table 3-5: add “upper respiratory tract” as footnote for Lungs

p. 3-10, Table 3-6: add volumes

p. 3-11, line 11: delete “or binding”

p. 3-12, line 6: add Beliveau et al. 2005 and Abraham and Weathersby 1994.

p. 3-14, table: move Ramsey and Andersen (1984) from ACSL row, last column to SimuSolv row, last column. Add Clewell et al. 2000 to ACSL row, last column.

p. 3-17, line 5: change “levels” to “amounts” in three places

p.3-18, line 17: after “profiles”, add (Lilly et al.1998)”

p. 3-20, line 23: after “zero”, add (Sarangapani et al. 2003a)

p. 3-22, line 4: change “the” to “hypothetical”

p.3-22, line 11: add “In actual practice, it is a matter of concern for sensitivity ratios for be greater than one in absolute value, since this results in the amplification of input error (Allen et al. 1996).

p. 3-22, Figure 3-3 caption: add “hypothetical” before “PBPK”

p. 3-24, line 6: add Sarangapani et al. 2003b, Gentry et al. 2003.

p. 3-24, line 14: add Gentry et al. 2002.

p. 3-24, line 18: add Clewell and Andersen 1996.

p. 3-24, line 23: change “/Q” to “/Qc”

p. 3-25, line 24: change Clewell and Andersen 1997 to 1987.

p. 3-28, line 26: change “suffices” to “may suffice”

p. 3-30, lines 29-31: delete

p. 4-1, line 7: delete “epidemiological”

p. 4-1, line 9: change “as well as” to “and/or”

p. 4-5, line 15: add Andersen et al. 1987.

p. 4-7, table: add acrylic acid ; nasal lesions ; average nasal concentration ; Andersen et al. 1999.

p. 4-7, table, butoxyethanol row: change “Levels” to “Concentration”

p. 4-7, table, methylmethacrylate row: change “amount metabolized/time” to “amount metabolized/time/volume nasal tissue” and delete 1999 reference

p. 4-8, table, top row: add “transferase” after “glutathioine

p. 4-8, table, TCDD row: change “Number” to “Fraction”

p. 4-8, table, TCE row: change “in” to “per L”

p. 4-8, table, VC row: delete “mg metabolite produced/L liver”

p. 4-9, lines 22 and 26: change Clewell and Andersen 1997 to 1987.

p. 4-12, line 26: change “is” to “can be”

p. 4-15, lines 11-12: delete “for unimodel, normal distribution (Naumann et al. 2001)”

p. 4-17, line 32: delete “Subsequently” and change “conventional” to “case of the RfD”

p. 4-19, line 11: change “simulate” to “estimate”

References:

Abraham, MH; Weathersby, PK. J Pharm Sci. 1994, 83, 1450-1456.

Allen BC, Covington TR, Clewell HJ. 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. Toxicology 111:289-303.

Astrand, P., and Rodahl, K. Textbook of Work Physiology. McGraw-Hill, New York, 1970.

Beliveau, M; Lipscomb, J; Tardif, R; Krishnan, K. Chem Res Toxicol. 2005, 18, 475-485.

Clark, LH, Setzer RW, and Barton, HA. 2004. Framework for evaluation of physiologically-based pharmacokinetic models for use in safety or risk assessment. Risk Analysis, 24(6):1697-1717.

Clewell HJ, Andersen ME. 1985. Risk assessment extrapolations and physiological modeling. Toxicol Ind Health 1:111 131.

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Clewell, H.J., Gentry, P.R., Covington, T.R., Sarangapani, R., and Teeguarden, J.G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Toxicol. Sci. 79:381-393.

Gentry, P.R., Hack, C.E., Haber, L., Maier, A., and Clewell, III, H.J. 2002. An Approach for the Quantitative Consideration of Genetic Polymorphism Data in Chemical Risk Assessment: Examples with Warfarin and Parathion. Toxicological Sciences 70:120-139.

Gentry, P.R. et al. 2004. Data for physiologically based pharmacokinetic modeling in neonatal animals: physiological parameters in mice and Sprague-Dawley rats. J. of Children's Health 2(3-4):363-411.

Gentry, P.R. et al. 2006. Physiological parameters for early life stages. Report of an ILSI working group. In preparation.

Lilly, P.D., Thornton-Manning, J.R., Gargas, M.L., Clewell, H.J., and Andersen, M.E. 1998. Kinetic characteristics of CYP2E1 inhibition in vivo and in vitro by the chloroethylenes. Arch Toxicol 72:609-621.

Mendez J, Keys A, 1960. Density and composition of mammalian muscle. Metabolism 9:184-188.

Ross R, Léger L, Guardo R, De Guise J, Pike BG 1991 Adipose tissue volume measured by magnetic resonance imaging and computerized tomography in rats. J Appl Physiol 70: 2164–2172

Sarangapani R, Teeguarden J, Andersen ME, Reitz RH, Plotzke KP. 2003a. Route-specific differences in distribution characteristics of octamethylcyclotetrasiloxane in rats: analysis using PBPK models. Toxicol Sci. 71(1):41-52.

Sarangapani, R., Gentry, P.R., Covington, T.R., Teeguarden, J.G., and Clewell, H.J. 2003b. Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15(10):987-1016.

Gary L. Ginsberg

ES, 2nd Page (2nd para) and later in the document – 3 aspects of PBPK models are called out as being essential to include. However, there are others. For example, PBPK model structure be must be appropriate to the exposure route (oral vs. dermal vs. inhalation vs. lactation vs. trans-placental) and MOA (only parent compound simulated or both parent compound and metabolites; GSH depletion or other defense mechanisms simulated?). Further, a good general principle is that the PBPK model must include all major elimination pathways (renal, exhalation, liver metabolism, as appropriate).

ES- same para as last entry, last sentence – "available animal-alternative algorithms" – the word animal seems out of place given that many of the algorithms in the appendix are for humans as well as animals.

Page 2-1, last sentence of 1st para and Page 2-2 to 2-3: assertion is made that internal dose metric provides a better relationship to toxic effect than does external dose. While this is typically true and may be self-evident to some, the text needs to say why this should be so. For example, it can be stated that internal dose is obviously closer to biologically effective dose than is the applied dose, and that internal dose and applied dose may not be directly proportional due to saturable processes, distributional phenomena, secondary clearance pathways that are active at higher doses, etc.

Page 2-1, 2nd para – 3rd sentence – seems illogical – I think what it is trying to say is that orally absorbed compounds tend to exert their effects at systemic rather than local sites so PBPK models have focused on modeling systemic compartments. However, the sentence is poorly written. The next sentence appears to be referring to classical one and two compartment models in the first clause, with the more highly evolved modeling referring to PBPK. This sentence should be unpacked into one or several paragraphs which gives appropriate background for the more basic models and their potential utility in risk assessment (e.g., mercury, dioxin), and why the more complex PBPK models are needed in other applications.

Page 2-1 – last para – obviously the document is focusing on modeling of systemic sites and steering clear of local dosimetry at portal of entry. It would probably be best to state this more clearly and then refer to the RfC methodology for particles and gases, the more refined CFD models, and the oral models of g.i. dosimetry (e.g., Frederick, et al., 1992) as important applications for chemicals which produce contact site toxicity but will not be the focus of this document. Then state whether certain principles developed in this document would tend to apply to the contact site modeling approaches.

Page 2-4 – top – states that default approach is to use lifetime AUC in target tissue as the most appropriate dose metric for chronic exposure. However, how is the lifetime AUC derived – running the model for 2 years for a rat or 70 years for a human? That would be require large time steps that may miss important periods of greater exposure when there may also be windows of susceptibility that affect chronic risk. Therefore, the concept of windows of vulnerability should be included when considering a lifetime AUC approach.

Page 2-5, 2nd para – replace the word “current” with peak or Cmax??
Last 2 paras of 2-5: appears to be describing optimal dataset vs. limited dataset with need for PBPK modeling. It would be helpful to set up discussion this way. Lead in phrase “the most robust PK dataset needed for RA would consist of …” is awkward and indirect.

Page 2-7, 2nd para, last line – what is a non-compartmental model?? Even one compartment models are “compartmental”. This sentence appears to, once again, gloss over the important distinctions between non-physiological compartmental models vs. PBPK models. .

Page 2-12 – Box of bullet points – consider changing “dosimetric adjustment factor” to “cross-species” AF to be more specific and contrast this bullet from the one that follows.

Page 2-10, last para – a more complete discussion of how the default RfC methodology addresses cross-species dosimetric adjustments via PBPK modeling is needed. That methodology relies upon a steady state equation in which the HEC calculation is simplified down to a ratio of the animal to human blood/air partition coefficient. The current document should present this and describe whether there are situations in which RfC derivation should involve more complete PBPK modeling.

Page 2-16 – Interspecies Extrapolation for carcinogens – this section should make the point that there are no uncertainty factors in carcinogen toxicity assessment with the interspecies scaling factor the only factor used to modify the slope factor derived from animal studies. Therefore, PBPK models are valuable to extend the dose response analysis to human receptors that may have unusual exposure and pharmacokinetics (e.g., children, elderly) to address intraspecies variability, and to use PBPK models to improve upon the interspecies scaling factor approach. The existing text does not make these points.

Page 2-18, top para, 2nd sentence – "However …" This sentence is unnecessary – PK is not expected to adjust for PD differences across species or individuals under any circumstances. This does not diminish the value of PBPK model adjustment of cancer risk assessments for dosimetry differences at different life stages.

Page 2-20 –first sentence – very broad statement about circumstances under which PBPK models wouldn't be needed. Should add the caveat: and if the same relationships between external and internal dose exist across species, age groups, dose routes, etc., then wouldn't need PBPK modeling.

Page 2-20 – second set of numbered points – consider changing "potential dose" to "intake dose".

Page 3-1 to 3-2 – model purpose – should include the use of PBPK models to help interpret MOA by distinguishing between parent compound and metabolite as active toxicant. Ginsberg and Rao 2000 publication is a good example of this for MTBE. Discussion in Section 4 (page 4-5) gets at this but this purpose or utility should also be stated up front.

Page 3-1, end of first para – need to include another step in PBPK model evaluation: "evaluation of model predictiveness against external datasets".

Page 3-7, top para – general principles are described for a variety of important topics – binding, metabolism. However, much more can be stated, especially with regards to how metabolism is represented. No mention is made of Km or Vmax or possible involvement of multiple CYPS having different activity. Ping-pong mechanism needs definition.

Page 3-9, Table 3-3. Should include rapidly perfused tissues as a line in the table since many models have a simplified lumped compartment of this nature.

Page 3-12, Section 3.4.3 – Biochemical Parameters – should describe how in vivo data are used to derive metabolic constants – how backfitting (optimization) techniques are iteratively used.

Should also discuss issue where multiple parameters need backfitting (e.g., absorption coefficient, metabolic constants) and how this can lead to uncertain parameter estimates (Getting the right answer for the wrong reason).

Page 3-18 – "structure may be inadequate" not "is inadequate". Figure 3-2 uses log scale. Text should make the point that linear scale highlights data to model differences more clearly and should be used over log scale if possible.

Page 3-20, end of 1st full para – while visual inspection is a convenient evaluation procedure commonly used, the method of residuals is a relatively easy approach that is also used and should be described.

Page 3-20, next para – level of complexity evaluation – set one parameter to zero and see if it impacts model outcome – this isn't so simple as removing one parameter (e.g., a tissue compartment) may require adjusting the size of another compartment to maintain physiological sense; in this way would be changing more than one parameter.

Page 3-23 – bottom – should spell out what is meant by "individual-specific" parameters – are these values for actual people or values picked from a random distribution.

This section (Variability Analysis) should describe possible form of M-C results – normal, lognormal, multimodal.

Page 3-26 – should describe how uncertainty analysis is run – how is M-C analysis used differently here than in variability analysis?

Page 3-29, top – description of parallelogram approach for CFC and halothane a bit confused – needs rewording and clarification (e.g., The human CFC model was assumed to behave as well as the human halothane model because they were similarly developed and calibrated from in vitro data.

Page 3-30, 4th and 5th bullets – there is no apparent difference between them.
6th bullet is a new rule not stated previously in Section 3 and is not well developed.

Page 3-31 – there is no final bullet for variability, sensitivity, uncertainty. For completeness, one (or several) should be provided.

Page 4-1 – should add another bullet stating that the PBPK model should be reflective of the MOA in terms of simulating key activation/detoxication steps, stores of key factors such as GSH or metallothionen, and estimating concentrations in toxicologically important tissues.

Page 4-3 – the list of bullets should include one evaluating the fit to external datasets to determine predictive nature of the model. This listing overlaps with the information presented in Section 3 and may not be needed or just be merged into Section 3.

Page 4-9 – 1st full para, last sentence – what chemical is refererred to in this example where rat and mouse kidney tumor data could not be reconciled? Is this simply a matter of species differences in pharmacodynamics? This is what is implied on the basis that PK evaluation using various dose metrics couldn't resolve the difference.

Page 4-9, last para – the term dose-dependence used in a confusing manner. I think they are referring to non-linearities – better to use that term if this is what is meant. Dose dependence often implies something else.

Page 4-10, 1st para under Interspecies Extrap – the protocol described leaves out the iterative model fitting step that is often needed to "tweak" parameters to obtain optimum fit to the calibration dataset. That should come before the 4th sentence. Also, in the next step, it would be good to clarify what is meant by chemical-specific parameters (biochemical parameters such as metabolic and transport rates, partition coefficients).

APPENDIX F
OBSERVER COMMENTS

Comments by Eric Hack

Toxicology Excellence for Risk Assessment (*TERA*) was sponsored by the Department of Defense to prepare oral comments for this peer review meeting. The comments provided are those of *TERA*.

General comments

This document gives a good description of PBPK modeling, and covers the basics of how it can be used to reduce uncertainty in risk assessment. The compilations of existing models for kinetics and dynamics are also useful. The document will have merit in that it will help increase the understanding and acceptance of the need for quantitative risk analysis methods among qualitative risk assessors. EPA should be applauded for undertaking this effort.

Addressing the issues highlighted below will add to the value of the document.

Significant Issues:

There were a number of issues (such as the application of PBPK models to use data to replace uncertainty factors) that were addressed briefly in Chapter 2, but much more thoroughly in Chapters 3 and 4. It would be useful to note in Chapter 2 that these issues are addressed in more detail later. It would also be useful to include one integrated discussion of some topics, such as the use of PBPK models for interspecies and intraspecies extrapolation or variability and uncertainty analysis, since some people may use the document more as a lookup document.

While the document does mention the term chemical-specific adjustment factors (CSAFs) in Section 2.6.3, it would be useful to use this term, and to cite the IPCS (2005) guidance when the specific examples are provided in Chapters 3 and 4. This discussion should note that one may be able to obtain in vivo data (e.g., blood concentration) to address interspecies differences in pharmacokinetics, it is very unlikely that one could obtain enough samples to adequately characterize human variability in kinetics, and so the PBPK model is essentially the only way to do so (as discussed in IPCS, 2005). The Gentry et al. (2002) article cited in the detailed comments carried the calculations for dose variability resulting from polymorphisms through to the calculation of CSAFs. I was glad to see the IPCS 2001 citation, but it would be useful to include the citation in the text (e.g., rather than only citing the Naumann paper), in the context of the discussion of uncertainty factors. In addition, the 2001 document has been superseded by the finalized (2005) document, at
http://www.who.int/ipcs/methods/harmonization/areas/uncertainty/en/index.html

In the model evaluation section, the discussion of assessing model fit is oversimplified. Visual fitting and a criterion that the predictions should be within 2 standard deviations of the mean is described, but other goodness of fit metrics also exist. For example, Krishnan et al. (1995) proposed a PBPK index based on root mean square of the error between predictions and data. The description of the 2 standard deviations rule should be offered as perhaps one measure of goodness of fit, but it is too simplistic to be relied upon to evaluate all PBPK models under all circumstances.

The sections on uncertainty and variability analysis needs more detail. More description of the methods and especially the issues associated with the interpretation of the results of these analyses and their use in risk assessment would be helpful. For example, the importance of accounting for parameter correlations in MC or MCMC analyses, issues with the direct use of MCMC results in estimating dose metric distributions, the impact of model error on the results of MCMC analyses, or the proper handling of mixed group and individual observations in MCMC analysis.

More discussion of the use of surrogates for target doses should be included. Using surrogates, such as blood concentrations rather than target tissue concentrations, the use of parent chemical concentrations or flux through a metabolic pathway instead of toxic metabolite concentrations, is sometimes necessary when the knowledge of the mode of action is more detailed than the kinetic knowledge, or when the toxic moiety cannot be measured directly.

Discussion of fetal or neonatal modeling should be included in the document. This is an important contribution of PBPK modeling to the assessment of developmental toxicity.

There is a list of possible dose metrics is given in Box 2-1. This choice is critical for dose metric estimation, and more discussion of when each choice is appropriate would be helpful.

Although it is mentioned later in the document, the description of the uses of PBPK models in chapter 2 should include mode of action hypothesis formulation and testing.

Editorial and minor comments:

Using PBPK for duration adjustment may also be useful for the oral route, not just inhalation. It can be used to account for gavage vs. drinking water dosing, or dosing 5 days/week vs. 7 days/week.

P. 2-4, line 5. There are multiple Clewell et al. 2002 papers in the references. Which one is cited?

P 2-7, line 9. Should mutagenic be replaced with genotoxic?

P 2-8, line 32. Specify excess risk above background in the BMDL definition.

P 2-10, line 29. Define flow-limited perfusion.

Section 2.5.5. Should use standard notation for interspecies UF (i.e. UF_A) and the intraspecies variability factor (i.e. UF_H).

P 3-6, Table 3-2. The equations are incorrect. The rate of change in the cellular matrix should be $[PA]*(C1/V1 - C2/P_{tissue:blood})$, and this term is subtracted in the equation for the change in blood.

Section 3.4.1. Consider adding a citation for compilation of rodent neonatal physiological parameters:
Gentry, P.R., Haber, L.T., McDonald, T.B., Zhao, Q. Covington, T., Nance, P., Clewell III, H.J., Lipscomb, J.C., and Barton, H.A. 2004. Data for physiologically based pharmacokinetic modeling in neonatal animals: physiological parameters in mice and sprague-dawley rats. Journal of Children's Health. 2(3-4): 363-412.
ILSI also has a major project underway for compiling human and rodent neonatal physiological parameters.

Section 3.5. Since this section includes ways to verify that the model is coded properly, this is a good section for saying that a mass balance calculation should be included and checked.

Table 3-7. I believe there is an X-windows graphical user interface for MCSim. I was surprised to see no mention of the EPA software (is it under development). Even if under development, it would be useful to mention it.

P 3-18. There are some inconsistencies with the discussion of model evaluation. On line 12, the document says that the visual evaluation approach says nothing about the adequacy of the model structure or parameters. I disagree with this statement. On lines 16-17, it correctly says that inadequacies in the model structure can be inferred using this approach. Later, on page 3-20, line 19, the document says that visual inspection is the best approach to model evaluation.

Figure 3-3. It should be emphasized that one should also consider the uncertainty in the different parameters. For example, even though the model is sensitive to blood:air PC, if this parameter is very well known, then it will be changed very little in an uncertainty analysis, and will result in little uncertainty about the dose metric calculations. On the other hand, VMax is often known with little certainty, and although the model is less sensitive to this parameter, it will be very much more widely in an uncertainty analysis and contribute much more to the uncertainty in the dose metrics.

P. 3-23, lines 3 to 27. This is a very good point, and it is nice to see it given some discussion.

P. 3-23, line 12. Should this be “compensate to some degree for the change in **breathing rate”**?

P. 3-24, line 13. While we appreciate the citation of Haber et al. 2002, this paper only provided the background information about the polymorphisms. The PBPK/Monte Carlo simulation was published as
Gentry, P.R., C.E. Hack, L. Haber, A. Maier and H.J. Clewell, 3rd. 2002. An Approach for the Quantitative Consideration of Genetic Polymorphism Data in Chemical Risk Assessment: Examples with Warfarin and Parathion. Toxicol Sci. Nov, 70(1):120-39.

P. 3-24, line 31. Point out that one of the advanatages of the MCMC approach is that the posterior parameter distribution obtained is a joint, multivariate distributions that accounts for correlations between the parameters.

P. 3-27, line 24. Include physiological parameters in the bulleted list of minimal data required to build a model.

P. 3-28, line 13. Expand that the critical study is the critical study used for development of the risk value (RfC, RfD, or cancer unit risk).

P. 4-1, lines 21-23. This is very simplified. I guess the peer review is expected to capture all of the model evaluation criteria described earlier? Bullet 2 above includes 'and evaluated for its structure and parameters'.

Box 4-1. There are bullets in this box that were not in the chapter 3 summary (e.g., major sites of storage).

Figure 4-4. A description of how the 4-hour inhalation exposure duration was selected is needed in the text describing this figure, or in a footnote.

Section 4.3.4. Add a caveat regarding extrapolation over very long durations and pharmacodynamic factors that may dominate (see Clewell, Andersen, and Barton, 2002).

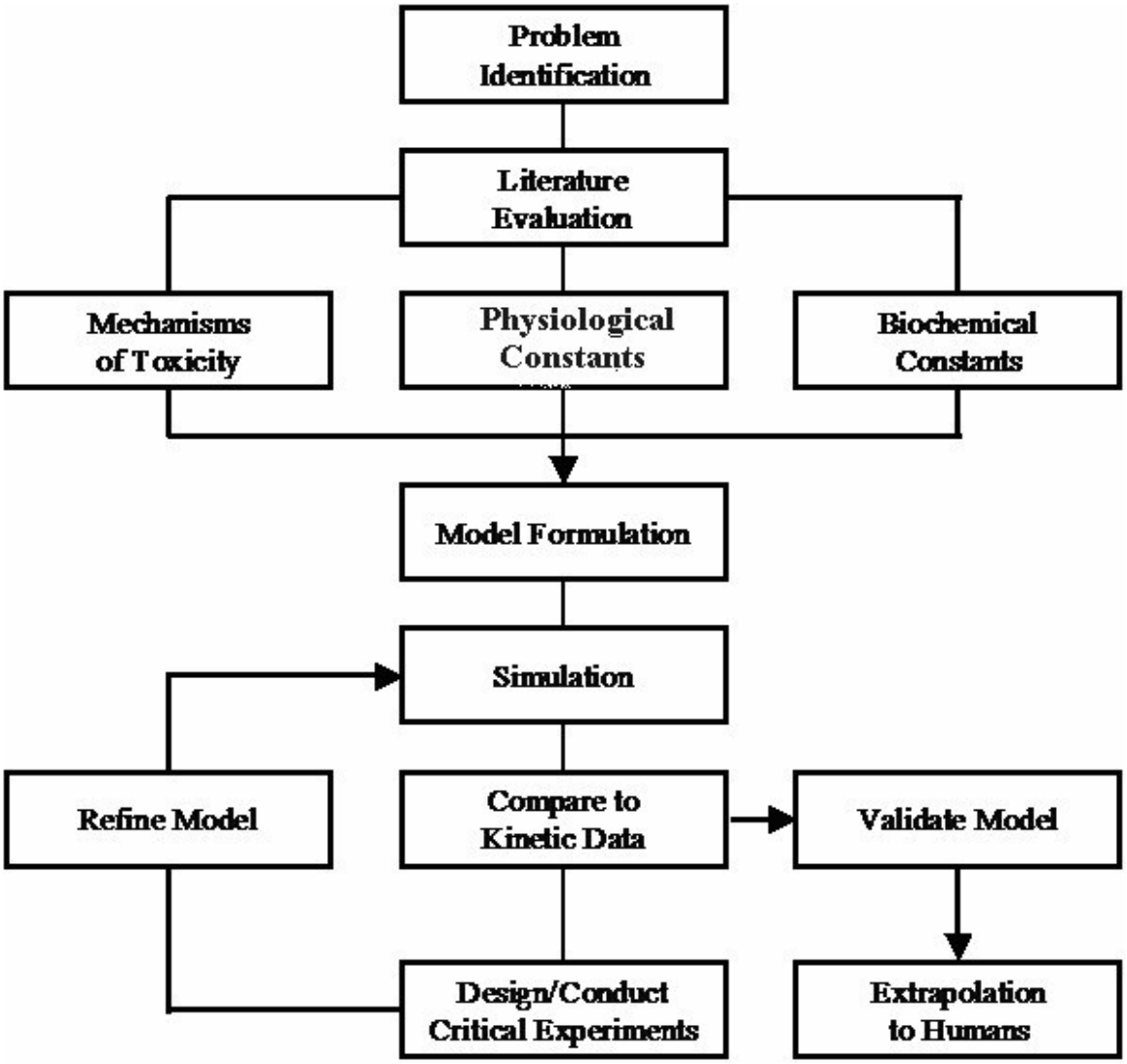
Section 4.3.5. Consistent with other language in the document, intraspecies variability sounds more appropriate as a header than intraspecies extrapolation.

P. 4-15, line 12. Why does the distribution have to be a unimodel normal distribution? I understand why you would want a unimodel distribution, but it does not have to be normal.

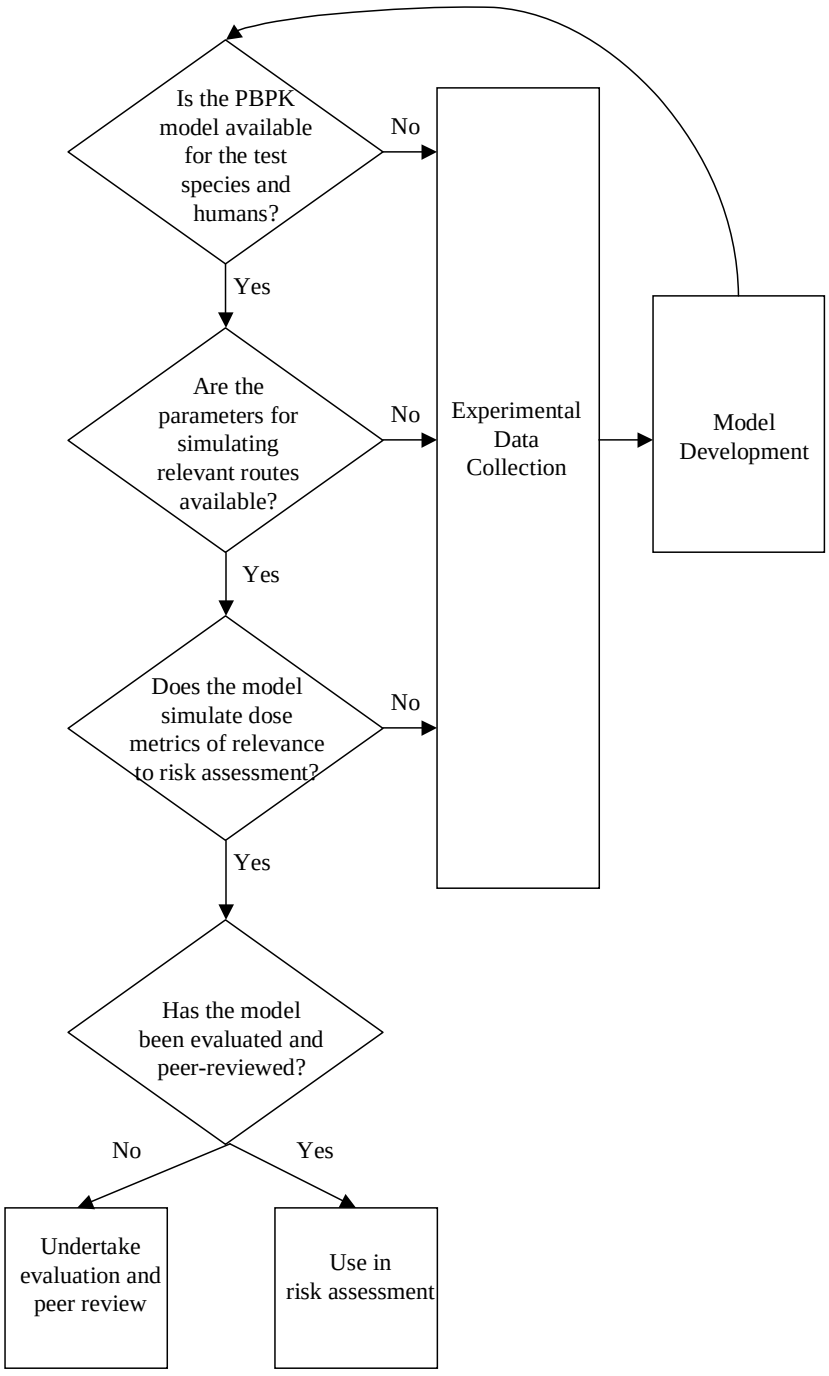
P. 4-18. It would be useful to use vinyl chloride as a case example for the requirements and capabilities of PBPK for route-to-route extrapolation (mentioned earlier, but not with an example.) First, VC met the requirement that systemic toxicity was the concern, and that the same endpoint was applicable for the oral and inhalation routes. Second, use of route-to-route allowed the use of a better study for the derivation of the RfC. A NOAEL was available for the oral route, but not for the inhalation route.

APPENDIX G
ADDITIONAL REFERENCES AND SUGGESTIONS

PBPK Modeling Process Flowchart:



Proposed Figure 4-1 Modification:



Validation of Dose Metric Prediction:

Ideally, a PBPK model should be compared with data that is informative regarding the parameters to which the dose metric predictions are sensitive. This pre-supposes the use of sensitivity and uncertainty analysis to identify the parameters of concern (those that have the most influence on the dose metric estimate and are the least certain). For example, validation of a human model based solely on parent chemical data would not necessarily provide confidence that the model could be used to predict a metabolite dose metric. The use of parent chemical kinetic data to validate model estimates of metabolism in the human can be highly misleading, because it is often the case that the metabolism parameters have little impact on parent chemical concentrations, while other uncertain parameters (e.g., ventilation rate, blood flow, fat content) can strongly influence model predictions of parent chemical kinetic behavior. Time-dependent sensitivity analysis could be conducted with the model under the conditions of the "validation" exposure to determine whether the metabolic parameter values are identifiable from the data.

Calculation of Dose Metrics:

An important consideration in risk assessments conducted with a PBPK model is that the critical study (the study showing effects at the lowest exposure) cannot always be selected on the basis of administered dose or exposure concentration. This is because the relationship of the HEC or HED to the administered animal dose depends on the selected dose metric, which may vary from one endpoint to another, and the nature of the exposure (species, route of administration, vehicle, duration, etc.). Instead, the pharmacokinetic model is used to calculate the appropriate dose metrics for each of the endpoints of concern in each study (Barton and Clewell, 2000). To calculate the dose metrics, the model parameters are set to those for the species in the toxicity study, whether an experimental animal study or a human study. In the case of developmental studies, it will be necessary to estimate parameters for a pregnant female or neonate rather than an average adult, and physiological and biochemical parameters may have to be time-dependent. To the extent possible, study-specific data on animal strain, body weights, age, and activity should be used in selecting parameters for the model. The experimental parameters in the model are then set to reproduce the exposure scenario performed in the study, and the model is run for a sufficient period of time to characterize the animal exposure to the chemical and, if necessary, its metabolites.

There are often a number of options regarding the way in which the model should be run to characterize the dose metric Clewell et al. 2002a). These will depend upon the dose metric(s) selected (e.g., peak vs, average), the nature of the chemical (e.g., volatile vs. persistent), and the nature of the risk assessment (acute vs. chronic, cancer vs. noncancer). Usually, the desire is to estimate an average daily dose metric, such as the average daily AUC. (Note that the average daily AUC is the same metric as the time-weighted average concentration, differing only by a factor of 24.) In general, the averaging period in the case of cancer is typically taken to be the lifetime, while the averaging period in the case of noncancer risk assessment is usually considered to be the duration of the exposure or, perhaps, a critical window of susceptibility.

For short-term exposures, the model must be run for an appropriate period, which depends on the dose metric being used and the timing of the measurement of toxicity in relation to the period of exposure. For short exposure, this is easily done by running the model for the total duration of the exposure (or exposures, for repeated exposure studies) to obtain dose metrics. If the animals were held for a post-exposure period before toxicity was evaluated, the model must be run either till the end of the post-exposure period or for a sufficient duration to ensure that the parent chemical has been completely cleared from the body or, for a dose metric based on a metabolite, a long enough time to ensure the complete clearance of the metabolite. On the other hand, if neurological tests were performed immediately at the end of the exposure period, then the dose metric should be determined at, or up to, that time. The resulting dose metric obtained for the total duration of the exposure (including any post-exposure period) can then be divided by the number of days over which the experiment was conducted in order to derive the average daily value.

The same approach (running the model for the total duration of the study) can be used to calculate dose metrics for longer-term exposures. This approach would typically be necessary for models that describe changes in physiology or biochemistry during different lifestages (*e*.g., children, elderly). However, an alternative approach, which is often attractive for modeling of chronic exposures with time-invariant model parameters, is to estimate the steady-state dose metric. There are two principal methods for calculating a steady-state estimate. In the first, the model is run until steady state is reached and then the dose metric is calculated by subtraction. For example, in the case of a chronic oral or inhalation exposure conducted 5 days per week, the model can be run consecutively for 1 week, 2 weeks, 3 weeks, and so on. To calculate the average daily AUC for a given week, the value at the end of the previous week is subtracted from the value at the end of the current week and the result is divided by 7. This process is repeated until the change in the dose metric from one week to the next is insignificant. For continuous exposures, the comparison can be made on a daily basis instead of weekly. The other method for estimating the steady-state dose metric is to estimate it from a single day exposure. The model is run for a single-day exposure plus an adequate post-exposure period to capture clearance of the parent compound or relevant metabolite. This value of the single-day dose metric is then modified by the necessary factors to obtain an average daily value (*e*.g., by multiplying by five-sevenths in the case of the 5-day per week exposure just described). This method is faster, but is only approximate if the system is not linear. Typically, it is sufficiently accurate for estimating average daily AUC when exposures are below the onset of any nonlinearities. It can be checked against the first method described to determine its accuracy in a particular case.

The dose metric calculations needed are determined by the method to be used for the noncancer or cancer analysis. If the NOAEL/UF method is being used in a noncancer risk assessment, a dose metric only needs to be calculated for the NOAEL or LOAEL exposure for a particular study and endpoint. On the other hand, if dose-response modeling is going to be performed, such as in the Benchmark Dose approach, dose-metrics must be calculated for all exposure groups. The dose metrics are then used in the dose-response model in place of the usual exposure concentrations or administered doses. It is important to remember that when this is done, the result of the dose-response modeling will also be in terms of a value of the dose metric rather than an exposure concentration or administered dose. Dose-response modeling is more properly conducted on the dose metrics, since it is expected that the observed effects of a chemical will be more simply and directly related to a measure of target tissue exposure than to a measure of administered dose.

In order to convert an animal dose metric (e.g., at the Benchmark dose) to an equivalent exposure concentration or administered dose, the pharmacokinetic model must be either run repeatedly, varying the exposure concentration or administered dose until the dose metric value is obtained. In the case of calculating the acceptable human exposure corresponding to a given toxicity study, the physiological, biochemical, and exposure parameters in the model are set to appropriate human values and the model is iterated until the dose metric obtained for the human exposure of concern, often continuous or daily lifetime exposure, is equal to the dose metric obtained for the toxicity

study, or, once again by dose estimation by a line-search regression. The dose metric should be calculated in an analogous way to the dose metric for the toxicity study; that is, if the dose metric in the toxicity study was expressed in terms of an average daily value, the dose metric used for calculating the associated human exposure should also represent an average daily value. When a steady-state dose metric is used in both an experimental animal and the human, it should be noted that the calculation of a steady-state dose metric in the human generally requires running the model for a much longer period of time than in the animal. For short-term exposures, where the model has been run for the total duration of the toxicity study and the average dose metric value has been calculated, the dose metric used for calculating the associated human exposure should usually be obtained for an exposure over the same time period. An exception to this rule is the case where it is anticipated that the short-term exposure of concern for the human may represent a short-term excursion against a background of chronic exposure. In this case, a more conservative approach may be preferred, in which a steady-state dose metric calculation is used for the human.

Model Evaluation:

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

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

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

In some cases it may be considered necessary or preferable to use all of the available data to support model development and parameterization. Unfortunately, this type of modeling can easily become a form of self-fulfilling prophecy: models are logically strongest when they fail, but psychologically most appealing when they succeed (Yates, 1978). Under these conditions, model evaluation can be particularly difficult, putting an additional burden on the investigators to substantiate the trustworthiness of the model for its intended purpose. Nevertheless, a combined model development and verification can often be successfully performed, particularly for models intended for interpolation, integration, and comparison of data rather than for true extrapolation.

Parameter “Fitting”:

When parameter estimation has been performed by optimizing model output to experimental data (“fitting”), the investigator must assure that the parameter is adequately identifiable from the data (Carson et al., 1983). Moreover, the practical reality of modeling biological systems is that regardless of the complexity of the model there will always be some level of "model error" (lack of homomorphism with the biological system) which can result in systematic discrepancies between the model and experimental data. This model structural deficiency interacts with deficiencies in the identifiability of the model parameters, potentially leading to mis-identification of the parameters. Due to the confounding effects of model error and parameter correlation, it is quite possible for an optimization algorithm to obtain a better fit to a particular data set by changing parameters to values that no longer correspond to the biological entity the parameter was intended to represent. It is usually preferable, prior to performing an optimization, to repeatedly vary the model parameters manually to obtain a sense of their identifiability and correlation under various experimental conditions, although some simulation languages include routines for calculating parameter covariance or for plotting joint confidence region contours. Estimates of parameter uncertainty obtained from optimization routines should be viewed as lower bound estimates of true parameter uncertainty since only a local, linearized variance is typically calculated. In characterizing parameter uncertainty, it is probably more instructive to determine what ranges of parameter values are clearly inconsistent with the data than to accept a local, linearized variance estimate provided by the optimization algorithm. Although MCMC parameter estimation may lead to systematic errors, it might be useful in calculating parameter uncertainty.

Model Verification:

It is important to differentiate model verification and model validation. In brief, model validation deals with building the right model, while model verification deals with building the model right (Balci 1997). The accuracy of transforming the chemical-biological system into a model specification (e.g. the model diagram or equations), and the accuracy of converting the model representation from a diagram or equations into an executable computer program is evaluated in model verification. Model validation, on the other hand, is substantiating that the model, within its domain of applicability, behaves with satisfactory accuracy.

Verification of a PBPK model involves evaluation of the biological plausibility of the model structure and parameters as described in the documentation, and the mathematical correctness of the equations. PBPK model verification also involves examination of the model code to assure that it mathematically implements the model as described in the documentation. This examination includes checking for correctness of statements and functions, and correct order of statement execution (for languages that are not self-sorting). Improper statement order in a numerical integration code can result in a model that appears to run normally but gives the wrong results. A problem common to some graphic model representations is the inadvertent mis-specification of a parameter as local vs. global in one of the compartments, again resulting in a model that appears to run normally but gives the wrong results.

Whether a code- or graphical-based model is used, it is preferable that the language produce as one possible output the set of equations that constitute the PBPK model. Code-based representations ease the task of insuring that the model is actually constructed as described in the documentation. To facilitate model verification, the model code should be organized and commented in such a way as to facilitate understanding by individuals other than the original program developer. In the case of a model intended for use in a risk assessment application, it is imperative to provide documentation of the particular parameter values and simulations that are required to reproduce any validation runs and dose metric calculations. This usually entails the provision of step-by-step directions, either using the language's scripting capability or in separate documentation, that allow reproduction of the validation plots and dose-metric calculations by following specific directions or by invoking specified program blocks.

Model Documentation:

Adequate documentation is critical for evaluation of a model. The documentation for a PBPK model should include sufficient information about the model so that an experienced modeler could accurately reproduce its structure and parameterization. Usually the suitable documentation of a model will require a combination of one or more "box and arrow" model diagrams together with any equations which cannot be unequivocably derived from the diagrams. In fact, for simple models a well-constructed model diagram, together with a table of the input parameter values and their definitions, is all that an accomplished modeler should need in order to create the mathematical equations defining a PBPK model. The model diagram should be labeled with the names of the key variables associated with the compartment or process represented by each box and arrow. All tissue compartments, metabolism pathways, routes of exposure, and routes of elimination should be clearly and accurately presented. The model diagram should also clearly differentiate blood flow from other transport (e.g., biliary excretion) or metabolism, and arrows should be used where the direction of transport could be ambiguous.

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

Interpretation of the model diagram is supplemented by the definition of the model input parameters in the corresponding table. The definition and units of the parameters can indicate the nature of the process being modeled (e.g., diffusion-limited vs. flow-limited transport, binding vs. partitioning, saturable vs. first-order metabolism, etc.). The values used for all model parameters should be provided, with units. If any of the listed parameter values are based on allometric scaling, a footnote should provide the body weight used to obtain the allometric constant as well as the power of body weight used in the scaling. Any equations included to supplement the diagram should be dimensionally consistent and in a standard mathematical notation. Generic equations (e.g., for tissue "i") can help to keep the description brief but complete.

Model Calibration:

A critical issue in the use of Bayesian approaches, such as Markov Chain Monte Carlo (MCMC) analysis, for the "calibration" of PBPK models in risk assessment applications is whether the posterior distributions for the PBPK parameters should be used to estimate dose metrics in place of the prior estimates based on the scientific judgment of the model developers. One concern is that the Bayesian approach may give undue priority to the particular studies included in the Bayesian analysis (as compared to potentially more appropriate studies that may have been used to inform the various parameters in the model, but which were not amenable to incorporation in the MCMC analysis.

Another consideration is whether the subject population in the data sets included in the MCMC analysis is representative of the population for which the risk assessment is being performed. For example, the subjects in controlled human exposures may be at rest, and the MCMC my correctly estimate a relatively low ventilation rate; however, this ventilation rate may not not be appropriate for the activity level in the general population. Therefore, calculations of dose metrics and uncertainty/variability analyses performed in support of an environmental risk assessment would more properly use a ventilation rate suitable for the general population, not the posterior obtained from the experimental subjects.

The greatest value of MCMC analysis is its ability to characterize the variability and uncertainty in the model predictions, and as such is most appropriately used in the risk characterization segment of a risk assessment. Its use in the dose-response assessment is more problematic.