Report of the Peer Consultation of Harmonized PBPK Model for Trichloroethylene

Peer Consultation Organized by
Toxicology Excellence for Risk Assessment
(http://www.tera.org/peer/vccep)

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Executive Summary

An independent panel of expert scientists met in a public meeting on June 29, 2004 to provide comment on a draft harmonized physiologically based pharmacokinetic (PBPK) model for trichloroethylene (TCE). This peer consultation meeting was conducted by Toxicology Excellence for Risk Assessment (*TERA*) and the effort was completed at the request of the United States Air Force (USAF) and the United States Environmental Protection Agency (EPA).

TERA is facilitating and providing support to a USAF-EPA workgroup developing a harmonized PBPK model for TCE and its metabolites. PBPK modeling is a useful method for improving the accuracy of chemical risk assessment. Since TCE metabolism varies in different tissues and organs, and the active chemical species responsible for the adverse effects in various organs may differ, PBPK models are useful in describing the kinetics of TCE and its metabolites in biological systems. The toxicokinetics of TCE and its metabolites in the body have been studied extensively in many laboratory animal species, and in humans. Based on these data, several PBPK models have been developed and published. The intent of the overall project is to use the best science and updated data to derive a comprehensive harmonized TCE PBPK model as an input for ongoing TCE risk assessment activities.

Expert peer consulters knowledgeable in the areas of TCE toxicokinetics, toxicodynamics, and PBPK modeling and calibration methods provided independent critical comments on the draft harmonized TCE PBPK model and associated documentation (hereinafter referred to as the document). This brief summary describes the major discussion topics and conclusions of the panel members.

Completeness of Toxicokinetic Data Used

A panel member suggested that the document include a comprehensive inventory of all toxicokinetic data sets for TCE (including human, mouse and rat data sets) to assist in verifying that the model had been appropriately derived and validated using all of the relevant existing data. Several panel members also suggested additional data that could be useful to enhance aspects of the PBPK model. Additional sources of data noted as potentially useful for enhancing the model included:

- A human study of TCE inhalation exposure by Lapare et al. (1995) (provided to authors at the meeting)
- Data on GST pathway metabolites presented in several published studies (provided by panel member at the meeting)
- Unpublished studies on chloral metabolism in humans (provided by panel member at the meeting)
- Published data on binding of TCE metabolites to liver and kidney tissue of rats and mice (Eyre et al., 1995a,b)
- Literature on kinetics of alcohol metabolism in the lung and liver

Modeling of the Glutathione Conjugation Pathway

There was general agreement that, given uncertainty in the choice of the appropriate dose metric and in the kinetics of individual steps in the glutathione (GSH) conjugation pathway, that it would be preferable to use a simpler model based on less specific estimates of the overall metabolic pathway that can be more clearly validated. Panel members noted that the decision to add detail in the specific kinetic pathways should consider the risk assessment context. However, panel members preferred using dose metrics based on internal dose rather than external exposure. Based on these considerations, it was suggested that one approach would be to start with flux through the GSH conjugation pathway, and add more specificity as the data allow. Panelists noted that using flux through the GSH conjugation pathway may overestimate formation of reactive metabolites in humans, based on information (noted by the authors) suggesting that the activity of beta-lyase (the enzyme that activates dichlorovinylcysteine - DCVC) is lower in humans than in rodents. However, concern was expressed by several members that it is unclear whether flux is a health-protective metric in light of uncertainties about the metabolism pathways for GSH-conjugate metabolites, including the role of flavin monoxygenase (FMO). Therefore, the implications of using this measure should be further considered.

Several issues and additional lines of consideration were noted by the panel members. It was suggested by a panel member that additional data on levels of GSH conjugates and mercapturic acids be evaluated to estimate flux through the GSH conjugation pathway. Information on disposition of GSH conjugates in the liver versus kidney should be evaluated to clarify assumptions in the model regarding the ultimate delivery of all GSH conjugation pathway metabolites to the kidney. Potential sources of human variability in flux through the GSH conjugation pathway noted by panel members included effects of diet on sulfhydryl pools, and polymorphism of genes encoding glutathione-S-transferase (e.g., null genotype of *GST*-mu) and N-acetyltransferase (*NAT*).

Modeling of Chloral in the Lung and Liver

The panelists agreed with the conclusions in the document that there does not appear to be a compelling need to include systemic chloral generation in the assessment of lung tissue dose. The panel also agreed that if important uncertainties regarding interspecies differences in chloral formation and clearance in the lung and issues surrounding systemic distribution of choral from the liver could not be resolved based on available data, an alternative approach could reasonably be used. The suggested simplifying approach was to use arterial blood concentration of TCE as the dose metric for the lung, assuming lung chloral production and clearance scale proportionately across species. The panel discussed issues surrounding chloral production and clearance in detail as described in the following paragraph.

A panel member noted that including estimates for chloral kinetics in the liver would add flexibility to the model for use of data generated from dosing studies with chloral itself, and that this dose metric would be important for certain endpoints that may be attributable to the chloral metabolite. Two panel members suggested that there may be sufficient data in the literature regarding the following processes to develop a model for estimating chloral kinetics in the liver:

(1) estimates of kinetic parameters for alcohol dehydrogenases, (2) the impact of trichoroethanol (TCOH) and trichoroethanol glucuronide (TCOG) levels on the reverse reaction to form chloral, and (3) the role of cellular redox state on rates of chloral formation, since TCOH may be cleared as a zero-order process under the prevailing redox conditions. Some panelists suggested that data on these parameters may be estimated from the body of data on general alcohol metabolism, although one panelist noted that due to substrate specificities data would be needed for choral itself. It was suggested that additional data from an unpublished study of chloral administered to humans (provided by a panel member at the meeting) may be useful to evaluate chloral kinetics in the liver.

Modeling of Dichloroacetic Acid

A panel member commented that it would be appropriate to simplify the modeling of the DCA pathway if the current uncertainties in the data result in poor confidence in the resulting dose metric estimates. Several other panelists agreed with this suggestion. An alternative suggestion for a simplified approach included using flux through the relevant portion of the oxidative pathway. For example, one such metric could be derived using some fixed proportion of the total oxidative metabolites normalized to liver volume. Several panel members suggested calculating the amount of TCE exposure that would be needed to generate a sufficient amount of DCA in the liver to raise a concern for liver effect, based on data from available DCA toxicity studies in experimental animals. This approach, coupled with further mode of action studies, would help to determine the degree to which more detailed modeling of DCA kinetics should be pursued. Uncertainty regarding the accuracy of DCA measurements in existing studies was noted as an important issue, with the extent of DCA formation from TCE in exposed humans of specific concern. One panelist recommended that the authors further explore the variability in DCA measures in the human exposure studies (where detectable DCA levels were found in only a few of the tested subjects). One panel member suggested that this difference between low levels of DCA in humans and higher levels in experimental animals may reflect differences in glucuronidation capacity, affecting the proportion of TCOH metabolized to TCOG versus DCA. This person further suggested evaluating correlations among TCE metabolites in the individual study subjects to assess the source of the variability.

Trichloroacetic Acid Binding in the Blood

Several panel members commented that the proposed modeling approach is an appropriate use of the *in vitro* data to estimate *in vivo* kinetics. In response to a specific point raised in the panel charge, several panel members noted that, due to multiple binding sites and the large albumin binding pool in the blood, there would be little concern about competition for binding at the high doses (e.g., Rolan, 1994; Sansom and Evans, 1995), as used in the cancer bioassays). It was noted that some noncancer studies (e.g., Buben and O'Flaherty, 1985) may have used TCE concentrations that resulted in internal TCA doses below those of the cancer bioassays. As a result, it should be confirmed that the proposed model captures the binding activity observed *in vitro* and accounts for dose-dependent changes in binding *in vivo* that may occur at low concentrations (in the range of those that occur in low-dose noncancer studies).

Overall Model Structure and Parameterization

Several points were raised by panelists in discussing the appropriateness of the overall model structure. One issue was the authors' use of only a gastrointestinal (GI) tract compartment for drinking water exposure, but the inclusion of stomach and duodenum compartments for "oral dose" (i.e., gavage exposure). The authors explained that the stomach and duodenum were kinetic compartments only (not containing any tissue volumes), and are used to capture the kinetics of absorption from gavage, while drinking water intake is modeled as zero-order uptake. Several panel members commented that for longer-term drinking water studies it would be generally appropriate to ignore the daily temporal variability in intake. One panelist noted that this issue could be important if the model were used to estimate dose metrics in alternative short-term scenarios. A second issue raised by some panel members was the need to include to the degree possible specific kinetic descriptions of key metabolic steps, rather than using approaches based on proportions of total metabolite generation. Nevertheless, simplifying approaches were considered reasonable in some cases (e.g., addressing GSH conjugation, DCA formation and metabolism, and chloral kinetics in the lung).

Several panel members also suggested further evaluation of the appropriate approach for selecting Vmax of CYP2E1-mediated oxidation of TCE in humans, since a wide range of values was suggested based on different human exposure studies and *in vitro* measurements. The panel discussed the merit of using different approaches to select the appropriate value for this parameter (e.g., use of a value that results in best fit of newer data sets, selection of a value that corresponds with prior estimates for this parameter and optimizing the model fit by changing other parameters, or relying on the model calibration effort to optimize the value for this parameter). Some panel members noted that variability in the human data sets is an underlying cause of the uncertainty in this parameter estimate. Differences in the low end of the range of estimated values have an impact on the model fit, while estimates in the high range are within the region where metabolism becomes flow-limited and thus have a limited impact on the model fit.

Specific issues regarding model structure raised in the charge questions were also addressed. One panel member commented that there does not appear to be a need to include a diffusion-limited fat compartment in the model structure, noting that keeping the model simple is appropriate if adding complexity does not improve overall model predictions. Another panel member provided unpublished data from a chloral study that may be useful in evaluating the role of enterohepatic circulation in explaining apparent peak TCA concentrations.

Model Calibration

The panel discussed the merit of calibrating the model using a Markov-Chain Monte Carlo (MCMC) approach. One panel member encouraged the authors to use MCMC to calibrate the model and to update all parameters simultaneously using all available data sets. Other panel members generally agreed that an attempt should be made to do this type of analysis, with individual panel members having differing levels of confidence in interpretation of results generated by this approach. The panel members advised critical and cautious implementation of the technique, and noted that more research is needed in how to apply MCMC analysis for PBPK model calibration. A number of issues and uncertainties were raised by individual panel

members in the discussion for consideration by the authors, such as the importance that the authors:

- Ensure that the intended use of the MCMC analysis is clear. Is it to parameterize the model or to evaluate uncertainty in the parameter estimates? If it is used for estimating uncertainty, then consistency with basic biology knowledge and application to appropriate populations should be considered (e.g., test subjects do not represent the full variability of the entire population). Conventional Monte Carlo modeling was suggested as an approach to address general population variability.
- Consider alternative approaches of using all data to calibrate the model. Consider cross-validating the model by sequentially pulling out individual data sets to test the model, versus an alternative approach of holding back a single data set to validate the model outputs.
- Consider alternative approaches of allowing the MCMC to update all physiologic and kinetic parameters using meaningful biologically-based limits versus restricting updating to kinetic or uncertain parameters.
- Consider whether the order of sequential updating impacts the resulting posterior distributions.
- Determine the impact of limitations in the MCMC modeling software for weighting different data sets.

Key Data Gaps and Research Needs

Panel members were asked to provide suggestions regarding key data gaps and research needs to improve the basis for the draft TCE PBPK model. Data gaps and potential experimental approaches were noted by individual panel members, with suggestions focusing on approaches that would fill high-priority data gaps most efficiently. These suggestions included:

- Characterize the kinetics of FMO metabolism of GSH-conjugation pathway metabolites.
- Develop *in vitro* dose-dependent kinetic data for TCOH glucuronidation and alcohol dehydrogenase activities and characterize the enzymes responsible for these activities in mouse and human lung cells. The results would inform interspecies differences in lung chloral generation and clearance.
- Need to better evaluate the formation of DCA from TCE. A first step is to follow up on the studies of Guengerich to better characterize the rates of DCA formation from TCE in microsomal preparations.
- Additional mode of action studies (e.g., comparative toxicogenomic studies of TCE and its metabolites in tumor tissues or TCA dosing studies in peroxisome proliferation receptor knock-out mice) to determine the potential contribution of DCA to TCE induced tumorigenesis may be useful. Further evaluation of the dynamic components of TCE toxicity would help to focus resources on aspects of kinetics that are most likely to have the greatest impact.

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1 Background

Toxicology Excellence for Risk Assessment (*TERA),* a non-profit organization dedicated to the best use of toxicity data in risk assessment is facilitating and providing support to a United States Air Force (USAF) - United States Environmental Protection Agency (EPA) workgroup developing a harmonized physiologically-based pharmacokinetic (PBPK) model for trichloroethylene (TCE). The workgroup was composed of scientists from the USAF and EPA, with additional technical expertise from *TERA* and independent scientists. As a part of this effort, *TERA* organized and convened a peer consultation panel meeting of independent expert scientists on June 29, 2004. The meeting's purpose was to comment on a draft report harmonizing the various PBPK models. The comments expressed at the meeting will be used to improve the harmonized TCE PBPK model; the model then will be made public to facilitate its use and consideration in ongoing TCE risk assessment activities and risk assessments.

The peer consultation meeting followed a standard *TERA* process, beginning with a close examination of the supporting documentation and important references by the expert panel prior to the meeting. At the meeting, the authors of the model briefly presented their work. Copies of the document authors' presentations are provided in Appendix A. The panel then systematically discussed the draft PBPK model, including a discussion of key issues identified in the context of panel charge questions (which form the structure of the subject headings in these technical meeting notes). Full discussion and participation were encouraged to solicit input from the expert panel members; however, because this was a peer consultation, no effort was made to reach consensus on the issues. The meeting was open to the public, and a number of observers were present. Meeting observers were offered the opportunity to provide written or oral comments for panel consideration.

In order to maintain the independence of this peer consultation, *TERA* efforts in facilitating development of the model were separated from the work of organizing the peer consultation by assigning different staff members to each project. Therefore, the staff members who participated in development of the harmonized model were not responsible for organization of the peer consultation, including the selection of panel members, development of the charge for the panel, and preparing this meeting report.

TERA, as the independent group convening the peer consultation, was solely responsible for selection of panelists for the peer consultation of the TCE harmonized PBPK model. The primary criteria for selection were knowledge and experience in PBPK modeling and TCE metabolism. *TERA* carefully balanced affiliations and backgrounds of the members to provide a wide range of views and perspectives. Members of this panel are from universities, government agencies, non-profits, and consulting agencies for government and industry. More information about panel selection and conflict of interest and bias considerations is found in the meeting background document in Appendix B.

This meeting report contains a summary of the authors' presentations, panel discussions, and individual panel member suggestions and recommendations.

1.1 Sponsoring Organizations

United States Air Force (USAF)
United States Environmental Protection Agency (EPA)

1.2 Workgroup/Author Team

Dr. Jerry Blancato, EPA
Dr. Weihsueh Chiu, EPA*
Mr. Harvey Clewell, ENVIRON*
Ms. Tammie Covington, ENVIRON
Dr. Michael Dourson, *TERA*
Dr. Jeff Fisher, University of Georgia*
Mr. Eric Hack, *TERA*
Dr. John Lipscomb, EPA
Dr. Dave Mattie, USAF
Dr. Miles Okino, EPA
Mr. Fred Power, EPA
Dr. Jay Zhao, *TERA*

*Presenters

1.3 Peer Consultation Panel Members

Dr. Hugh Barton, Office of Research and Development, EPA
Dr. Richard J. Bull, MoBull Consulting
Dr. Hisham El-Masri, Agency for Toxic Substances and Disease Registry
Dr. Michael Gargas, The Sapphire Group
Dr. Lynne Haber, *TERA*, Chair
Dr. Gregory L. Kedderis, private consultant
Dr. Kannan Krishnan, University of Montreal

1.4 Conflict of Interest Disclosures

After a brief welcome by *TERA*, each panel member introduced him or herself and noted whether they had additions or changes in their disclosure statements. (Copies of panel members' biosketches and conflict of interest and bias disclosure statements were provided to all attendees and are found in Appendix B).

2 Introduction

Dr. Lynne Haber, as Chair of the panel presented ground rules for conduct of the meeting, noting that the discussion of issues would be limited to panel members. Two authors sat at the table to answer panel questions and ask clarifying questions of the panel members. In order to avoid the appearance of undue influence on the panel, all parties were asked to refrain from discussing

issues related to this consultation with panel members during the breaks unless a panel member initiated the discussion. Panel members were asked to summarize any substantive conversations for the rest of the panel and observers when the meeting reconvened after the break. Prior to the meeting, the authors and panel had been reminded that they should not discuss the model or issues with one another outside of this meeting.

The discussion period began with the authors making several short presentations and a demonstration of the model. The purpose of the presentations was to provide context for the modeling effort and highlight the salient points and issues, and to give the panel the opportunity to ask clarifying questions of the authors. The meeting Chair, Dr. Haber, then led the panel in their discussions, covering the issues and questions from the panel charge. Individual recommendations, comments, and suggestions for each charge question were made and the Chair noted if there was agreement amongst panel members or not.

3 Presentations

3.1 Presentation by Document Authors

The document authors gave short presentations summarizing the results of an effort to develop a harmonized PBPK model for TCE. This harmonized model was intended to integrate and update earlier generation models that were developed independently by groups led by Dr. Jeff Fisher and Mr. Harvey Clewell. Mr. Harvey Clewell gave an overview of TCE metabolism followed by a short chronology of previous PBPK modeling efforts for TCE. Applications of TCE PBPK modeling in the context of U.S. EPA's risk assessment process for TCE were also noted. The framework for the current effort to develop a harmonized model was described, with emphasis on updates and changes compared to earlier generation models. Dr. Jeff Fisher provided an overview of the harmonized model, including examples of model fitting to various data sets. Specific results addressing the significance of DCA inhibition of its own metabolism in the context of TCE dosing and data on TCA binding in the blood were presented. These presentations were followed by a brief demonstration of the model. Several questions were asked during the course of the presentations to clarify various technical points on the slides, but substantive clarifying issues were deferred to the panel discussion and have been incorporated into the notes under the relevant charge questions.

3.2 Public Participation

The meeting was open to the public. A list of attendees is found in Appendix C. Interested parties were offered the opportunity to provide written or oral comments for panel consideration. No written comments were received. One observer from a state agency asked the panel whether the harmonized model includes compartments for male reproductive tissues and the developing fetus and how the model could best be used to estimate dose metrics for the male reproductive and developmental effects of TCE. A panel member commented that for compounds that have reactive metabolites such as TCE, tissue-specific metabolism is important, which makes addressing this issue an important one. However, based on the limited prior work in this area the appropriate dose metric for TCE's developmental effects is unclear. The panel member was

unsure as to whether the data on male reproductive effects are sufficient to select a dose metric, and also questioned whether there are sufficient data to parameterize the model and to generate dose estimates for both humans and animals. A document author also responded to the observer's question. This document author commented that prior work (Barton and Clewell, 2000), discusses issues in selecting a dose metric for TCE when there are uncertainties regarding the mode of action. This author further suggested that for developmental and reproductive effects for most volatile organic compounds, blood concentration is generally an appropriate dose metric. A panel member agreed with this general recommendation, and noted there may be some data available on fetal versus maternal blood TCE concentrations in animals, which could be reviewed to refine this recommendation.

4 Panel Discussion

The panel discussion was organized according to a series of charge questions. Discussions surrounding each of these topics are presented below. The individual charge questions are provided in Appendix D.

4.1 Charge Question 1 - Other Available Data

A panel member suggested that the document include a comprehensive inventory of all toxicokinetics data sets for TCE (including human, mouse, and rat data sets) to assist in verifying that the model had been appropriately derived and validated using all the relevant existing data. The authors noted that a reference table of the studies used in developing and validating the model has been developed, but was not included in the documentation. One panelist commented that some data sets had very few data points, and that the authors should consider how the availability of only minimal data would impact the Markov Chain Monte Carlo (MCMC) calibration.

Several panel members also suggested additional data that could be useful to enhance aspects of the PBPK model. Additional sources of data noted as potentially useful for enhancing the model included:

- A human study of TCE inhalation exposure by Lapare et al. (1995). This reference was provided to authors at the meeting.
- Data on GST pathway metabolites presented in several published studies (e.g., blood GSH conjugate levels). Several references were provided to the authors at the meeting.
- Unpublished studies on chloral metabolism in humans. These studies were provided by a panel member at the meeting.
- Published data on binding of TCE metabolites to liver and kidney tissue of rats and mice (Eyre et al., 1995a,b).
- Literature on kinetics of alcohol metabolism in the lung and liver

4.2 Charge Question 2 -Modeling of the Glutathione Conjugation Pathway

One panel member opened the discussion on modeling the glutathione (GSH) conjugation pathway by commenting that, in the absence of detailed metabolic information, flux through the GSH conjugation pathway could be described as a first order process that could be used as a dose surrogate. It was suggested by this panel member that additional data on levels of GSH conjugates and mercapturic acids be evaluated to estimate flux through the GSH conjugation pathway, and several studies containing such data sets were provided to the authors. The use of this simplified approach is supported by the fact that GSH conjugation represents a very small portion of TCE metabolism relative to oxidative metabolism, and at such low levels significant nonlinearity or consideration of saturable kinetics or GSH turnover and synthesis would not need to be modeled. There was general agreement among panel members that, given uncertainty in the choice of the appropriate dose metric and in the kinetics of individual steps in the GSH conjugation pathway, it would be preferable to use less specific dose metrics for the overall metabolic pathway, if these alternative metrics can be more clearly validated. Panel members noted that the decision to add detail in the specific kinetic pathways should consider the risk assessment context, but it would be preferable to rely on internal dose metrics rather than external exposure to TCE. Based on these considerations, it was suggested that one approach would be to start with flux through the GSH conjugation pathway, and add more specificity as the data allow.

The panel also discussed whether the available data support a more detailed modeling approach of using dichlorovinylcysteine (DCVC) formation as a dose metric. A panel member commented that if DCVC levels are correlated with total thioacetylating metabolites, then it would be reasonable to use DCVC as the dose surrogate, with additional specificity in modeling not required. A document author commented that clearance of GSH conjugates and metabolites in urine could be used to calibrate flux though the pathway. However, the proportion of DCVC metabolism via beta-lyase (which activates DCVC) versus via N-acetyltransferases (which generate metabolites excreted in the urine) has not been verified *in vivo*. One panel member noted that the dose metric of DCVC formation could be estimated based on urine GSH conjugate levels and kinetic parameter estimates for beta-lyase activity. However, this panel member also expressed concern regarding the lack of data to adequately validate DCVC formation as a dose metric. Another panel member indicated that using *in vitro* enzyme kinetic data to estimate kinetic parameters for beta-lyase and N-acetyltransferase would be appropriate if possible, particularly if renal cancer is an outcome of concern. However, a document author noted that due to a lack of dose-response data for these enzyme activities, kinetic parameters (Km and Vmax) cannot be estimated. Given these uncertainties another panel member suggested that the authors build the model only to the level of detail that can be clearly validated, which requires a clear communication of what is and is not known. This panel member referred the authors to earlier papers published as a Special Issue in *Environmental Health Perspectives* (such as Barton and Clewell, 2000) to evaluate how well different dose metrics compare to tumor responses as a means to assess the need to pursue and include in the harmonized model various toxicokinetic pathways. Several other panel members noted agreement with this general approach.

A document author asked the panel whether GSH conjugate production as an alternative dose metric should be adjusted for species differences based on allometric scaling, or using species

differences in *in vitro* enzyme kinetics. One panel member noted that if a scaling approach were used, it would be important to estimate at low doses the proportion of metabolites that move through the activation pathway. Two panel members noted another difficulty in relying on species-based kinetic estimates - kidney toxicity is greater in mice than in rats given the same dose (Eyre at al., 1995a,b), even though kinetic data suggest that rats have a greater rate of metabolism in one step in this pathway as compared to mice. This suggests that species differences in dynamics (responsiveness at a given tissue dose) or additional kinetic differences (e.g., GSH conjugate production and downstream metabolism) have not been fully explored. Therefore, dose metrics derived based only on GSH pathway metabolites may not predict adverse kidney responses. An author confirmed that these uncertainties support using the more general approach of determining GSH conjugation pathway flux.

An author noted that estimating flux though the pathway using TCE minus all oxidative products would provide an upper bound estimate of the dose. A panel member noted that this estimate could be validated by comparing it to estimates of GSH pathway flux approximated from GST enzyme kinetics or NAT pathway metabolite levels in urine. This panel member noted that the latter approach, based on measured GSH pathway metabolites in urine, would give a lower-bound estimate of flux through the GSH pathway.

Panel members also noted that it is unclear whether the use of GSH pathway flux would lead to health protective estimates due to limitations in extrapolating kinetics across species and the apparent toxicodynamic differences between rats and mice. A document author noted however, that beta-lyase activities are lower in humans than rats, suggesting that humans would form less of the toxic metabolite. Therefore, estimating flux in rodents after adjusting with allometric scaling would tend to be health protective for humans. Panelists noted that using flux through the GSH conjugation pathway may overestimate formation of reactive metabolites in humans, based on the lower beta-lyase activity in humans than in rodents. However, several panel members suggested that the authors consider the implications of using GSH pathway flux since it is unclear whether it is a health protective metric.

One important area of uncertainty in modeling the GSH conjugation pathway discussed by the panel was the role of flavin monoxygenases (FMO). The panel discussed the potential importance of FMO as an alternative metabolic activation pathway for DCVC that was not included in the model. A document author noted that recent data suggest (e.g., Lash et al., 2003) that FMO activity may be a major pathway for DCVC metabolism, and may play a greater role than beta-lyase in humans. However, both beta-lyase and FMO activities are lower in humans than in rats and GSH conjugate excretion is significant in humans. These observations suggest that DCVC metabolism pathways other than beta-lyase are important in humans. The document author commented that if FMO is an important enzyme for the metabolism of DCVC, then the current model description of metabolism in the kidney is lacking an important consideration. Another reviewer commented that data may be available for liver FMO activities, but GSH conjugates are excreted via bile to the gut and are reabsorbed and distributed systemically to the kidney. Therefore, FMO activity in other organs may be an important data gap. Several additional panelists commented on difficulties raised by the absence of data on FMO activity. A document author noted that there are no data to determine the split between FMO and beta-lyase metabolism of DCVC, since urine metabolites do not provide information on metabolites formed

via these pathways. Another panelist noted that the relative importance of FMO versus beta-lyase is a critical issue since it impacts the ability to extrapolate between rats and humans in terms of DCVC activation. Another panelist noted that assuming a constant proportion of reactive metabolite generation may not be appropriate, noting there could be significant non-linearities at low doses.

The panel further discussed the use of total GSH conjugates formed in the liver as the estimate of dose to the kidney. The authors confirmed that the data support the conclusion that all GSH conjugates eventually are taken up by the kidney, but the specific kinetics are complicated due to factors such enterohepatic circulation. One panelist commented that the choice of the authors to model GSH metabolites based on metabolism in the liver would be a reasonable simplification, since metabolism of TCE in the kidney likely represents a much lower level of total activity as compared to the liver. Another panel member commented that using liver conjugates to represent kidney dose is likely a good assumption based on liver and kidney binding data in rats and mice, although the panel member noted that these data would need to be reconfirmed[1]. Furthermore, it was suggested by a panel member that disposition of GSH conjugates in the liver versus kidney should be evaluated to clarify assumptions in the model regarding the ultimate delivery of all GSH conjugates to the kidney.

Panel members identified several potential sources of variability in the GSH conjugation pathway. One panel member noted that human variability in flux through the GSH conjugation pathway may be affected by dietary impacts on sulfhydryl pools. Other panel members discussed the role of genetic polymorphisms. For example, an author noted that N-acetyltransferases are polymorphic, although the isoform that metabolizes DCVC is unclear. Another reviewer noted that glutathione-*S*-transferase mu (GSTM) may be an important GST isoform in this pathway, and that a significant portion of the population lacks activity of this enzyme (i.e., have a null *GSTM* genotype).

4.3 Charge Question 3 - Modeling of Chloral in the Liver and Lung

The panelists discussed issues regarding estimating chloral dose in the lung. Several panel members agreed with the conclusions in the document that there is not a compelling need to include systemic chloral generation in the assessment of lung tissue dose. One panel member noted that the selected approach is reasonable based on the data in animals that show little circulating chloral after TCE dosing and the data in humans that indicate an absence of measured chloral in human blood. A document author noted that the literature suggests that local production of choral dominates systemic chloral doses to the lung, which supports the focus of the model on lung rather than liver generation of chloral as a measure of lung tissue dose.

[1] Post meeting comments, summarizing key results from these studies, were provided by the panel member. The referenced studies (Eyre et al. 1995a,b) indicated that the vast majority of radioactivity associated with protein is metabolically incorporated. However, some acid labile adducts are also observed. The binding of these acid labile adducts correlates very well with cytotoxicity whether DCVC or TCE is administered. Mice are much more sensitive to such binding from DCVC and twice as sensitive to binding from TCE. In other words, there are quantitative differences in the two segments of the activation to DCVC. This binding is closely related to the degree of cytotoxicity that is measured by cell replication and by histopathological scoring. Finally, the binding to protein from TCE is significantly higher in the liver of both mice and rats than in the kidney. The binding of DCVC is much more selective for the kidney in the mouse than the rat.

The document author noted that mixed function oxidase activity in the lung could be used is to estimate choral generation. This approach would reflect the overall number of metabolically active cells per unit of lung tissue. A panel member noted that the current model presents dosimetry based on chloral in the lung without indicating specific target cell populations (e.g., Clara cells), and this potential subpopulation of target cells represents a small fraction of the total lung tissue. Another panel member remarked that there is uncertainty in estimating chloral dose in the lung and that a dose metric based on chloral cannot be estimated without considering clearance pathways as well. This panel member further noted that it is unclear if the lung tissue dose is driven more by chloral production from TCE or the rate of TCOH removal by glucuronidation (which affects the rate of the reverse reaction to choral from TCOH). This panel member noted that the chloral levels observed in mice are due more to rates of TCOH clearance than chloral production itself. A panel member suggested that lung doses could be estimated assuming the same enzyme kinetics for lung choral generation as for the liver (for which kinetic data are available). This estimate could be further adjusted based on differences in MFO activity among species. This panelist asked if CYP2E level scales across species. A document author commented that CYP2E levels tend to scale with relative proportion of metabolically active cells in the lung (Sarangapani et al., 2002).

The panel also discussed alternative dose metrics for the lung. A panel member noted that it would not be sufficient to look just at TCE metabolism without considering downstream metabolism to chloral. This panel member noted that rate of chloral formation may be more accurately predicted than the other possible dose metrics. However, if this estimate is too uncertain for the intended risk assessment applications, then other metrics of potential dose may be adequate. Another panel member noted that ignoring chloral detoxification pathways would decrease accuracy of the dose estimates at steady state. A document author also commented that using only the production of chloral in the lung may be problematic if both the production and removal of chloral do not scale across species. This is an important uncertainty since good predictions of removal kinetics are not available. A panel member agreed that the kinetics of removal of chloral is an important consideration, since accumulation of glucuronide metabolites will affect the steady state chloral levels.

The Panel Chair summarized that based on the discussions, none of the panel members were strongly suggesting that systemic generation of chloral for estimating lung doses be included in the model. Rather, they thought the production rate of chloral in the lung should be further considered as a dose metric, although several difficulties would need to be considered. Based on these considerations, the panel members all thought that if important uncertainties regarding interspecies differences in chloral formation and clearance in the lung could not be resolved based on available data, an alternative simplifying approach could reasonably be used. The suggested simplifying approach was to use arterial blood concentration of TCE as the dose metric, assuming lung chloral production and clearance scale proportionately across species.

With regard to the general model structure regarding chloral, a panel member commented that the current structure, which estimates TCA and TCOH production from TCE, is reasonable in the absence of specific data on the rates of metabolism of the choral intermediate. This reviewer also noted that in studies of other compounds, aldehydes are difficult to measure in the blood, suggesting that they convert rapidly to downstream products (TCOH in this case). However, this

panelist noted that including estimates for chloral kinetics in the liver would add flexibility to the model and would improve the use of data generated from dosing studies with chloral itself. This dose metric could be important for endpoints attributable to the chloral metabolite. For example, recent studies on the immunotoxicity and neurotoxicity of chloral might be useful for dose-response if the TCE model included choral kinetics. On the other hand, in the case of neurotoxicity studies observed effects may correlate better with TCOH than chloral, suggesting that the current proposed TCE model may be sufficient for this consideration.

Two additional panel members also suggested that adding specific estimates of chloral production and elimination is an area where the model could be improved. These panelists noted that adding these kinetic descriptions may be particularly important if exposures lead to conditions other than steady state (where significant dose-dependent differences in kinetics are most likely). One commented further that having an approach based on enzyme kinetics would be preferred over to the proposed approach of estimating concentrations of metabolites as a fraction of the total oxidation pathway, particularly if having an accurate prediction of TCA is needed. This panel member noted that there may be sufficient data in the literature regarding estimates of kinetic parameters for alcohol and aldehyde dehydrogenases to enhance the existing model. In addition, data on chloral formation from TCE in human liver microsomes provides *in vitro* estimates that could be used to estimate kinetic parameters in the harmonized model. Two panelists suggested that relevant enzyme kinetics may be estimated from data for other alcohols. Another panelist noted, however, that due to substrate specificities and the multiple classes of aldehyde dehydrogenases that exist, metabolism data would be needed for chloral itself rather than using a surrogate approach. This panelist expressed concern about including specific kinetic estimates for choral in the model noting prior difficulty in modeling reactive aldehyde concentrations for other related chemicals. A document author commented that in an earlier mouse model for TCE metabolism the liver compartment did include kinetics estimates for chloral production, but no adequate data are available for estimating the required kinetic parameters for alcohol and aldehyde dehydrogenases in humans. This document author suggested that the unpublished data on choral metabolism as well as the *in vitro* data noted by the panel members be evaluated further to determine if choral could be described using an enzyme kinetics approach.

A panel member commented that the impact of trichoroethanol (TCOH) and trichoroethanol glucuronide (TCOG) levels on the reverse reaction to chloral would need to be considered. The role of cellular reduction-oxidation (redox) state on rates of chloral formation is important since redox state drives TCOH clearance (which may be a zero-order process due to the prevailing redox state[2]), and conversion of TCOH back to chloral is a source of chloral kinetics. Existing

[2] Post meeting discussion of two panel members expanded on the rationale for concluding that cellular redox state may be a critical driver for TCOH kinetics. These panel members agreed that it is appropriate to raise the issue of the redox status of the cells and parallels in this process to that of ethanol metabolism. One panel member made the argument that as for other alcohols, the redox conditions in vivo will be the critical determinate of TCOH kinetics, rather than kinetic parameters (e.g., Km values). However, another panel member noted that it remains unclear if the conditions for interconversion of trichloroethanol and chloral are necessarily such that what occurs for ethanol (at high concentrations following consuming alcohol) is appropriate to TCOH and Chloral. It is entirely possible that TCOH and Chloral interconversion would be controlled through redox conditions, but it is equally plausible that other factors may have more impact (e.g., TCOH metabolism to TCOG). Nevertheless, the redox status and the ethanol comparison are important issues to evaluate for their utility to modeling the metabolites of TCE.

literature is available on this topic for other alcohols, and could provide useful data for estimating chloral kinetics. This panelist noted that at low doses the impact of TCOH glucuronidation as well as polymorphisms may have little impact on choral generation, since the rate of the reaction may be driven by redox conditions (at least in the liver). Another reviewer commented that at low doses oxidative metabolism of TCE in the liver may be limited by blood flow, which would argue for using a simpler model structure.

A panelist also commented that the model may not account for realistic sources of human variability. For example, variability in choral concentrations should account for potential polymorphism in aldehyde dehydrogenase, which would be greater than variability in CYP2E1, which has no functional polymorphism. Another reviewer commented that there is also significant variability in glucuronidation activity that would affect choral levels. Additional data from an unpublished study of chloral administered to humans (provided by a panel member at the meeting) may be useful to evaluate chloral kinetics. A third panelist questioned whether any sensitivity analysis was done to determine which parameters are driving chloral kinetics, and noted potential difficulties in the model calibration effort when evaluating multiple pathways with uncertain parameters.

4.4 Charge Question 4 - Modeling of Dichloroacetic Acid

Two panel members commented that inclusion of DCA in the model needs to be thoroughly considered since it appears to have a different mode of action than TCA (with regard to liver tumor formation), and available mode of action studies suggest that the TCA is not the only chemical that plays a role in observed liver effects. Several panel members suggested calculating the amount of TCE exposure that would generate a sufficient amount of DCA to induce liver toxicity as identified in DCA toxicity studies. This could be used to bound the calculations and determine whether generation of DCA from TCE is a concern for liver carcinogenicity. A document author noted that effects in the liver have been reported at low concentrations (in the range of detection limits) in some DCA studies, suggesting that DCA may continue to be of interest for TCE risk assessment. Two panel members commented that further mode of action studies would also help to determine the degree to which more detailed modeling of DCA kinetics should be pursued. For example, one panel member suggested that a mode of action study utilizing a toxicogenomics approach may be useful to further evaluate if the mode of action for TCE is more like that of TCA or DCA.

Uncertainty regarding the accuracy of DCA measurements in existing studies was noted as an important issue in modeling DCA kinetics. The reliable determination of the extent of DCA formation from TCE in humans (as opposed to DCA formed as an artifact of *ex vivo* conversion of TCA to DCA) was noted as a specific concern. A document author commented that work is ongoing in developing an analytical method for DCA, but that based on current methods DCA has not been accurately determined in the presence of TCA. One panelist recommended exploring the basis of variability in DCA measures in the human exposure studies (where detectable DCA levels were found in only a few of the tested subjects). A panel member suggested that observed differences in measurable DCA levels may reflect differences in glucuronidation capacity, affecting the proportion of TCOH metabolized to TCOG vs. DCA. This panel member further suggested evaluating correlations among TCE metabolites in the

individual study subjects to assess potential sources of the variability. A document author commented that there was a large degree of variability observed for other metabolites in addition to DCA in the human toxicokinetic studies.

A panel member noted that the author's decision to remove TCA as a direct source of DCA formation was appropriate. This panelist commented that there is evidence that DCA arises from TCE via a dichloroacetyl chloride intermediate. In discussing other sources of DCA generation *in vivo*, a panel member asked whether there is evidence for dehalogenation of TCA to DCA. Another panel member commented that in isolation the dehalogenation reaction can occur, but the rate is very low. TCOH and chloral can also be dehalogenated, but the rates of these reactions are not known. Regarding estimates of kinetic parameters for DCA formation, one panel member noted that limited information on DCA half-life is available and there is evidence for the existence of the acid chloride formation. However, these data are not adequate to fully describe DCA kinetics. Furthermore, the lack of adequate tracking of DCA fate in the glyoxylate pool hampers the direct evaluation of DCA kinetics *in vivo*.

A panel member commented that if the relevance of the DCA measurement is unclear and the data are inadequate to generate an estimate of DCA based on limited and uncertain kinetic data, then it would be appropriate to simplify the model. This panel member suggested a simplified approach such as using flux through the relevant portion of the oxidative pathway as an alternative dose metric. The panel discussed this suggestion. One panelist commented that an overall oxidative mass balance approach would not be adequately specific, given the potential need for a dose metric relating DCA exposure to mouse liver tumors. To better account for DCA, this panel member suggested estimating DCA formation by subtracting other measured metabolites from the overall oxidative pathway flux, and using this estimate of DCA as compared to dose-responses for DCA studies to evaluate the need for refinements in the modeling approach[3] A second panel member agreed with this approach of estimating DCA production, and preferred this approach to the less specific metrics based on overall flux through the oxidative pathway. A document author further commented that selecting a dose metric upstream of DCA concentration in the liver may also be preferred if the DCA production pathway, rather than DCA itself, is the cause of liver effects. Another panel member suggested that TCA levels could serve as a dose metric for estimating the oxidative pathway. Based on these discussions several panel members agreed that an appropriate dose metric for DCA could

[3] The panel member in post meeting comments added additional discussion of this approach, where the maximum amount of DCA that could be formed as an intermediate in the metabolism of TCE can be estimated by subtracting the TCE that is eliminated as TCA and TCOH, and TCOH-G from the total flux through the oxidative pathways. It is possible that other pathways exist to convert TCE to the glyoxylate and subsequent oxidative products, but certainly DCA can be converted to these products (Tong et al., 1998 and a number of subsequent papers from these same authors – P. Board and M. Anders). The amount of DCA formation is counterbalanced by the very rapid rate of DCA clearance that is seen at low doses (i.e., doses lower than those that produce significant inhibition of glutathione transferase zeta). The pseudo steady-state levels of DCA that might be achieved with these levels of formation and elimination can be estimated from data in the literature. The amount of DCA that could be formed can then be compared to the dose that would be obtained from drinking water containing DCA. Extrapolation of these data downward to low doses of TCE that are encountered in the environment would place an upper limit on the carcinogenic activity of DCA that may arise from any give dose of TCE. Predictions of DCA levels obtained in this way provide an idea of how sensitive methods analytical methods must be to demonstrate a smaller contribution of DCA from TCE metabolism.

be derived using some fixed proportion of the total oxidative metabolites (as estimated based on the considerations above) normalized to liver volume.

4.5 Charge Question 5 - Trichloroacetic Acid Binding in the Blood

Several panel members commented that the proposed model is an appropriate use of the *in vitro* data to estimate *in vivo* kinetics. Several panel members commented that it is very appropriate to use *in vitro* tissue binding data as a surrogate for estimating partitioning *in vivo* and to then base dose-response on the derived estimates of free versus total TCA. Panel members raised the question as to whether tissue dynamics such as varying blood flow to the tissues or other factors related to tissue distribution *in vivo* would be accurately accounted for using partitioning data generated *in vitro*. The document authors commented that the *in vitro* data should be adequate since it was being used only to describe transient binding in blood, which occurs with fast on/off rates. Therefore, other tissue dynamics would have limited impact on free versus bound TCA in the blood. Another panel member agreed with the document author on this point and further indicated support for the modeling approach that was proposed.

The panel also discussed the dose-dependence of plasma protein binding. In response to a specific point raised in the panel charge, several panel members noted that due to multiple binding sites and the large albumin binding pool in the blood there would be little concern about competition for binding at the high doses used in cancer bioassays. Although, another panel member commented that individuals who have a reduced pool of such low affinity sites, such as those with defects in albumin production due to underlying liver disease, might have different TCA binding proportions in the blood. A panel member agreed that dose-dependent changes in free versus bound TCA would not be a major concern in the presence of a large pool of low affinity binding sites. However, the presence of a smaller population of high affinity binding sites could impact the degree of binding at low doses. Another panel member commented that based on the apparent Kd values, there appear to be relatively high affinity sites, where a greater portion of the TCA will be bound and less available to tissues at low doses. Therefore, one would expect the dose-response relationships dependent on tissue levels of TCA are likely to be sublinear in the low dose range. Since dose-response data from cancer bioassays for TCE are at much higher doses than the Kd, dose-dependent plasma binding would not be a concern for these cancer studies. However, it was noted that some noncancer studies (e.g., Buben and O'Flaherty, 1985) have used TCE concentrations that result in low TCA levels in the blood, suggesting that low dose TCA binding rates can be important, and could affect the interspecies extrapolation. The Panel Chair summarized that panel members generally agreed with the proposed modeling approach for TCA binding, but suggested confirming that the model captures the binding activity observed *in vitro* and accounts for dose-dependent changes in binding *in vivo* that may occur at low concentrations (in the range of those that occur in low-dose noncancer studies).

4.6 Charge Questions 6 and 7 - Overall Model Structure and Parameterization

Several points were raised by panelists in discussing the appropriateness of the overall model structure. One issue raised by a panelist was the authors' use of only a GI tract compartment for drinking water exposure, but the inclusion of stomach and duodenum compartments for "oral dose" (i.e., gavage exposure). The authors explained that the stomach and duodenum were

kinetic compartments only (not containing any tissue volumes), and are included to capture the kinetics of absorption from gavage, while drinking water intake is modeled as zero-order uptake (assuming a constant daily intake rate, which ignores daily temporal patterns of exposure). The document authors considered this approach appropriate based on the assumption that absorption occurs quickly relative to other kinetic processes and that this was the best available approach in the absence of specific data on temporal patterns of drinking water consumption. A panel member noted that EPA has some data on temporal intake patterns for drinking water in animal studies. Several panel members commented that for longer-term drinking water studies it would be generally appropriate to ignore the daily temporal variability in intake. One panelist noted, however, that this issue could be important if the model were used to estimate dose metrics in alternative short-term scenarios or for acute studies where steady state is not reached.

A second issue raised by some panel members was the need to include specific kinetic descriptions of key metabolic steps, rather than using approaches based on proportions of total metabolite generation. However, simplifying approaches were considered reasonable in some cases (e.g., addressing GSH conjugation pathway, DCA formation and metabolism, and chloral kinetics in the lung). A document author noted that in other published second generation rodent models (such as Fisher, 2000), specific Michaelis-Menton enzyme kinetics were used in some cases, but were not included in the proposed harmonized model due to the lack of data for the corresponding enzyme kinetics in humans.

Several panel members also suggested further evaluation of the appropriate approach for selecting Vmax of CYP2E1-mediated oxidation of TCE in humans, since a wide range of values was suggested based on different human exposure studies and *in vitro* measurements. One panel member commented that the selection of a value that is lower than that used by other prior models (e.g., the use of 3 to 5 in the current model versus 10 to15 in earlier efforts) might reflect the overall partitioning among the pathways that was used in the proposed model. A document author acknowledged that the data on Vmax are quite variable and further noted that it is unclear why a large difference in *in vivo* and *in vitro* estimates has been observed. One panelist commented that regardless of the variability in the Vmax estimate, this may have little consequence if the clearance of TCE is driven by flow limited conditions. This panelist noted that the newer data (Fisher et al., 1998) does not correspond with TCE measurements observed in earlier data sets, and using the estimated Vmax of 3 derived from this newer data does not provide an adequate fit to other data. This panelist further commented that the use of a value of 3 for Vmax makes the clearance slower, which would not be consistent with the flow-limited metabolism of TCE, which has largely been considered to be the case to date. This panelist commented that if flow limited conditions do not adequately address the rate of oxidative metabolism of TCE then a range of values may be needed (from 3 to 15). A document author commented that differences in the low end of the range of estimated Vmax values have an impact on the model fit, while estimates in the high range (such as those observed in *in vitro* studies) are within the region where metabolism becomes flow-limited

Since Vmax estimates impact the overall model fit, issues regarding the variability in the underlying data used to generate these estimates were discussed. A document author noted that the data themselves vary and result in different estimates of the amount of TCE metabolized. Other panel members also noted that different predictions generated from the human data sets are

an underlying cause of the uncertainty in this parameter estimate. The panel reviewed several figures in the document. One panel member noted differences in the amount of metabolites identified in separate studies (e.g., comparison of TCOH/TCOG estimates in Figure 14 to those in Figure 18). This panel member commented that human inhalation studies conducted under conditions of light exercise are preferred, since they tend to generate more consistent results. This panel member also noted that if reported chamber concentrations in key inhalation studies were not accurate, the Vmax estimates derived on the basis of these studies might need to be adjusted. Another panelist suggested that the variability in the data shown in Figure 14 of the document may reflect two independent samples (based on the data visually falling on two separate curves in Figure 14B, particularly after the 8 hour time point) and Vmax derived from the lower of the two data sets would be near 8 (not unlike the value of 10 used as an estimate based on earlier studies). Furthermore, this reviewer noted that variability in TCOH and TCOG data were not large; only TCA was variable. This reviewer commented that fitting the upper TCA curve from this Figure may have had an overly important impact on the selection of the human kinetic parameters. Given the variability in data sets, various panel members suggested several options for selecting the appropriate value for the Vmax for CYP2E1 oxidation of TCE in humans. Options included use of a value that results in the best fit of newer data sets, which resulted in the Vmax estimate lower than in previous modeling efforts. A second option was to select a value that corresponds with prior estimates for this parameter (in the range of 10-15) and optimize the model fit by changing other parameters. The authors noted they had done this by varying parameters such as ventilation rate. A third option was to rely on the Markov Chain Monte Carlo (MCMC) calibration effort to optimize the value for this parameter.

Other issues regarding model structure raised in the charge questions were also addressed by the panel. One panel member commented that there does not appear to be a need to include a diffusion-limited fat compartment in the model structure, noting that keeping the model simple is appropriate if adding complexity does not improve overall model predictions. Another panel member provided unpublished data on chloral metabolism in humans that may be useful in evaluating the role of enterohepatic circulation in explaining apparent peak TCA concentrations.

4.7 Charge Question 8 - Model Calibration

The panel discussed the merit of calibrating the model using a Markov-Chain Monte Carlo (MCMC) approach. A panel member opened the discussion by commenting that in using MCMC it is important to realize that the results are model and data dependent, and should be used and interpreted in the context of the model and data for which it was generated. Difficulties in selecting various estimates of Vmax for TCE oxidation identified from the MCMC calibration conducted by Bois et al. (2000a,b) of earlier independent models by Clewell et al. (2000) and Fisher (2000) were highlighted as an example. Panel members generally agreed that an attempt should be made to do a MCMC calibration, with individual panel members having differing levels of confidence in the interpretation of results generated by this approach. A document author favored using the approach, but asked to what extent judgment should be used to interpret and apply the results of a MCMC calibration, particularly when many of the input parameters are uncertain. A panel member advocated the use of MCMC when parameter estimates are uncertain; noting that if one has confidence in the parameters then a simpler Monte Carlo approach would be adequate. A document author further noted that many data sets do not give

information on rodent or human variability in parameters and the correlation between them, and therefore, an MCMC approach is useful to evaluate uncertainty. Several other panel members expressed concern about letting the statistical tool overshadow the biology knowledge.

It was also suggested by several panel members that posterior estimates be judged cautiously, with one panel member suggesting that it might be useful to hold back a data set to validate the posteriors. Several panel members and authors commented on the idea of calibrating the model with all data sets simultaneously versus holding back data for validation of the MCMC posterior predictions. Some panel members or authors favored using MCMC to calibrate the model and to update all parameters simultaneously. It was noted that using all available data sets reduces bias in data selection. Furthermore, checks and balances in the statistical method allow for the evaluation of issues such as autocorrelation. Others commented on the need to provide a critical appraisal of the data sets included in the MCMC calibration. Since prior distributions are based on the input data, culling of data sets before running the analysis is a point of control in the process. It was also suggested by several panel members that resulting posteriors be evaluated carefully to ensure they are consistent with the overall understanding of the biology. For example, model predictions can be compared to well-characterized physiological parameters (blood flows, tissue volumes, etc.). Another possibility for validating the MCMC calibration suggested by the document authors was to use some type of cross validation approach in which data sets are excluded one data set at a time. A panel member noted that cross-validation is a common practice in some modeling procedures, such as in developing quantitative structure activity relationships.

Several panel members suggested that restricting parameters that are more certain, such as well-defined physiological parameters, is reasonable. The merits of allowing the MCMC calibration to update physiological parameters with and without restrictions were discussed, with opinions presented for and against this approach. A concern was raised that the resulting posteriors for physiological parameters for a small test population in a study group should not replace the values chosen based on scientific judgment that are thought to reflect a larger general population of interest. Another panelist commented that it is important to move toward doing a better job of estimating uncertainties in dose metrics, and MCMC is a reasonable tool for starting to do this. However, this panel member cautioned that in interpreting the results it is important to distinguish between characterizing uncertainty versus variability. This panel member suggested that the technique has been employed successfully with individual data available, but that it is important to ensure characteristics of studied individuals correspond to those in the population of interest. Based on these discussions a document author suggested that the harmonized PBPK model be updated using physiological parameters based on the priors and kinetic parameters based on the MCMC calibration posteriors. Several other panel members agreed that certain physiological parameters should be restricted based on scientific knowledge.

Panel members advised critical and cautious implementation of the technique, and noted that more research is needed in how to apply MCMC analysis for PBPK model calibration. A number of issues and uncertainties were raised by individual panel members in the discussion for consideration by the authors, such as:

- Ensure that the intended use of the MCMC analysis is clear. Is it to parameterize the model or to evaluate uncertainty in the parameter estimates? If it is used for estimating uncertainty, then consistency with basic biology knowledge and application to appropriate populations (e.g., test subjects do not represent the full variability of the entire population) should be considered. Conventional Monte Carlo modeling was suggested as an approach to address general population variability.
- Consider alternative approaches of using all data to calibrate the model. Consider cross-validating the model by sequentially pulling out individual data sets to test the model, versus an alternative approach of holding back a single data set to validate the model outputs.
- Consider alternative approaches of allowing the MCMC to update all physiologic and kinetic parameters using meaningful biologically-based limits versus restricting updating to kinetic or uncertain parameters.
- Consider whether the order of sequential updating impacts the resulting posterior distributions.
- Determine the impact of limitations in the MCMC modeling software for weighting different data sets.

4.8 Charge Question 9 - Key Data Gaps and Research Needs

Panel members were asked to provide suggestions regarding key data gaps and research needs to improve the basis for the draft TCE PBPK model. One panel member commented that it would be valuable to end up with a clear description of data gaps and unresolved issues to better target additional studies. Specific data gaps and potential experimental approaches were noted by individual panel members, with suggestions focusing on approaches that would fill high priority data gaps most efficiently. These suggestions included:

- Characterize the kinetics of FMO metabolism of GSH-conjugation pathway metabolites.
- Develop *in vitro* dose-dependent kinetic data for TCOH glucuronidation and alcohol dehydrogenase activities and characterize the enzymes responsible for these activities in mouse and human lung cells. The results would inform interspecies differences in lung chloral generation and clearance.
- Need to better evaluate the formation of DCA from TCE. A first step is to conduct additional studies to address to better characterize the rates of DCA formation from TCE in microsomal preparations, building on the published work of Guengerich and colleagues.
- Additional mode of action studies (e.g., comparative toxicogenomic studies of TCE and its metabolites in tumor tissues or TCA dosing studies in peroxisome proliferation receptor knock-out mice) to determine the potential contribution of DCA to TCE induced tumorigenesis may be useful. Further evaluation of the dynamic components of TCE toxicity would help to focus resources on aspects of kinetics that are most likely to have the greatest impact.

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Appendix A - Author Presentations

Harmonized PBPK Model for TCE

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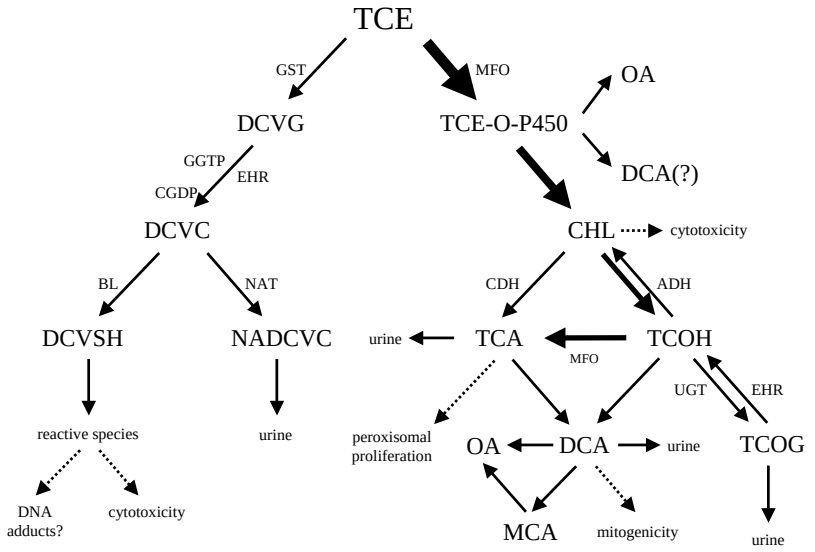
Metabolism of TCE (Lash 2000)



History:

- Previous models (EHP 2000) for liver dosimetry were based on different data sets
  - Clewell: primarily published data
    - Mouse:Templin (aqueous gavage), Prout (corn oil), Fisher (inhalation)
    - Human: Muller, Monster, Stewert
  - Fisher: primarily new studies
    - Mouse: Abbas (iv, corn oil gavage), Greenburg (inhalation)
    - Human: Fisher (individual data)
- Recalibration of each model by Bois was performed with essentially the same data used in development of that model
- Model differences reflected different data sets used for development
- Models also differed in some minor elements of structure
  - Separate description of portal and arterial blood flows to liver (Clewell)
  - Multi-compartment models for water soluble metabolites (Fisher)
  - Urinary excretion: secretion from kidney (Fisher) vs. glomerular filtration of plasma (Clewell)

Uses of PBPK Modeling Considered in EPA 2001 Risk Assessment for TCE

- Cross-species extrapolation for cancer risk estimates
  - Mouse liver tumors – TCA, DCA
  - Rat kidney tumors – DCVC (not used)
  - Mouse lung tumors – Chloral (not used)
- Cross-species extrapolation for noncancer risk estimates
  - Mouse and rat liver effects – TCA
  - Rat kidney effects – DCVC
  - Rat developmental eye effect – TCA (not used)
  - Rat and human neurological effects – TCE, TCOH
- Cross-route extrapolation of human inhalation epidemiological study results – TCA

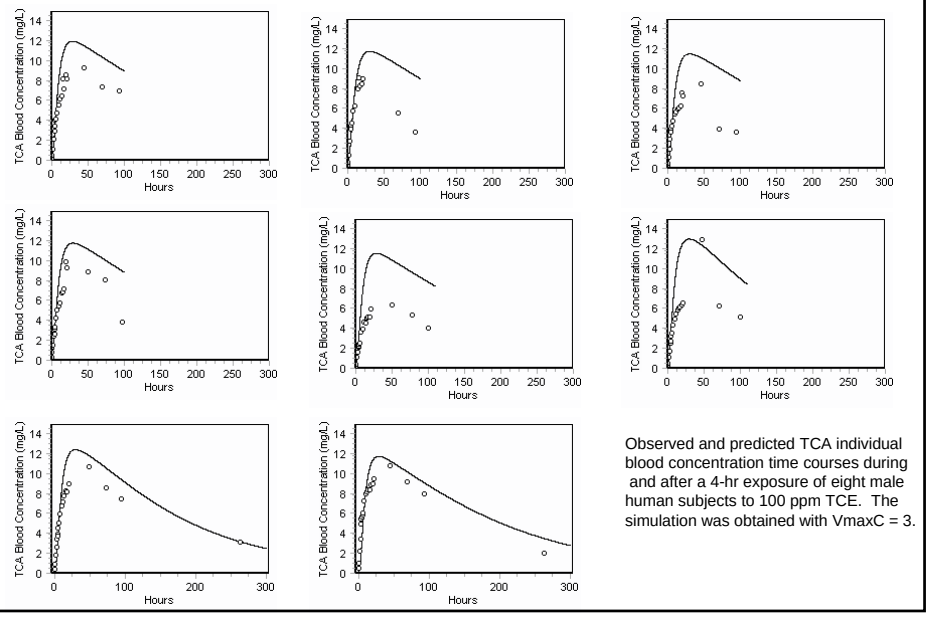
Development of Harmonized Model:

- Developed “consensus” structure
  - Multi-compartment description of TCA based on Fisher (2000)
  - Kidney and lung description based on Clewell et al. (2000)
- Updated physiological parameters
  - Consistent with Brown et al., EPA defaults, BW**3/4 scaling
- Re-evaluated distribution and metabolism parameters
  - TCE parameters based primarily on Fisher et al. (1981)
  - Kidney and lung parameters taken from Clewell et al. (2000)
  - Incorporated new data on TCA plasma binding in mice and humans (Lumpkin et al. 2003)
  - Liver parameters re-estimated using all kinetic data from both previous model development efforts
  - Evaluated feasibility of DCA dosimetry
- Next step: MCMC “calibration” of harmonized model

Harmonization of TCE Models

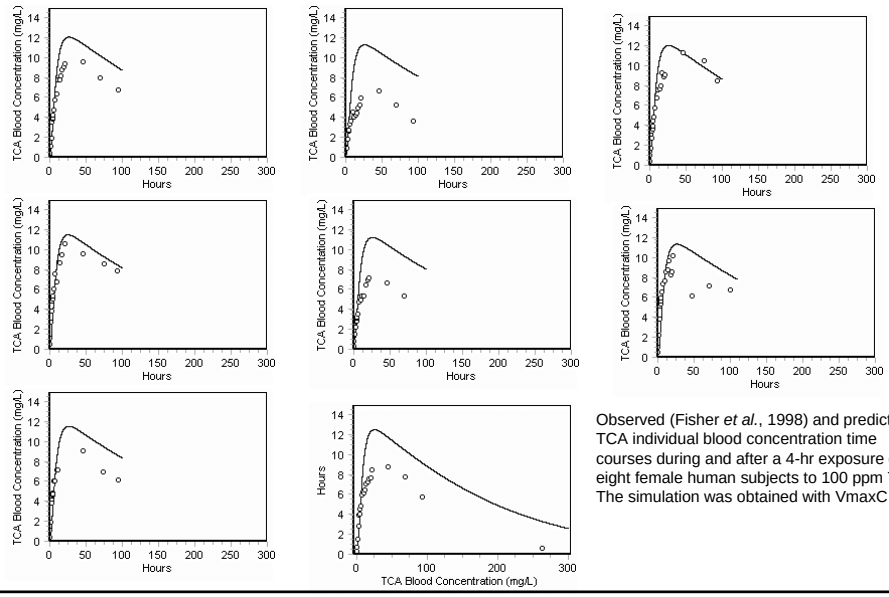
Jeff Fisher and Deborah Keys
University of Georgia
June 29, 2004

Human simulations with harmonized model using data from Fisher et al., 1998, TAP



Observed and predicted TCA individual blood concentration time courses during and after a 4-hr exposure of eight male human subjects to 100 ppm TCE. The simulation was obtained with VmaxC = 3.

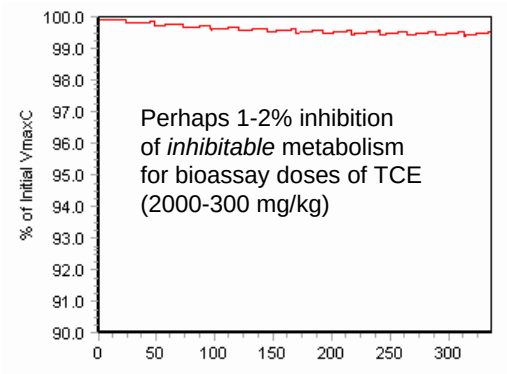
Human simulations with harmonized model using data from Fisher et al., 1998, TAP



Observed (Fisher *et al.*, 1998) and predicted TCA individual blood concentration time courses during and after a 4-hr exposure of eight female human subjects to 100 ppm TCE. The simulation was obtained with VmaxC = 3.

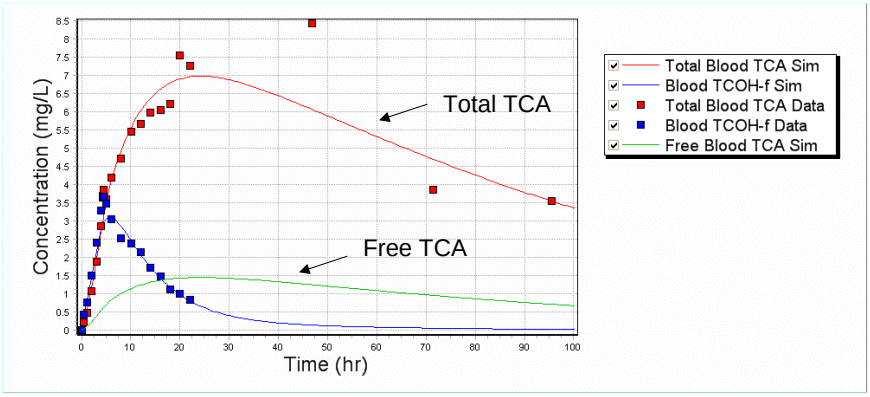
Does DCA inhibit it own metabolism as a metabolite of TCE?

- Using DCA data from Abbas and Fisher (1997) (blood levels=0.01 to about 3 ug/ml) and recent DCA model (Keys et al., 2004, submitted)

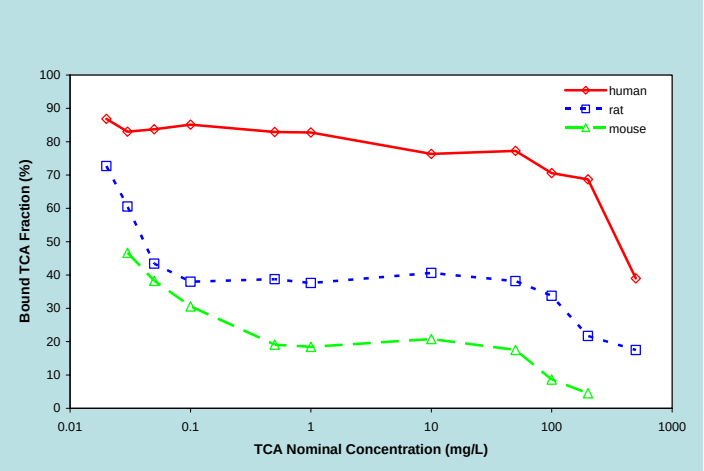


Human PBPK Model – influence of binding In Humans (not harmonized model)

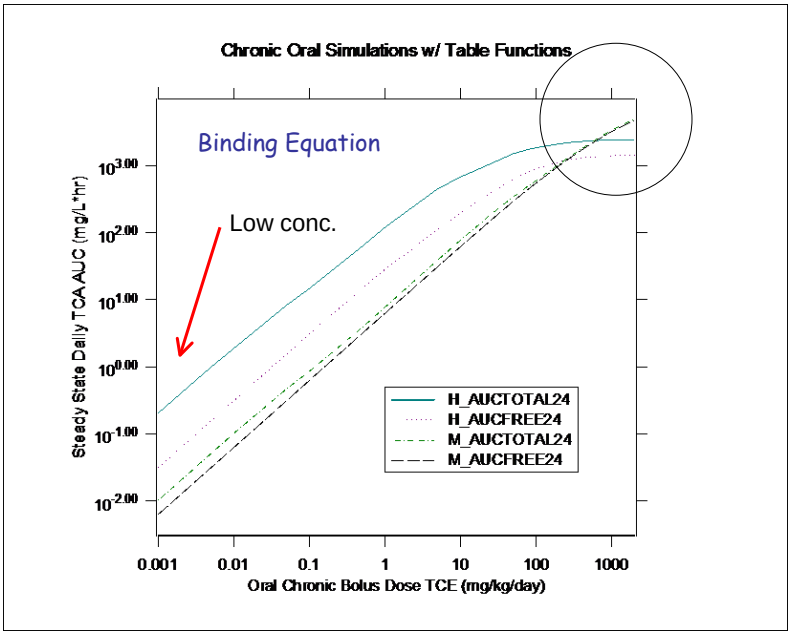
•Concentrations of TCA (•) and TCOH-f (◦) in blood of male exposed to 100 ppm TCE for 4 h for the individual male subject with the lowest TCA blood area under the curve.

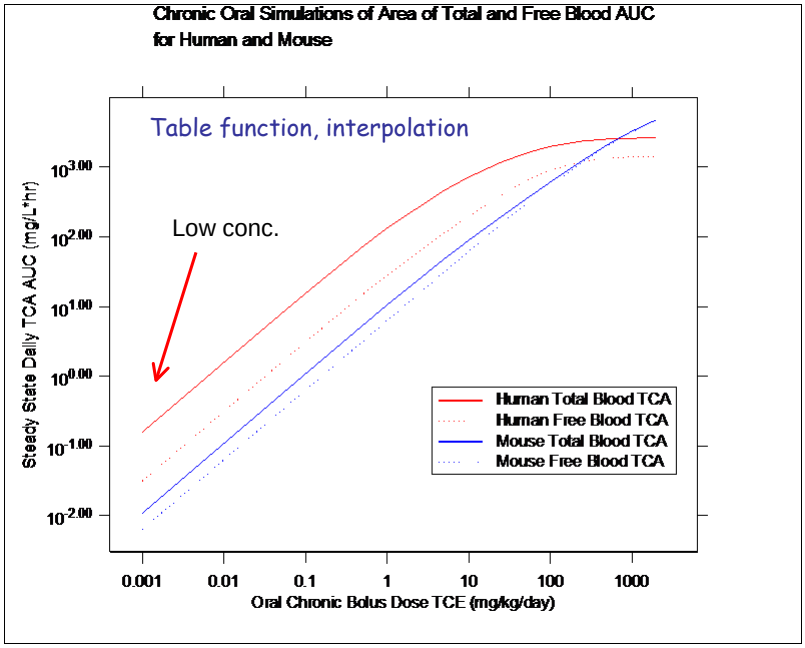


Lumpkin et al., 2003, in vitro binding study



Describe binding with equation vs Table function?





Critical Issues for Discussion:

1) Potential use of PBPK model: endpoints and recommended dosimetrics.

2) Written questions to panel and panel questions about the written questions

Appendix B - Background Information for Meeting

Peer Consultation of Harmonized PBPK Model for Trichloroethylene
Kingsgate Conference Center, University of Cincinnati
June 29, 2004

Background

Toxicology Excellence for Risk Assessment (*TERA*) is receiving funds from the U.S. Air Force (USAF) (through a subcontract with ManTech) to coordinate this peer consultation meeting on physiologically-based pharmacokinetic (PBPK) modeling of trichloroethylene (TCE) and its metabolites. This process is intended to assist in the protection of the public's health from exposure to this chemical by providing an open and transparent discussion of PBPK modeling issues.

Peer consultation is a process of seeking advice and input on a work product; this process could occur at one or more points during the development of a work product but often occurs at an early stage. The goal is to obtain information and analysis from experts, stakeholders, and/or members of the public to improve the work product. Peer consultation is different from peer review, which is a process of reviewing a final work product to determine if it is technically sound. Often a peer consultation panel is seeking individual opinions, which may or may not converge, while the peer review panel operates by consensus to reach a common opinion.

The outcome of this peer consultation will be used to make improvements to the harmonized TCE PBPK model; the model then will be made public to facilitate its use and consideration in risk assessments. *TERA* anticipates the results of the peer consultation and resulting harmonized model serve as important input into future TCE risk assessment efforts, including subsequent independent peer reviews and consultations.

Participants

The USAF and the U.S. Environmental Protection Agency (US EPA) are jointly sponsoring the scientific workgroup that has developed the harmonized PBPK model for TCE and its metabolites based on the full range of available science and data. The products from this joint workgroup will serve as important input to ongoing TCE risk assessment activities, including a planned multi-agency consultation with the National Academy of Sciences on TCE science issues as well as EPA's revised TCE human health risk assessment. This workgroup is composed of scientists from the USAF and EPA, with technical expertise from *TERA* and independent scientists. The participants from each organization are listed below:

U.S. Air Force (USAF)
Dave Mattie

U.S. Environmental Protection Agency (EPA)
Jerry Blancato
Weihsueh Chiu
John Lipscomb
Miles Okino
Fred Power

University of Georgia

Jeff Fisher*

ENVIRON
Harvey Clewell*
Tammie Covington*

Toxicology Excellence for Risk Assessment (*TERA*)
Michael Dourson
Eric Hack
Jay Zhao

* Participation sponsored by USAF

TERA's Disclosures as Peer Consultation Coordinator

TERA is a non-profit 501(c)(3) corporation that conducts research and documentation in risk assessment for public and private sponsors. *TERA* is currently working on other projects with author team organizations. For EPA *TERA* is preparing or peer reviewing several risk assessment documents and conducting issue-specific work (e.g., collecting physiological parameters for neonatal animals), and participating on the Candidate Contaminant List workgroup for the Office of Water. *TERA* has a cooperative agreement from EPA to develop and conduct peer consultations. None of the current or previous work for EPA is on TCE, but *TERA* has prepared drinking water criteria documents for EPA on dichloroacetic acid (DCA) and trichloroacetic acid (TCA). *TERA* also has worked jointly on projects with ENVIRON (and with the K.S. Crump Group while they were part of ICF Kaiser); a current project is to conduct Markov Chain Monte Carlo analysis of a PBPK model. *TERA* has collaborated with the USAF on research on health effects and assessment of perchlorate.

TERA is intentionally maintaining a separation in assigning work to our scientists working on the TCE issues and our peer consultation staff, in order to ensure the objectivity of the peer consultation process. Several *TERA* staff members have been facilitating these efforts on the harmonized PBPK model and other issues related to the scientific data for TCE. Different *TERA*

employees have organized this peer consultation, including the selection of panel members, development of the charge for the panel, and drafting of the meeting report.

Selection of Panel

The primary criteria for selecting members for the TCE panel were knowledge and experience in PBPK modeling and TCE metabolism. This knowledge and expertise is essential for providing the authors with meaningful advice on the harmonized model at this stage of its development. Therefore, *TERA* concluded that the need for specific expertise outweighed potential concerns regarding bias that might arise from previous or current work related to TCE. The panel members selected do not have conflicts of interests (consistent with *TERA* policy on Conflict of Interest and Bias – http://www.tera.org/peer/COI.html), although most have done previous work on TCE and each brings a background and history of affiliation with various types of organizations. *TERA* has been careful to balance affiliations and backgrounds of these members (i.e., biases) to provide a wide range of views and perspectives. Members of this panel are from universities, government agencies, non-profits, and consulting agencies for government and industry.

TERA, as the independent group convening the peer consultation, was solely responsible for selection of panelists for the peer consultation of the TCE harmonized PBPK model. *TERA* did however ask the author team for suggestions of experts for *TERA* to consider and also to identify issues and questions for the charge.

TERA determined that in order to provide a complete and thorough review of the document it was important to locate scientists with experience in the following key subject areas:

- Metabolism of TCE
- PBPK modeling, evaluation of structure and extent to which model can describe the data sets with a consistent set of model parameters
- TCE metabolites and their effects
- Modeling of TCE and metabolites
- Use of PBPK in risk assessment
- Risk assessment

TERA's conflict of interest policy (see http://www.tera.org/peer/COI.html) identifies the following situations as examples of those that could create a real or perceived conflict of interest.

- Working for an organization that sponsors or contributes to the document to be reviewed,
- Having direct personal financial investments potentially benefiting from the outcome of the review, or
- Authoring or providing significant comments on the document.

Having defined COI generally in our policy, *TERA* then determines specific situations that could be considered a conflict for each individual meeting. Prior to selecting the TCE harmonized PBPK model panel members, *TERA* determined that the following would be conflicts of interest or sources of unacceptable bias and therefore reason to exclude a candidate from selection.

- Member of the author team.
- A significant contributor to the harmonized model. Providing data that are used in the model would not be a conflict.
- Individuals working in the same immediate office as the members of the author team (i.e., EPA/ORD/NCEA/NERL; USAF/AFRL/HEPB, ENVIRON, and the Department of Environmental Health Science, College of Agricultural and Environmental Sciences, University of Georgia) would not be allowed on the panel.
- Individuals with a financial stake in the results of the peer consultation. It is not practical and may not be possible to identify all the companies that manufacture TCE, use TCE currently or in the past, or have environmental liability regarding TCE and query candidates about their financial holdings or relationships with every party; therefore, *TERA* asked candidates directly if they have a financial stake in the outcome of the peer consultation.

The *TERA* COI policy also discusses bias. For these reviews, "bias" means a predisposition towards the subject matter under consideration that could influence the candidate's viewpoint. Examples of bias would be situations in which a candidate:

- Has previously taken a public position on subjects to be discussed, or
- Is affiliated with an industry, governmental, public interest, or other group with a partiality regarding the subjects to be discussed.

Most scientists with technical expertise in areas relevant to a risk assessment document will have existing opinions about some of the subject matter, and therefore, may be considered to have some degree of bias. These biases are considered in panel selection decisions to determine if they will interfere with the panel member's objectivity for the review. However, each panel member is representing his or her own personal views at the meeting and not that of an employer or other organization to which he or she may be affiliated.

Disclosures

None of the panel members has had direct involvement with the development of the harmonized PBPK model for TCE. However, the panel includes a number of experts in TCE metabolism and PBPK modeling who have been involved in development of previous models and some of the data used for this model. Each of the panel members has indicated that they are not aware of any personal financial holdings that have any connection to TCE (including manufacture, use, or environmental liability) or to the harmonized model, nor do they have any known financial stake in the outcome of this peer consultation. In addition each has certified that he or she does not believe he or she holds any personal values or beliefs that would preclude him or her from conducting an objective scientific evaluation of the materials to be reviewed and is not aware of any other factors that may create an actual or perceived conflict of interest or bias for his or her participation in this peer consultation.

The members of this panel were selected for their expertise in developing and evaluating PBPK models on TCE and other compounds, metabolism of TCE, kinetics of TCE and metabolites, and use of models in risk assessment. Each panel member brings his or her own perspectives

resulting from their employment, affiliations, and experience. *TERA* has attempted to balance potential biases by forming a panel with scientists from diverse backgrounds, including government, consulting for industry and non-industry, and academia. In addition, *TERA* selected scientists who have done research on TCE, as well as those who have not.

Individual panel biographical sketches and disclosure statements are found below.

Biographical Sketches of Presenters

Dr. Hugh A. Barton

Dr. Hugh A. Barton received his Ph.D. in Applied Biological Sciences from the Toxicology Program at MIT in 1988. He is currently a toxicologist, and formerly Branch Chief, in the Pharmacokinetics Branch of the Experimental Toxicology Division of the US EPA's National Health and Environmental Research Laboratory in Research Triangle Park, NC. He is an adjunct Assistant Professor in the Curriculum in Toxicology at the University of North Carolina at Chapel Hill. Dr. Barton develops models and supporting data on tissue dosimetry and mode of action for use in biologically based dose-response analyses for chemical toxicity. He specializes in the use of state-of-the-art techniques, including physiologically based pharmacokinetic (PBPK) and pharmacodynamic modeling, to address the low dose, interspecies, and inter-route extrapolations that critically determine the development of chemical-specific dose-response values used for estimating risks. Recently, he has used modeling for evaluating noncancer toxicities, including reproductive and developmental effects, and for assessing dose-response and mechanisms of endocrine active compounds. Pharmacokinetic modeling to which he has contributed include: vinyl chloride, trichloroethylene, dichloroacetate, bisphenol A, and acetate esters and their metabolites (e.g. butyl acetate, butanol, butyraldehyde, and butyric acid). Pharmacodynamic models were developed for the male and female central hormonal axis (e.g. feedback between testosterone/estradiol and LH/FSH) and estrogen receptor-mediated gene regulation in the uterus.

Upon completion of his Ph. D., Dr. Barton worked at ENSR Consulting and Engineering where he planned and implemented risk based environmental management for toxic chemicals at contaminated waste disposal sites and manufacturing facilities. He was an Adjunct Assistant Professor at Boston University School of Public Health, from 1990 - 1993, teaching Principals of Toxicology. In 1991 he joined the Toxic Hazards Research Unit of ManTech Environmental Technology, Inc. at the Air Force Toxicology Division, Wright-Patterson AFB. There he directed research in xenobiotic metabolism, trichloroethylene carcinogenicity, PBPK modeling of mixtures, and risk assessment for total petroleum hydrocarbons (TPH). From 1995 - 1999 he was Principal Toxicologist with the K.S. Crump Group of ICF Kaiser where he developed pharmacokinetic and pharmacodynamic models focusing on issues of dose response for endocrine active compounds. He has published more than 25 articles in the scientific literature on xenobiotic metabolism, PBPK and PD modeling, endocrine disruption, dose response assessment, and risk assessment, with approximately 10 publications in peer-reviewed literature on TCE kinetics, modeling, and cancer and noncancer risk assessment.

Disclosure

Dr. Barton is currently employed by the US EPA. Dr. Barton has informally provided comments and discussed TCE issues with other offices at EPA as requested, but he is not formally part of the EPA group working on the TCE risk assessment. He formerly worked for ManTech Environmental as a contractor to the USAF and for ICF Kaiser as a member of the group currently at ENVIRON. He has conducted research on TCE, TCA, and DCA pharmacokinetics or pharmacokinetic modeling in relation to cancer and noncancer dose-response analysis while

with ManTech Environmental. He participated in meetings of the TCE Issues Group and the 1995 Williamsburg meeting on TCE while with ManTech. While with ICF Kaiser, he participated in a TCE literature review for a law firm. In October of 2001, while employed by EPA, Dr. Barton provided public comments to the EPA Science Advisory Board (SAB) on EPA's Draft Trichloroethylene Health Risk Assessment document (dated August 2001). The main focus of Dr. Barton's personal comments on the EPA document was that use of a combined point of departure would make it difficult to use a mode of action approach and pharmacokinetic modeling in a non-cancer analysis.

Dr Barton has developed models for TCE (Barton et al., 1995, Toxicol Appl Pharmacol 130: 237-247) and DCA (Barton et al., 1999, Toxicol Lett 106:9-21). He has also utilized the Clewell et al. model for dose response analyses (e.g., Barton and Clewell, 2000, Environ Health Perspect 108(Suppl 2):335-342).

Dr. Barton was selected for the panel for his experience in developing PBPK models, including models for TCE and metabolites.

Dr. Richard J. Bull

Dr. Richard J. Bull has a Ph.D. in Pharmacology from the University of California, San Francisco Medical Center. For 34 years, he has been involved in research related to environmental health. His field of study is toxicology and he has been most heavily involved with human health effects of drinking water contaminants. He has published extensively on the modes of action of halogenated solvents and byproducts of the disinfection of drinking water.

Dr. Bull worked with the Environmental Protection Agency's Health Effects Research Laboratory in Cincinnati, Ohio for fourteen years, where he directed the Toxicology and Microbiology Division at Cincinnati and was in charge of the EPA's health research programs in water. He joined Washington State University as a professor/scientist in the College of Pharmacy in 1984. In 1994, he accepted a position at Pacific Northwest National Laboratory (PNNL). At both institutions his research focused on establishing the mechanisms of action of environmental chemicals data in ways that will facilitate accurate across-species and low-dose extrapolation of health effects data, with an emphasis on mechanisms of carcinogenesis by halogenated solvents. Dr. Bull left PNNL in 2000 to return to Washington State University to work with the Department of Energy on their Low-Dose Radiation Research Program. As of May of 2003, Dr. Bull devotes all his time to his company MoBull Consulting.

Dr. Bull has served on numerous committee and advisory bodies, including those for the World Health Organization, International Agency for Research on Cancer, EPA's Science Advisory Board, Santa Anna River Water Quality and Health Science Advisory Panel, and National Research Council (NRC) Committees. Dr. Bull had conducted research and published over 40 articles on trichloroethylene and its metabolites. He was a state of the science author commissioned by the U.S. EPA and wrote a paper entitled "Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate" (2000, Environ Health Perspect 108(Suppl 2):241-59). Dr. Bull is on the editorial board of *Toxicology*.

Disclosure

Dr. Bull worked for the U.S. EPA from 1970 to 1984. Dr. Bull has worked with Irvin Schultz, who is also a kineticist, while at Pacific Northwest National Laboratory and together they have informally collaborated with Jeff Fisher on projects. While he and Dr. Schultz have published pharmacokinetic data on trichloroethylene and metabolites, he does not suggest that they have a competing model to the two models being evaluated and used in the harmonized model. Both the models have however, depended to varying degree on data generated in Dr. Bull's laboratory and that of Irvin Schultz. Dr. Bull worked as a consultant (now terminated) to a law firm to whom he provided literature on the health effects of TCE and provided guidance to the lawyers taking a deposition. He did not serve as an expert witness, nor make public statements.

In addition to the state of the science article in *Environmental Health Perspectives*, Dr. Bull has published extensively in the scientific literature on a variety of topics directly or indirectly related to metabolism of TCE and the effects of a number of its metabolites. He has also participated in a number of workshops and symposium addressing issues related to risk assessments for TCE. Dr. Bull has noted that "in the course of my career, I have probably made statements that support positions taken by the USAF while at other times supported the views of EPA."

Dr. Bull was selected for the panel because of his expert knowledge of the metabolism of TCE.

Hisham El-Masri, Ph. D

Dr. Hisham A. El-Masri is the director of the Computational Toxicology Laboratory at the Agency for Toxic Substances and Disease Registry (ATSDR) in Atlanta, Georgia. He has held this position since 1999. He received his M. Sc. degree in Environmental Engineering from California State University in 1989. He was awarded a Ph. D. degree in Environmental Health from Colorado State University at Fort Collins in 1994. After graduation, he joined the Computational Biology and Risk Analysis Laboratory at the National Institute of Environmental Health Sciences as a research fellow. Upon completion of his fellowship at NIEHS, Dr. El-Masri had worked as a biomathematical modeler for Novigen Sciences, Inc. before joining ATSDR. Dr. El-Masri's research and publications are in the area of computational toxicology to further advance methods for risk analysis and help in investigations of chemical mixture toxicity. He had served in several scientific advisory boards as an expert in biologically-based computational methodologies.

Disclosure

Dr. El-Masri works for ATSDR. Dr. El-Masri holds an unpaid adjunct faculty position at the University of Georgia, where he teaches 5-6 lessons for one course which is offered every other year. While he was at Colorado State University he developed a model for the interaction between TCE and 1,1-DCE in the mid-1990s (funded by the USAF). He also presented a poster

at a Society of Toxicology meeting, which looked at the application of default uncertainty factors as applied to some solvents, including TCE.

Dr. El-Masri was selected for the panel for his expertise in PBPK modeling and Markov Chain Monte Carlo techniques.

Michael L. Gargas

Dr. Michael L. Gargas, Ph.D., is a toxicologist Managing Principal of *The Sapphire Group*™, with over 26 years of related environmental experience. Dr. Gargas oversees and prepares human health risk assessments, conducts toxic tort support investigations, serves as an expert witness, interacts with regulatory agencies, and addresses critical toxicological issues through applied and basic research on behalf of clients. His clients include private industry, trade associations, law firms, regulatory agencies, and private citizens. Dr. Gargas' area of expertise is in human health risk assessment and biochemical toxicology research with emphasis in the areas of inhalation toxicology, chemical metabolism, physiologically based pharmacokinetic (PBPK) modeling, and chemical dosimetry, with specific application of these approaches to risk assessments. Prior to joining *The Sapphire Group*™, Dr. Gargas served as a Principal Health Scientist with ChemRisk (a risk assessment and toxicology consulting firm), a senior research scientist at the Chemical Industry Institute of Toxicology (CIIT), and as a toxicology research scientist with the U.S. Air Force (as a civilian) and the U.S. Navy (on active duty).

Dr. Gargas received his doctorate in Biomedical Sciences (Toxicology Specialty) from Wright State University. Dr. Gargas has been honored by the Society of Toxicology with the Frank R. Blood Award, the Department of the Air Force Invention/Patent Award (Co-Inventor) for an *In Vivo* Dermal Absorption System for Rats, Invention No. 15, 859 (U.S. Patent Number: 4,582,055) and the Outstanding Technical Civilian of the Year Award, from the Air Force Aerospace Medical Research Laboratory. Dr. Gargas has served on the editorial board of *Toxicology and Applied Pharmacology*. Dr. Gargas has been invited to present numerous guest lectures on toxicology and risk assessment topics and is an Adjunct Assistant Professor of Toxicology at Wright State University, serving as director for a graduate course in biokinetics and toxicology. He has published numerous book chapters and publications on a wide range of health and toxicologic topics.

Disclosure

Dr. Gargas worked for the USAF as a civil servant from 1981-89 conducting toxicological research at Wright Patterson Air Force Base, including studies with TCE. He has conducted research and published papers with Fisher and Clewell regarding metabolism and pharmacokinetic modeling of trichloroethylene. He currently consults to a confidential (industrial) client on TCE issues. His task is to keep them informed of progress on the U.S. EPA reassessment of TCE. He also conducted a comparison of risk values for the same client that included TCE (2000-2001). Dr. Gargas consulted to the Trichloroethylene Users Group in the mid-1990s on a project that used a PBPK model to recalculate the U.S. EPA cancer potency factor. Dr. Gargas is currently working on a project on chloroethane PBPK modeling for the EPA, but he is not aware of any other project the Sapphire Group is working on for EPA, USAF,

ENVIRON, or *TERA*. He has previously served on *TERA* panels, most recently receiving an honorarium for his VCCEP acetone panel participation.

Dr. Gargas was selected for the panel for his extensive experience in PBPK modeling and familiarity with TCE.

Lynne T. Haber

Dr. Lynne Haber is Research Program Manager for Toxicology Excellence for Risk Assessment (*TERA*). She received her Ph.D. in Molecular Biology from the Massachusetts Institute of Technology in 1990. She has more than 12 years of experience in developing risk values for government agencies and industry and conducting research related to risk assessment methods. She has developed more than 20 noncancer and cancer assessments for EPA's Integrated Risk Information System (IRIS) for EPA program offices (including ones using PBPK models), for other government agencies, and for private sponsors. Her current interests are in the application of mechanistic information in risk assessment and in methods for extending the dose-response curve to low doses. Other recent work includes research on children's risk issues, consideration of mode of action in cancer risk assessment, incorporating data on polymorphisms into risk assessment, and development of scientifically-based occupational exposure limits. Dr. Haber was the coauthor for an analysis of the effect of genetic polymorphisms on human variability in dose, using PBPK and Monte Carlo modeling. Dr. Haber worked for ICF Kaiser/Clement from 1991-1998 and as a Staff Scientist for the Illinois Legislative Research Unit. Dr. Haber has served on National Academy of Science panels and has served on peer review panels for EPA, the Department of Defense, and the Ontario Ministry of the Environment.

Disclosure

Dr. Haber works for Toxicology Excellence for Risk Assessment (*TERA*), but has not been involved in the efforts to develop the harmonized PBPK model. While with *TERA* and with ICF Kaiser/Clement she helped develop risk assessment documents for the U.S. EPA, including drinking water criteria documents on DCA and TCA. While with ICF Kaiser she served as the contract manager for work performed by Clewell and colleagues on TCE that became part of the 2000 EHP volume, but she had minimal technical involvement. She has worked on a joint project with scientists of the K.S. Crump Group for the U.S. EPA collecting physiological parameter data for neonatal animals.

Dr. Haber was selected for the panel for her experience in applying PBPK models and data to risk assessments and chairing expert panel meetings.

Gregory L. Kedderis

Dr. Gregory L. Kedderis is currently a consultant in biochemistry, pharmacology, and toxicology. He received his Ph.D. degree in biochemistry in 1982 from Northwestern University Medical and Dental School, Chicago, IL. He was a postdoctoral fellow at the Chemical Industry

Institute of Toxicology (CIIT) in Research Triangle Park, NC from 1982 to 1984 and subsequently joined Merck Sharp & Dohme Research Laboratories in Rahway, NJ as a senior research biochemist. Dr. Kedderis returned to CIIT as a staff scientist in 1988, where he was Director of the Chemical Carcinogenesis Research Program (since 1998) and the Division of Biochemistry and Molecular Genetics (since 2000) until 2002. He is also a Visiting Research Professor in the Nicholas School of the Environment and Earth Sciences and in the Integrated Toxicology Program at Duke University, Durham, NC. Dr. Kedderis is author or co-author of 67 publications. He has served on the editorial boards of *Fundamental and Applied Toxicology*, *Drug Metabolism and Disposition*, *Cell Biology and Toxicology*, *Chemico-Biological Interactions*, *Archives of Toxicology*, and the *Journal Pharmacology and Experimental Therapy*. His research interests include the relationship between chemical dosimetry and biological effects, mechanisms of toxicity of drugs and xenobiotics, and mechanisms of genotoxicity and chemical carcinogenesis. Dr. Kedderis is a member of the Society of Toxicology, the Chemical Substances Threshold Limit Values Committee of the American Conference of Governmental Industrial Hygienists, and the National Occupational Research Agenda Cancer Research Methods Team.

Disclosure

Dr. Kedderis was a coauthor of a recent paper (Lipscomb et al., 2003) that addresses interindividual variability in metabolic capacity using TCE as an example. Their modification of the Fisher model was to use better estimates of metabolic capacity and a statistical distribution of that activity. This work was supported by funding from a cooperative agreement with EPA (2000-2003), that was entitled "Development of Chemical-specific Human Physiologically Based Pharmacokinetic Models for Adults and Children" with TCE and chloroform as examples. The objective of the work was to evaluate the impacts of interindividual differences in bioactivation and adult-child differences in bioactivation on internal dosimetry using PBPK models.

Dr. Kedderis was selected for the panel because of his expertise in pharmacokinetics and enzyme kinetics on multiple chemicals.

Dr. Kannan Krishnan

Dr. Kannan Krishnan is Professor of Occupational and Environmental Health at the University of Montreal where he is also the Director of the Human Toxicology research group (TOXHUM). He was a Post Doctoral Research Fellow at the Chemical Institute of Toxicology (CIIT) from 1990-1992. He has been the leader of the risk assessment methodologies theme team of the Canadian Network of Toxicology Centres (1994 – 2001), and Vice President of the Biological Modeling Specialty Section of the Society of Toxicology (2001-2002). Dr. Krishnan is a member of the U.S. National Academy of Sciences (NAS) Sub-committee on Acute Exposure Guideline Levels (2001-2004). He is currently a temporary advisor for the World Health Organization for developing a document on the scientific principles for the health risk assessment for children. His primary expertise is in the areas of pharmacokinetics, PBPK modeling, risk assessment methods, Quantitative Structure Activity Relationship (QSAR)

modeling and mixture toxicology. He has been a peer reviewer of several IRIS updates, risk assessments, mixture risk assessment supplemental guidance and efforts on interactions for US EPA and on toxicological profiles of chemicals, interaction profiles involving Environmental contaminants and mixture risk assessment guidelines for ATSDR. Dr. Krishnan received his P.D. in Public Health from the University of Montréal. He has been on the editorial boards of *Toxicological Sciences*, *International Journal of Toxicology*, *Journal of Applied Toxicology*, and the *Journal of Children's Health.*

Disclosure

Dr. Krishnan has applied a published human PBPK model (Lapare and coworkers at the University of Montreal) to derive the fraction of TCE absorbed following dermal and inhalation exposures of adults and children of various age groups (6, 10 and 14 years) for the Drinking Water Division of Health Canada. He is currently receiving funding from the U.S. EPA to develop a document on the appropriate use of PBPK models in risk assessment.

Dr. Krishnan was selected for the panel for his expertise in pharmacokinetics and PBPK modeling for multiple chemicals.

Biographical Sketches of Presenters

Harvey J. Clewell III

Harvey J. Clewell III is a professional research manager with over twenty-five years of experience in environmental quality research, toxicology research, and hazardous materials management. He has gained an international reputation for his work in the application of physiologically based pharmacokinetic (PBPK) modeling to chemical risk assessment and pharmaceutical safety assessment. He has played a major role in the first uses of PBPK modeling in cancer and non-cancer assessments by EPA, ATSDR, OSHA, and FDA, for such chemicals as methylene chloride, trichloroethylene, vinyl chloride, and retinoic acid. He served for 20 years as an officer in the U.S. Air Force; his duties included Deputy Director of the Air Force Toxic Hazards Research Unit, Director of Hazardous Materials Safety for the Air Force Aeronautical Systems Center, and consultant to the Air Force Surgeon General on Chemical Risk Assessment. He is currently a Principal with the ENVIRON Health Sciences Institute.

Jeffrey W. Fisher

Dr. Fisher has a B.S. degree in biology from the University of Nebraska at Kearney, a M.S. degree in biology from Wright State University, and a Ph.D. in Zoology/Toxicology from Miami University. He has worked as a research toxicologist for 18 years specializing in development and application of physiologically based pharmacokinetic (PBPK) models for risk assessment. He worked for 25 years in a toxicology laboratory at Wright-Patterson AFB and for the last four years at the University of Georgia. Dr. Fisher is currently a Professor and Department Head in the Department of Environmental Health Science. He teaches a graduate class in PBPK

modeling and trains graduate students in the application of PBPK models in toxicology and environmental health. Dr. Fisher has published over 50 papers on pharmacokinetics and PBPK modeling, including several PBPK models for trichloroethylene and its metabolites in laboratory animals and humans. He has also published PBPK models for different life stages for trichloroethylene and perchlorate and as well as PBPK models for other solvents. He has served on several panels and advisory boards for DOD, ATSDR, US EPA and non-profit organizations. He was a US delegate for NATO and a visiting scientist at CIIT and NIOSH.

Appendix C - List of Attendees

TCE Harmonization Model Peer Consultation - Attendees
June 29, 2004

Mr. Ganesh Balasubramanian
Water Quality and Treatment Division

Dr. Robert Benson
US EPA – Region 8

Dr. Weihsueh Chiu
US EPA

Mr. Harvey Clewell
ENVIRON Health Sciences Institute

Ms. Tammie Covington
ENVIRON Health Sciences Institute

Dr. Michael Dourson
TERA

Dr. Paul Dugard
HSIA

Dr. Jeffrey Fisher
Environmental Health Science

Mr. Eric Hack
TERA

Dr. Jeri Higginbotham
Kentucky Dept. for Environmental Protection

Mr. Brian Howard
Air Force Institute for Operational Health

Dr. Donald Hughes
Private Consultant

Dr. Katharine Kurtz
Environmental Programs Directorate

Dr. Brian Magee
AMEC Earth and Environmental

Dr. Andy Maier
TERA

Dr. David Mattie
AFRL/HEPB

Dr. David Morgott
Eastman Kodak Company

Ms. Jacqueline Patterson
TERA

Dr. Jan Storm
New York State Dept. of Health

Dr. Rod Thompson
Indiana Dept. of Environmental Management

Dr. Sheng-Wei Wang
UMDNJ

Mr. Edward Winner
Kentucky EPPC

Dr. Jay Zhao
TERA

Appendix D - Charge for Peer Consultants

Charge Questions
TCE Harmonized Model Peer Consultation
June 29, 2004

The 2001 draft EPA risk assessment of TCE used a number of PBPK models for TCE and its metabolites as part of its quantitative risk assessment. In particular, PBPK models were used for developing human equivalent dose metrics from rodent studies, conducting human route-to-route extrapolation, and characterizing human variability. There were a number of uncertainties surrounding the different models and the data used to develop them. The accompanying document describes a draft PBPK model that attempts to harmonize previous models as well as incorporate new scientific data. Input on this draft model is being solicited from a panel of experts on TCE, PBPK modeling, and the kinetics issues relevant to the TCE assessment. Additional background on the model development is provided in the accompanying model documentation.

For all of the charge question issues, the panel is being queried re what is possible given the available data. The peer consultants are asked to focus on providing any information and insight they have regarding additional data sources and approaches to the modeling, given the available data. We are not looking for input as to the structure of the ideal model if all desired data were available, although one of the charge questions does address key data gaps.

1. Are there additional studies or data (either published or unpublished) that should be incorporated into the model development?

2. Was the glutathione conjugation pathway portion of the model appropriately addressed? Consider both the model structure and the parameterization of the model. Given the available data, is the model structure adequate to describe this pathway? The proposed model assumes that all glutathione conjugate formation results in dichlorovinyl cysteine (DCVC) in the kidney, and that metabolism of DCVC in the kidney is either by beta-lyase to a reactive metabolite or by N-acetyl transferase to a nontoxic metabolite that is excreted in the urine. Is a more complete description needed for this pathway? Are there other data on the metabolism of trichloroethylene (TCE) by the glutathione pathway that should be included? Is metabolism of DCVC in the kidney by flavin mono-oxygenases (FMO) an important factor? Should excretion of DCVC from the kidney into the urine be included? Is there too much uncertainty in this aspect of the model to consider its use to estimate kidney dose metrics for a TCE risk assessment? If so, what would be a reasonable approach for estimating kidney dose metrics?

3. Is the model of chloral in the lung adequate? Chloral is generated in both the lung and the liver, but the authors included only chloral generation in the lung in the model. Is this appropriate? The authors noted in the discussion that local generation of chloral appears to be the dominant source of the lung concentrations of chloral observed in the mouse, since the concentrations of chloral in the lung following oral dosing with TCE were much greater than the concentrations in the blood (from metabolism in the liver). This suggests that not

including systemic chloral in the model may not be a problem. In addition, systemic chloral was not included in the model due to the inability to implement that portion for the human model. Would including systemic chloral for the animal model, but ignoring it for the human model be conservative or non-conservative? Was the choice not to include systemic chloral an appropriate judgment? The issue has been raised regarding whether there are dose-dependent differences in the degree of local versus systemic generation of chloral. This issue arises based on analogy to styrene, for which local metabolism makes a higher contribution at lower concentrations, and has a lower contribution at higher concentrations, due to saturation of local metabolism (Sarangapani et al. 2002). Since the data on TCE found that local generation of chloral dominates at the high concentrations used in the animal study, and the model authors noted that local metabolism will also dominate for humans at the low environmental exposures of interest, they were less concerned about saturation of local metabolism for TCE. Do you agree with the authors' decision? Is the proposed approach for modeling of local chloral production and clearance an acceptable approach, given the available data and what is known about interspecies scaling of aldehyde dehydrogenase (ALDH) kinetics in the lung?

Are you aware of data on the relationship between CYP2E1 and ALDH levels in human vs. mouse lungs that can be used in the model? Is there too much uncertainty in this aspect of the model to consider its use to estimate lung dose metrics for a TCE risk assessment? If so, what would be a reasonable approach for estimating lung dose metrics?

4. The authors have noted difficulties in the modeling of dichloroacetic acid (DCA), due to a number of factors. In some analyses, DCA was formed from TCA as an artifact, so DCA levels measured do not reflect true *in vivo* levels. In addition, because the metabolism of DCA is self-inhibitory, DCA data after multiple dosing in experimental animals would be needed to characterize the DCA generation from TCE. Data on DCA production from TCE in humans are also limited. When DCA production was modeled as a fraction of the rate of metabolism by the P450 pathway in the liver, using the empirical volume of distribution and half life, the predicted time-course for DCA after TCE dosing was not consistent with the best available data (Abbas and Fisher, 1997). Even for these data, which were collected in such a way as to minimize *ex vivo* conversion of TCA to DCA, the concentrations of DCA paralleled those of TCA, suggesting that DCA was being generated from TCA at a level of about 2%. The authors have therefore concluded that it is not currently feasible to confidently model the production of DCA from TCE *in vivo*. Is there a way of describing DCA production and metabolism, given the currently-available data? Is there too much uncertainty in this aspect of the model to consider its use to estimate liver dose metrics for a TCE risk assessment? If so, what would be a reasonable approach for characterizing dose metrics for DCA?

5. TCA binding in the blood is modeled using empirical equations derived from *in vitro* studies. Measured partition coefficients for total TCA between tissues and blood are then converted to partitions for free TCA between tissues and plasma, assuming that all TCA in the tissue is free and using an estimate of the free fraction in plasma from the *in vitro* binding studies. Is this appropriate, given that the fraction bound may not be a constant? Was the calculation of

serum vs. blood concentrations appropriately addressed? Does DCA binding in the blood need to be considered? If so, how?

6. Overall, is the PBPK model structure correct? Is the model adequate for describing the toxicokinetics of TCE and its metabolites for the purposes of the TCE risk assessment? In particular, can the model adequately describe all of the data sets with a consistent set of model parameters? Were the correct metabolites modeled?

7. Are there any other changes you would recommend to the model structure or parameterization? For example, the general TCE model has the capability to describe the fat compartment as a diffusion-limited tissue, but this option was not exercised. Was this an appropriate choice? Is the enterohepatic circulation of TCOH appropriately described?

8. Should the kinetic parameters be calibrated? Should they be re-estimated using Markov Chain Monte Carlo? How many data sets should be used? Which are the key data sets? Are there alternative approaches that should be considered?

9. What key studies would you recommend to fill data gaps?

References:

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