UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

The Honorable Lisa P. Jackson
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Subject: Review of EPA's Draft Assessment entitled *"Toxicological Review of Trichloroethylene"* (October 2009)

Dear Administrator Jackson:

EPA's Office of Research and Development (ORD) requested the Science Advisory Board (SAB) to conduct a peer review of EPA's draft Integrated Risk Information System (IRIS) assessment entitled, "*Toxicological Review of Trichloroethylene"* (October 2009). This draft document responded to the National Academy of Sciences (NAS) 2006 recommendations published in a report entitled *"Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues"* (National Research Council, 2006). In response to ORD's request, the SAB convened an expert panel to conduct this review. The SAB Panel was asked to comment on the scientific soundness of the hazard and dose-response assessments of trichloroethylene (TCE)-induced cancer and non-cancer health effects. Specifically, the SAB was asked to comment on the use of a physiologically-based pharmacokinetic (PBPK) model for dose and route of exposure extrapolation within species and across species; TCE metabolism and mode of action; the derivation of an oral reference dose (RfD) and inhalation reference concentration (RfC) for non-cancer toxicity; the weight of evidence of potential human carcinogenicity; and the estimated cancer oral slope factor and inhalation unit risk for TCE.

The SAB commends EPA for its comprehensive approach and responsiveness to the NAS recommendations. Overall, the SAB Panel supported EPA's scientific approaches to the risk assessment and found these to appropriately adhere to EPA's risk assessment guidelines. The SAB Panel made a number of recommendations aimed at enhancing the transparency of the draft assessment and strengthening the scientific basis for the conclusions presented. The SAB responses to the EPA's charge questions are detailed in the report. SAB major comments and recommendations are provided below:

- EPA has made significant changes that improve the existing PBPK model for TCE. The Panel supported the use of this updated PBPK model for dose- response assessment for the extrapolation of doses within species, across species and route-to-route extrapolation. The Panel also supported the use of the Bayesian framework for estimation and characterization of the PBPK model parameter uncertainties. The Panel made a number

of suggestions for better documentation of the model.

- The Panel found that the draft document adequately synthesizes the available scientific information to support a conclusion that TCE poses a potential human health hazard for non-cancer toxicity, including effects on the central nervous system, the kidney, the liver, the immune system, the male reproductive system, and the developing fetus.
- The Panel supported the selection of an RfC and an RfD based on multiple candidate reference values that fell within a narrow range rather than reliance on a single most sensitive critical endpoint. Although recognizing the kidney hazards of TCE, the Panel was concerned about the use of three candidate RfD/RfCs based on kidney effects as the primary basis for the RfD and RfC because of uncertainties regarding the relative rate of formation of toxic metabolites in humans vs. animals. The Panel recommends that EPA derive RfD/RfC values based on immunological endpoints and cardiac malformations.
- The Panel found that the EPA's meta-analyses for kidney cancer, lymphoma, and liver cancer were well-conducted, with results that bolster the weight of evidence for potential human carcinogenicity from TCE exposure. Accordingly, the Panel agreed with EPA's conclusion that TCE is considered to be "*Carcinogenic to Humans"* by all routes of exposure, based on convincing epidemiological evidence of a causal association between TCE exposure and kidney cancer, compelling evidence for lymphoma, and limited evidence for liver cancer. This conclusion is further supported by consistent evidence from animal studies and pharmacokinetic and metabolism information.
- EPA concluded that a mutagenic mode of action (MOA) was operative in TCE-induced kidney tumorigenesis. However, the Panel concluded that the available evidence also supports MOAs involving cell death and compensatory cell proliferation. The Panel agreed with EPA's conclusion that there is inadequate evidence for an MOA mediated by activation of peroxisome proliferator receptor-alpha for TCE-induced liver cancer in humans.
- Finally, the Panel supported EPA's approaches for deriving cancer inhalation unit risk and oral slope factors, including the use of default age-dependent adjustment factors to address susceptible populations. The Panel supported the use of the French occupational study (Charbotel et al., 2006) as the basis for estimating cancer unit risks, and the use of a default linear extrapolation from the point of departure for cancer dose-response assessment. The Panel, however, recommended inclusion of a more detailed discussion of assumptions used in the analysis to support the calculation of the unit risks.

The SAB appreciates the opportunity to provide EPA with advice on this important subject. The SAB urges EPA to move expeditiously to finalize the IRIS document for trichloroethylene. We look forward to receiving the Agency's response.

Sincerely,

Dr. Deborah L. Swackhamer, Chair	Dr. Deborah Cory-Slechta, Chair
EPA Science Advisory Board	SAB Trichloroethylene Review Panel

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ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criteria
ADAF	age-dependent adjustment factor
BMD	benchmark dose
BMDL	benchmark dose lower bound
BW	body weight
CI	confidence interval
cRfCs	candidate RfCs
cRfDs	candidate RfDs
DCA	dichloroacetic acid
DCVC	dichlorovinyl cysteine
DCVG	S-dichlorovinyl glutathione
DEHP	di(2-ethylhexyl) phthalate
EPA	Environmental Protection Agency
ESRD	end stage renal disease
GC-MS	gas chromatography-mass spectrometry
GSH	gluthione
HEC	human equivalent concentration
HED	human equivalent dose
HPLC-UV	high performance liquid chromatography-ultraviolet
idPOD	internal dose points of departure
IQR	interquartile range
IRIS	Integrated Risk Information System
LOAEL	Lowest Adverse Effect Level
MCMC	Markov Chain Monte Carlo
MOA	mode of action
NAG	N-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NOAEL	No Adverse Effect Level
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
ORD	Office of Research and Development
PBPD	physiologically-based pharmaodynamic
PBPK	physiologically-based pharmacokinetic
p-cRfC	PBPK model-based candidate RfCs
p-cRfD	PBPK model-based candidate RfDs
PERC	perchloroethylene
POD	point of departure
PPARα	peroxisome proliferator activated receptor alpha
RCC	renal cell carcinoma
RfC	reference concentration

RfD	reference dose
RR	relative risk
SIR	standardized incidence ratio
SMR	standardized mortality ratio
TCA	trichloroacetic acid
TCE	trichloroethylene
TCOH	trichloroethanol
UF	uncertainty factor
VSD	ventricular defects

EXECUTIVE SUMMARY

This report was prepared by the Science Advisory Board (SAB) Trichloroethylene Review Panel (the "Panel") in response to a request by EPA's Office of Research and Development (ORD) to review the Draft IRIS Toxicological Review of Trichloroethylene (TCE) (hereafter referred to as the draft document). The Panel deliberated on the charge questions (see Appendix A) during a May 10 – 12, 2010 face-to-face meeting and subsequent conference calls on June 24, 2010 and September 13, 2010. There were 12 charge questions that focused on: hazard assessment of non-cancer and cancer health effects, the use of a PBPK model for TCE and its metabolites for the derivation of a proposed oral reference dose (RfD), an inhalation reference concentration (RfC) for non-cancer endpoints, cancer weight of evidence classification, mode of action of TCE carcinogenicity, as well as inhalation and oral unit risks for TCE. This Executive Summary highlights the Panel's major findings and recommendations.

PBPK Modeling

The Panel commended the updated PBPK model (Evans et al., 2009; Chiu et al., 2009) for dose-response assessment. The Panel found that while the PBPK model was generally well presented, its description was incomplete in that mass-balance equations were not presented. The Panel provided suggestions to improve model documentation and clarity, including clearer descriptions of the strategy behind the model structure and the biological relevance of each model equation. Model assumptions need to be more clearly described and the consequences of potential violations of these assumptions should be discussed. In addition, a more detailed justification was needed for the handling of between-animal variability in the model. The Panel agreed that use of the Bayesian framework for estimation and characterization of the PBPK model parameter uncertainties was appropriate. However, a more thorough description was needed for the choice of prior distributions, the Bayesian fitting methodology, and the fit of the posterior distribution for each model parameter. The Panel also generally endorsed the hierarchical calibration approach that uses the posterior results in mice to establish the rat priors, and the rat posterior results to set the human priors. The Panel also recommended performance of a local sensitivity analysis to identify key model parameters that drive changes in modeling results.

Meta-Analyses of Cancer Epidemiology

The Panel agreed that EPA's updated meta-analyses for kidney cancer, lymphoma and liver cancer followed the NRC (2006) recommendations. The Panel agreed with EPA's conclusions that TCE increased the risk for the three cancers studied, based on appropriate inclusion criteria for studies, the methods of conducting the meta-analysis that included consideration of bias and confounding, and the robustness of the findings based on the tests for heterogeneity and sensitivity. The Panel also suggested performing a meta-analysis for lung cancer to further support the absence of smoking as a possible confounder.

Non-Cancer Hazard Assessment

EPA has provided a comprehensive synthesis of the available evidence regarding the effects of TCE and its major metabolites on the central nervous system, the kidney, the liver, the immune system, the male reproductive system, and the developing fetus. One issue of concern was the inconsistencies between reported levels of glutathione conjugation pathway metabolites. The Panel recommended that the impact of these divergent levels be more transparently presented. The Panel recommended inclusion of the potential for TCE-induced immune dysfunctions (i.e.immunosuppression, autoimmunity, inappropriate and/or excessive inflammation) to mechanistically underlie other adverse health endpoints.

Carcinogenic Weight of Evidence

The Panel agreed with EPA's conclusion that TCE is "*Carcinogenic to Humans"* by all routes of exposure. This is based on convincing evidence of a causal association between TCE exposure and kidney cancer, compelling evidence for lymphoma, and more limited evidence for liver cancer as presented in the draft document. The epidemiologic data, in the aggregate, were quite strong. The summary risk estimates from the meta-analyses provided a clear indication of a cancer hazard from TCE. In addition, both animal data and toxicokinetic information support the epidemiologic data.

Role of Metabolism

The Panel agreed with EPA's conclusion that oxidative metabolites of TCE were likely responsible for mediating the liver effects. The Panel recommended that EPA examine studies that provided quantitative assessment of trichloroacetic acid (TCA) and dichloroacetic acid (DCA) formation after TCE exposure. Dose-response modeling, similar to that performed for tetrachloroethylene, may be considered by EPA to provide scientifically-based information on relative contribution, or lack thereof, of TCA and/or DCA to the liver carcinogenesis effect of TCE.

EPA has provided a clear and comprehensive summary of the available evidence that metabolites derived from GSH conjugation of TCE mediate kidney effects. The Panel noted that uncertainties exist with regard to the extent of formation of the dichlorovinyl metabolites of TCE between humans and rodents. The issue of quantitative assessment of the metabolic flux of TCE through the GSH pathway vs. the oxidative metabolism pathway needs to be considered carefully. A more complete discussion of the strengths and limitations of the analytical methodologies used should be provided to address the large discrepancies in estimates of DCVG formation.

Mode of Action (MOA)

The Panel agreed that the weight of evidence supports a mutagenic MOA for TCE-induced kidney tumors. However, the Panel concluded that the weight of evidence also supported an MOA involving cytotoxicity and compensatory cell proliferation and including

these may more accurately reflect kidney tumor formation than does a mutagenic mechanism alone. The combination of cytotoxicity, proliferation and DNA damage together may be a much stronger MOA than any individual components.

The Panel agreed that the TCE-induced cancer and non-cancer effects in rodents are relevant to humans.

The Panel agreed that there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced human liver carcinogenesis. Recent data from animal models (Yang et al., 2007) suggest that activation of PPARα is an important but not limiting factor for the development of mouse liver tumors, and additional molecular events may be involved. The Panel viewed the MOA for liver carcinogenicity in rodents as complex rather than unknown. It is likely that key events from several pathways may operate leading to acute, subchronic and chronic liver toxicity of TCE.

Susceptible Populations

The Panel found EPA's hazard assessment provided a good review of potentially susceptible populations, and identified factors (genetics, lifestage, background, co-exposures and pre-existing conditions) that may modulate susceptibility to TCE carcinogenicity and non-cancer effects. However, the Panel disagreed with EPA's conclusion that toxicokinetic variability can be adequately quantified using existing data. The Panel recommended that exposure to solvent mixtures should be considered for potential co-exposures, since exposure to more than one chemical with the same target organ likely increases risk.

Selection of Critical Studies and Effects

The Panel supported the selection of a RfC and RfD based on multiple candidate reference values that lie within a narrow range at the low end of the full range of candidate reference values developed, rather than basing these values on the single most sensitive critical endpoint. The Panel expressed concerns about the use of several candidate critical studies and effects, specifically NTP (1988) [toxic nephropathy], NCI (1976) [toxic nephrosis], and Woolhiser et al. (2006) [increased kidney weights]. However, the Panel noted that uncertainties about the quantitative risk assessment based on kidney effects in NTP (1988), NCI (1976) and Woolhiser et al. (2006) did not indicate that there was uncertainty that TCE caused renal toxicity. As discussed previously, the three PBPK model-based candidate RfCs/RfDs (p-cRfCs/RfDs) for renal endpoints were based on an uncertain dose metric, especially in regard to the relative rate of formation of the toxic metabolite in humans and animals. Additional issues related to choice of toxic nephropathy in female Marshall rats from NTP (1988) included excessive mortality due to dosing errors and possibly other causes, and a high level of uncertainty in the extrapolation to the benchmark dose (BMD) due to the use of very high doses and a high incidence (>60%) of toxic nephropathy at both dose levels used. With respect to toxic nephrosis in mice from NCI (1976), the BMD analysis was not supported because the effect occurred in nearly 100% of animals in both dose groups, and because a high level of uncertainty is associated with extrapolation from the LOAEL at which nearly 100% animals were affected. Renal

cytomegaly and toxic nephropathy, which were not selected as critical effects, occurred at high frequency in all treated groups.

The Panel recommended that the two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) be considered the principal studies supporting the RfC. The Panel also recommended that the endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2009) and the cardiac malformations from Johnson et al. (2003) be considered as the principal studies supporting the RfD.

Derivation of RfD and RfC

The screening, evaluation, and selection of candidate critical studies and effects used for the development of the RfC and RfD were sound. The derivation of the points of departure (PODs) was generally appropriate. However, the BMD modeling results were uncertain for some datasets. For example, the log-logistic BMD analysis for toxic nephropathy in female Marshall rats in NTP (1988), shown in Figure F-10 in Appendix F, may greatly overestimate the risks at low doses. As discussed above, this modeling involved extrapolation from a high LOAEL at which a high percentage of the animals were affected.

EPA used PBPK-based dose metrics for interspecies, intraspecies, and route-to-route extrapolation. The Panel supported this approach for development of the RfC and RfD. The Panel noted that the candidate RfDs /RfCs for kidney endpoints were highly sensitive to the rate of renal bioactivation of the cysteine conjugate, DCVC, in humans relative to rodents. Candidate RfDs/RfCs developed using this dose-metric were several hundred-fold lower than RfD/RfCs for the same endpoints based on applied dose with standard uncertainty factors. The Panel noted that the uncertainties about the *in vitro* and *in vivo* data used to estimate the rate of renal bioactivation of DCVC were much greater than for other dose metrics [e.g. there are large discrepancies in the rates of human glutathione conjugation reported by Lash et al. (1999a) and Green et al. (1997a)]. These uncertainties should be clarified and should be the basis of a sensitivity analysis in the next update of the TCE draft risk assessment. The Panel also recommended that the rationale for scaling the dose metric to body weight$^{3/4}$, in conjunction with the interspecies extrapolation based on PBPK modeling, should be presented in a clearer and more transparent way.

Uncertainty Factors

The Panel agreed that, in general, the selection of uncertainty factors was clearly and transparently described and appropriate. EPA developed equivalent doses and concentrations for sensitive humans to replace standard uncertainty factors for inter- and intra-species toxicokinetics. The Panel concluded that the approach used, including the selections of Point of Departure (PODs) and the extrapolations from rodent to human, followed by consideration of the 99th percentile human estimates, was acceptable to address the sensitive population. In future work, the variability and uncertainty could be better characterized by considering other quantiles of the distribution.

Inhalation Unit Risk and Oral Unit Risk

In this assessment, EPA developed an inhalation unit risk and oral unit risk for the carcinogenic potency of TCE in accordance with the approach outlined in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a, b). The unit risks for renal cell carcinoma were based on a case control study published by Charbotel et al. (2006). The Panel found that the analysis of the Charbotel et al. (2006) data was well described and that the selection of this study to estimate unit risks was appropriate. However, more discussion is needed on whether or not it is necessary to adjust for exposure to cutting oils when computing an odds ratio or relative risk relating TCE exposure to kidney cancer. The Panel recommended that EPA take a closer look at the literature to determine if there are other studies which suggest that exposure to cutting oils is a risk factor for kidney cancer. EPA should also provide a more detailed discussion on the implication of assumptions made in their analysis. In addition, background kidney cancer rates in the United States were used in constructing the life table, although the Charbotel et al. (2006) data was based on a French cohort. A comparison of background cancer rates in France and the United States would be helpful in supporting their conclusions. The Panel supported the adjustment of the renal cell carcinoma unit risks to account for the added risk of other cancers, using the meta-analysis results and Raaschou-Nielsen et al. (2003).

The Panel agreed that human data, when available, should be preferred over rodent data when estimating unit risk since within species uncertainty is easier to address than between species uncertainty. The Panel supported the use of linear extrapolation from the POD for cancer dose-response assessment of TCE as a default approach. The Panel agreed that characterization of uncertainty and variability was appropriate, and was exceptionally strong in the PBPK models.

Age-Dependent Adjustment Factors (ADAFs)

The Panel agreed that application of ADAFs in the TCE analysis consistently followed recommendations in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a). All of the steps were clearly presented for inhalation exposure. However, the discussion for the oral exposure route was shortened and referred back to the inhalation section, making understanding of the example difficult to follow. Currently, EPA's IRIS assessment provide lifetime cancer risk drinking water concentrations for adults only. The Panel recommended that drinking water concentrations for specified cancer risk levels should also be derived for various age groups.

RESPONSES TO EPA'S CHARGE QUESTIONS

1. PBPK Modeling

Is EPA's updated PBPK model for TCE and its metabolites (also reported in Evans et al., 2009, and Chiu et al., 2009) clearly and transparently described and technically and scientifically adequate for supporting EPA's hazard characterization and dose-response assessment? Specifically, please address the PBPK model structure; Bayesian statistical approach; parameter calibration; model predictions of the available in vivo data; and characterization of PBPK model dose metric predictions, including those for the GSH conjugation pathway.

Response

1a PBPK model structure

According to the TCE Review Document (page 3-64), the version of the PBPK model published by Hack et al. (2006) consisted of many parameter values that differed by study, particularly in the case of metabolism. In addition, according to the authors, DCA metabolism in the lung compartment remained highly uncertain. Subsequently, the EPA made efforts to improve the 2006 model using an extensive analysis with different datasets to produce the PBPK model used in this risk assessment. The Panel found this PBPK model expansion seemed to accurately predict the internal dose in the target tissue. The Panel agreed that using a PBPK model did improve the quality of the predictions for risk assessment and anticipated that the current model will reduce uncertainties that resulted from the use of previous PBPK models.

The Panel found that, for the most part, the PBPK model was well presented in the TCE Review Document but also noted that improvement was still possible. For example, the conceptual representation of the PBPK model given in Figure 3-7 [page 3-69] was useful in understanding the changes made to the Hack (2006) model, but did not facilitate a full understanding of the model structure. Figure 3-7 could be expanded to also include the symbols used for the model parameters (e.g. blood flow and metabolic parameters along the appropriate arrows and volumes in the compartments).

The Panel agreed that the details provided in Appendix A fully explain how the population model was structured. However, the description of the PBPK model was incomplete in that the mass-balance equations are not presented. In parallel to presenting these equations, references should be given to Figure 3-7 (PBPK model structure) and Table A-4 (PBPK model parameters). A better description would facilitate a complete understanding of both the conceptual and mathematical structure of the model. The Panel suggested the following additions: 1) a more detailed explanation of how interspecies extrapolation was performed, especially the use of scaling equations, 2) graphical comparisons of prior vs. posterior distributions for all key parameters, and 3) fits and the graphs of the concentration-time profiles and the predictions of critical dose metrics. These additions can be made to either the master document or incorporated into Appendix A. Many of the desired graphics could be found in the

"linked documents" but these were overlooked by many reviewers because they were not part of the formal documentation. Placing many of these graphics alongside the model descriptions will improve both clarity and transparency.

On the issue of PBPK model structure, the Panel had some difficulty in fully understanding its structure, and also noted deficiencies in the mathematical descriptions for each compartment. With enough work and persistence, the structure was understandable, but these deficiencies will be a bigger issue for users who are not experts in PBPK modeling. The Panel made recommendations regarding improvements to the documentation of the PBPK model.

The Panel believed that the model documentation should also highlight any questionable assumptions and discuss the potential implications of these assumptions being wrong. The Panel observed that there remained a significant amount of variability between animals that did not seem to be accounted for in the final model. Because the raw data sets were not available to the Panel, it was difficult to determine if this was indeed the case. In addition, some analyses discussed by the Panel would appear to be computationally unfeasible. The Panel initially discussed extensions of the model which would avoid some of these problems (e.g., inclusion of animal-specific parameters), but decided that these extensions are computationally unfeasible given current resources.

Recommendations:

- Provide a better description of the final model structure and, in particular, provide a revised model structure diagram that identifies model parameters with model states and pathways (flows).
- Clarify the strategy behind the model structure and describe the biological relevance of each model equation.
- Document model assumptions and discuss the consequences of potential violations of these assumptions, e.g. impacts on bias and accuracy) .
- Provide a more detailed justification for how between animal variability is accounted for in the model.

1b Bayesian statistical approach

The Panel agreed with the EPA that use of the Bayesian framework for estimation and characterization of the PBPK model parameter uncertainties was appropriate. The general description of the Bayesian approach presented in the TCE review document was acceptable. The description of how uncertainty and variability are characterized was confusing mainly due to the inconsistent use of the terms "population" and "group." The description of the Bayesian model fit suffered from a lack of sufficient detail to provide complete transparency. Several model parameters entered the Bayesian estimation method with wide and uniform prior distributions. The large number of such parameters made the Markov Chain Monte Carlo (MCMC) chains longer, resulting in long time to convergence and wide posterior distributions. The Panel noted high variability in the posterior distributions of many model inputs and the

stated parameters. However the posterior distributions for many internal dose stated parameters were much less variable.

The Panel would have liked to see the extent to which posterior parameter distributions are correlated. If rodent parameters were correlated as might be expected, how this correlation was accounted for in human-specific model parameter estimates should be discussed.

Recommendations:

- Present better descriptions and/or details on the choice of prior distributions, the Bayesian fitting methodology and fit of the posterior distribution for each model parameter.
- Provide some information on correlations around posterior medians for species-specific parameters.
- Supply more information on the model ordinary differential equations and on the likelihood function used in the Bayesian estimation.

1c Parameter Calibration

Parameter calibration as described in the draft Document was accomplished via a hierarchical fitting approach that used the posterior results in mice to establish the rat priors and the rat posterior results to set the human priors. The Panel generally endorsed this hierarchical fitting approach.

Recommendation:

- Improve the quality and the description of the assumptions underlying the use of the hierarchical approach to parameter calibration. Help the reader to understand the extent to which these assumptions are used consistently throughout the parameter calibration process.

1d Model Fit Assessment and Dose Metric Projections

There were a very large number of parameters in the PBPK model which made critical review of the whole model and in particular identifying the key issues around model fit a significant challenge.

A review of Figures 3-9, 3-10, A-3 and A-4, suggested that the updated model has adequate fit. Table 3-45 was particularly useful, as were the graphs in the linked documents that provided detailed descriptions of how well the model fit for the individual in vivo studies. When evaluating the quality of each prior, the draft document focused on agreement of the interquartile ranges. In Figure 3.9 (page 3-107), the vertical axes changed from the Hack model fit to the updated model fit. This added a challenge to assessing model fit since the models were predicting two slightly different quantities [N-Ac(1,2-DCVC) excreted (ug) for the Hack model and N-Ac(1,2 or 2,2 -DCVC) excreted (ug) for the updated model].

As a measure of model goodness of fit, the draft document presented the residual error geometric standard deviations (Table 3-41, page 3-98). The Panel was not certain how to use this statistic. For example, what does it say about model fit when the residual error is GSD 2.7 for venous blood TCE? Does this indicate a good fit or poor fit? For people who are not familiar with the design of the PBPK model, it is hard to critically interpret the values in this table.

The Panel pointed out other issues related to the evaluation of the posterior distributions. Some of the posteriors were flatter than their priors, which was an unexpected result. In addition, in Table 3-36, (section 3.5.6.2), pages 3-88 to 3-89, the Panel observed that prior and posterior distributions of model parameters were almost identical and only in a few cases were the distributions different.

The Panel noted that a large number of studies were available to EPA for this review. Some of the rat studies were not used for parameter calibration and hence were used to assess the validity of the model, that is, to determine whether the fitted model was adequate to predict data from situations not specifically covered in the parameter estimation exercise. The Panel approved of this approach, finding that even a limited validation analysis improved the confidence of users in the final PBPK model and helped point to areas where the model may still be inadequate.

Recommendations:

- Move some graphical presentations from the linked graphics documents into the body of the report or into Appendix A.
- Incorporate more discussion on model fit and in particular indicate areas where the model fits well and areas where it did not fit well. Tie this discussion somehow to Table 3-41.
- Include graphs that show predicted versus observed values for all data points used in the analysis (one graph per endpoint).
- To help readers identify which parameters are better specified than others, provide a table of model parameters listed in reverse order by the width of their posterior variability (width of the IQR or width of 95% CI).
- Identify those parameters with very different prior and posterior distributions and discuss why this might be a reasonable result of the parameter calibration process. An alternative would be to provide a table where parameters are ranked based on the percent change of the posterior from the prior.
- Clarify which parameters are related to variability and which address parameter uncertainty. Separate the discussion of the two types of parameters.

1e Lack of an adequate sensitivity analysis

The charge to the Panel did not specifically address parameter sensitivity but the Panel did discuss the lack of and need for some form of sensitivity analysis. A common feature of PBPK models is that the output is highly sensitive to a few parameters (key parameters) and far less sensitive to the remaining parameters.

Recommendation:

- Perform a local sensitivity analysis, starting from the final fitted PBPK model, to assess how small changes in model parameter estimates impact predictions. Provide graphical presentations of the sensitivity of the model to changes in key model parameters in the final documentation.

2. Meta-analysis of cancer epidemiology

NRC (2006) recommended that EPA develop updated meta-analyses of the epidemiologic data on TCE exposure and cancer, and provided advice as to how EPA should conduct such analyses. Is EPA's updated meta-analysis of the epidemiologic data on TCE exposure and kidney cancer, lymphoma, and liver cancer clearly and transparently described and technically and scientifically adequate for supporting EPA's hazard characterization and dose-response assessment? Specifically, please address the standards of epidemiologic study design and analysis as they were applied to select studies for inclusion in the meta-analysis; the rationales for study relative risk estimate selections; the meta-analysis methods; and the characterization of the conclusions of the meta-analyses. [Note: The scope of this charge question only includes the meta-analysis methods and results and not the overall weight of evidence for TCE carcinogenicity, which is addressed as part of a subsequent charge question.]

Response

NRC recommended that EPA conduct a new meta-analysis and to (1) pay attention to essential design features; (2) include only studies where exposure is documented; (3) classify studies on objective characteristics; (4) assess study power for each; (5) combine cohort and case-control studies unless it introduces substantial heterogeneity; (6) test for heterogeneity; and (7) perform sensitive analyses.

The Panel agreed that EPA followed these principles in their meta-analyses for lymphoma, and cancers of the kidney and liver. The EPA approach was clearly and transparently described and technically and scientific appropriate for supporting EPA's hazard characterization and dose-response assessment. The Panel found EPA performed a thorough literature review and clearly developed a comprehensive listing of candidate studies for the meta-analyses. The strengths and weaknesses of each study were characterized and clearly presented in the draft document. Procedures for selection of studies for the meta-analyses were clearly described.

Studies selected for inclusion had clear indications of TCE exposure and included exposure assessments for each study participant. Exposure levels differed considerably among and within the studies, which was an advantage. Candidate studies were also evaluated based on study design, endpoints evaluated, TCE exposure assessment, follow-up procedures for cohort studies, interview type (for case-control studies), use of proxy respondents (for case-control studies), sample size, and statistical analysis. Information on these factors was clearly presented for each candidate study. Appropriate criteria for including and excluding studies from the meta-analysis were developed and carefully applied. Reasons for excluding studies were clearly stated. Studies included had cohort or case-control designs, appropriate evaluation of cancer incidence or mortality, adequate selection of study subjects, characterization of individual TCE exposure for each subject, and relative risk estimates for lymphoma or cancers of the kidney or liver adjusted for at least age, sex, and race. For example, studies where individual exposure to TCE could not be reasonably determined were excluded, even though some exposure to individuals in the group was a reasonable assumption. Although excluded studies likely included

some individuals who had exposure to TCE, exclusion was appropriate because inclusion would likely result in classification of some unexposed individuals as exposed, which would increase exposure misclassification and bias estimates of relative risk downward. The Panel found EPA carefully considered and described overlap between different studies (because of slightly overlapping study populations and extended follow-up of individual cohorts) and made appropriate selection of the results to include in the meta-analyses. The strengths and weaknesses of the meta-analyses were appropriately considered in the evaluation and interpretation of the results in relation to hazard characterization.

The Panel found that EPA discussed possible misclassification of exposure and disease for the studies included in the meta-analyses. EPA appropriately noted that most exposure assessment limitations would diminish relative risks and mute exposure-response gradients.

EPA indicated that in only one study were the interviewers blinded with regard to case/control status. Although it is desirable to attempt blinding for case-control studies, it is usually not possible to fully accomplish this because subject responses during the interview provide clues as to subject status. The Panel thought this was not a serious limitation.

The Panel found that EPA clearly described the statistical techniques used in the meta-analyses. Both random and fixed-effect models were used in the meta-analyses. This was useful to assess the accuracy of the underlying assumptions regarding study variation. The Panel agreed with EPA's reliance upon the random effects models for interpretation. Use of several approaches to evaluate heterogeneity provided a fuller characterization than would be available from any single technique. The potential for publication bias was appropriately evaluated. The robustness of the findings was highlighted based on the tests for heterogeneity and sensitivity. Results from the meta-analyses were fully and clearly presented in tables and figures.

Meta-analyses were performed only for lymphoma, and cancers of the kidney and liver. The text did not make clear why only these three were selected for the meta-analysis approach, although it was assumed this was because prior reviews of the literature had identified these cancers as possibly associated with TCE exposure. The Panel found it might be useful to have information on other cancers to provide evidence regarding possible confounding. For example, kidney cancer was associated with smoking. Most cohort studies lacked information on tobacco use. However, if there was confounding by smoking, there would have to be an excess of lung cancer and other tobacco-related diseases in the cohorts. Absence of an excess of lung cancer was very strong evidence that workers exposed to TCE did not smoke more than the unexposed, or comparison population. Although no studies had excess of lung cancer, a meta- analysis of lung cancer showing no association with TCE would document this conclusion regarding possible confounding. Smoking could not cause excesses of kidney cancer, liver cancer or lymphoma without also causing an excess of lung cancer. The lack of effect of TCE for lung cancer in individual studies provided convincing evidence that confounding by smoking is unlikely.

The Panel agreed that EPA carefully evaluated the data from the studies included in their review and results from the meta-analyses against standard epidemiologic criteria for causality,

i.e., consistency, strength of the association, specificity of the association, temporal relationship, exposure-response gradient, biologic plausibility, coherence, experimental evidence, and analogy. The document provided a full discussion of these issues.

Bias and confounding are concerns in epidemiologic studies. The Panel agreed that the draft document had a strong discussion on potential confounding. Age, gender and race were appropriate potential confounders to include in the meta-analyses and the meta-analyses included effect estimates that were adjusted. The potential for confounding was evaluated in a number of ways. Several of the case-control studies could directly adjust for potential confounding from important risk factors and provide directly adjusted relative risks. EPA also pointed out that many potential confounders, e.g., obesity, diabetes, tobacco, and hypertension in kidney cancer, were unlikely to be associated with the level of TCE exposure and, thus, were unlikely to confound. If these factors did confound, other cancers would be affected. Other occupational exposures were mentioned as possible confounders, e.g., other organic solvents, cutting fluids, and hydrazine. The link between most of these and the cancers of concern relative to TCE was weak or non-existent, so they were not strong candidates for confounding. Biases are also a concern in observational studies. In case-control studies, case-response bias and case or control selection bias are a concern, while in cohort studies biases associated with follow-up and exposure are a concern. No obvious bias that would occur across studies of different designs, in different countries, and with different exposure metrics falsely produced an association with TCE. The Panel did not think confounding or bias were likely explanations for the findings from the epidemiologic studies and meta-analyses.

The Panel agreed that the findings of several community studies although intriguing, were appropriately omitted from the meta-analyses due to large misclassification errors and lack of control for confounding, which would tend to bias estimates from the meta-analysis.

The Panel found that EPA appropriately discussed the changing classification of hematopoietic and lymphatic system tumors and selected lymphoma (predominately non-Hodgkin's lymphoma (NHL) as an outcome for meta-analysis. EPA specifically wanted to select studies with the best outcome definitions, rather than pick at studies where the hematopoietic cancers were grouped. (e.g. myeloid and lymphoid neoplasms together). EPA selected studies representing various groupings of NHLs (with some studies that included chronic lymphocytic leukemia) or focused on specific subtypes of NHL (including one study that focused on hairy cell leukemia), but did not include studies of Hodgkin lymphoma (if any such studies existed). Given that the EPA's intent was to conduct a meta-analysis with NHL as the outcome, the Panel felt that the terminology should be changed to 'non-Hodgkin lymphoma' instead of 'lymphoma', throughout the document. The term 'NHL' more accurately describes the intent of the analysis as well as the overwhelming majority of cases in the analysis, despite changing classification schemes. The focus of the meta-analysis on NHL and any indication in the meta-analysis where cases definition may diverge from classical NHL (as in studies that included chronic lymphocytic leukemia) should be clearly explained in both Appendix C and in the Hazard Characterization document (section 4.6.1.2.2).

The Panel agreed that appropriate approaches were used in the meta-analysis. Effect size (the relative risks or odds ratios) included in the meta-analyses were selected appropriately using

the most appropriate selection criteria. However the Panel has a few questions of clarification about the meta-analysis for kidney cancer.

There are a number of technical points that should be mentioned as footnotes to the meta analysis plots. First, the exact confidence intervals given in the original publications have been replaced with approximations. The Panel suggests that the explanation in Appendix C be reiterated in the main document. For reference, Appendix C, Table C-6 [pages C-26 to C-27] shows the actual SE(logRR) used to calculate the weights. In addition, Appendix C, page C-3, lines 14-20 explains the discordant confidence intervals in the figures. A second example is that a 20 year lag was used for the Zhao study while lags were either not given or not used in the other studies. Clarify the rationale for selecting the "20 yr lag" result from Zhao et al. (2005) and not selecting the "20 yr lag" result from Raaschou- Nielsen et al. (2003).

The Panel agreed with EPA's conclusions from the meta-analyses that TCE increased the risk for the three cancers studied. The Panel's agreement with EPA's conclusion was based on the strict and appropriate inclusion criteria, the methods of conducting the meta-analyses, including consideration of bias and confounding, and the robustness of the findings based on the tests for heterogeneity and sensitivity.

Recommendations:

- Provide a rationale for the three cancer sites selected for the meta-analysis. The rationale could be nicely summarized in a table.

- Consider including meta-analysis for lung cancer for confounding purposes or other sites for comparison for which some association with TCE exposure has been reported in epidemiologic studies, such as childhood leukemia and cervical cancer. It might also be possible to provide this information without a formal meta-analysis.

- Provide measures of heterogeneity such as the I^2 statistic for each meta-analysis. Although this information was provided and accurately explained in Appendix C, it was mischaracterized at several points in the primary document. For example, the summary of the kidney cancer meta-analysis on p. 4-167 of the primary document states that "there was no observable heterogeneity across the studies for any of the meta-analyses," but Appendix C indicates "the I^2 value of 38% suggested the extent of the heterogeneity was low-to-moderate." Non-significant heterogeneity is indeed observed heterogeneity.

- Evaluate the likely impact of converting odds ratios to relative risk estimates (i.e., using the method of Greenland (2004) or Zhang and Yu (1998), and decide if necessary to perform these conversions for the meta-analysis.

- Change the terminology regarding the meta-analysis results for 'lymphoma' to 'non-Hodgkin lymphoma' throughout the document.

3. Non-Cancer Hazard Assessment

Does EPA's hazard assessment of non-cancer human health effects of TCE logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions that TCE poses a potential human health hazard for non-cancer toxicity to the central nervous system; the kidney; the liver; the immune system; the male reproductive system; and the developing fetus, including the role of TCE in inducing fetal cardiac defects?

Response:

The Panel agreed that the EPA's TCE hazard assessment has clearly, accurately, logically and objectively represented and synthesized the available scientific evidence to support its conclusions that TCE poses a potential human health hazard for non-cancer toxicity. Specifically, the EPA has provided a comprehensive and thorough synthesis of the available evidence regarding the effects of TCE and its major metabolites in each of the tissues addressed in the charge question. This includes human epidemiological studies, animal studies, in vitro studies using renal cell cultures, and in vivo and in vitro metabolism studies.

3a Central Nervous System

TCE-associated auditory impairment was discussed in this section (4.3.2.3.). It is noted that auditory impairment is commonly seen with various autoimmune conditions and inflammation-based diseases and these were among the immune dysfunctions observed with TCE exposure.

3b The Kidney

In regard to the effects of TCE in the kidney, EPA had provided a thorough but clear description of these effects. One issue of concern here was the quantitative aspect of the GSH pathway metabolites. Dr. Wolfgang Dekant, in his public comment, suggested that data obtained using the "Reed method" overestimated the amount of DCVG produced. This HPLC method is characterized by variability and overall decline in retention times over the life of the HPLC column due to derivatization of amine groups on the column (Lash *et al.*, 1999b). Although data are limited, GSH pathway metabolite levels reported by methods that utilize ^{14}C TCE and radiochemical detection followed by mass spectrometry identification of the metabolites (Green et al, 1997a) are lower than those from reports using the "Reed method". In addition, studies using HPLC-MS/MS techniques with stable isotope-labeled DCVG and DCVC standards have also been used to detect GSH pathway metabolite levels (Kim et al, 2009). Based on the in vitro work presented in Table 3-23 (page 3-44 of the draft EPA document) determining DCVG formation by the "Reed method" in human, rat and mouse liver, one would expect mouse serum DCVG levels to be ~4-6 times lower than humans. However, using the HPLC-MS/MS technique of Kim et al., the peak DCVG serum levels are ~1,000 times lower in mouse serum than determined by Lash et al. (1999a) in human serum. Although differences in exposure routes, exposure doses, etc. should be

considered, this much larger than expected difference also suggests that the "Reed method" provides an overestimation of DCVG levels in humans. This could occur if the "Reed method" identifies non-specific derivatives as DCVG or other GSH pathway metabolites. Thus, interpretation of DCVG levels from the Lash et al. (1999a) paper should be made with caution.

It is noted that the focus on animal data in the EPA report is appropriate because human data on non-cancer kidney effects from TCE are limited by two factors. The first is outcome assessment. Due to the insensitivity of the clinical kidney outcomes such as glomerular filtration rate and end stage disease, human nephrotoxicant work often uses kidney early biological effect markers. Unfortunately, research to accurately determine the prognostic value of these biomarkers is fairly limited and data analysis in many of these studies is quite rudimentary often involving only a comparison of unadjusted mean values between an exposed and a control group. A range of biomarkers are used and results are frequently not entirely consistent as noted in Section 4.4. The second challenge is that human exposure often involves a mixture of solvents making determination of the impact of an individual solvent difficult. For example, the GN-PROGRESS retrospective cohort study in Paris, France, which examined the impact of solvents on risk of end stage renal disease (ESRD) and progression of glomerulonephritis, included patients with a wide range of solvent exposures. Solvent exposure was assessed by industrial hygienists from lifetime occupational histories collected by interview and a list of the 30 most common solvents. These authors noted an elevated risk for progression of glomerulonephritis to ESRD from TCE although numbers were small and did not achieve statistical significance (adjusted hazard ratio [95% CI] 2.5 [0.9 to 6.5]) (Jacob et al, 2007). These authors also did not discuss how they addressed exposure to solvent mixtures as they attempted to focus on specific agents.

3c The Liver

The only criticism noted for this section was the (perhaps unavoidable) repetitive nature of their coverage, as these issues appeared elsewhere in the document. Less repetition and better integration of these sections would improve the readability of the document.

3d The Immune System

It is noted that the children's exposure data and adverse outcomes are consistent with the immunotoxicity reported in the animal developmental models. It is noted that while TCE exposure can produce a range of immune dysfunctions, including immunosuppression, elevated risk of autoimmunity and dysregulation of inflammation, it is possible that the doses of TCE producing each category of adverse immune outcomes may differ. For example, most studies reporting autoimmune dysregulation used higher doses of exposure compared with at least some studies where immunosuppression was observed.

3e The Male Reproductive System

It is noted that male potency/sterility issues can be associated with inflammatory dysfunction in the testes produced by some environmental pollutants (usually associated testicular macrophage dysfunction) (see Pace et al., 2005). Since inflammatory dysfunction is associated with TCE exposure, this is an additional possible mechanism that may be associated with adverse outcome for male potency. For in utero exposure studies in rodents using lower doses of TCE and metabolites, where effects (carcinogenic and non-carcinogenic) can be observed transgenerationally, attention should be directed to epigenetic changes as possible MOA for TCE-mediated effects on the reproductive systems.

3f The Developing Fetus, Including the Role of TCE in Inducing Fetal Cardiac Defects

It is noted that the type of cytokine dysregulation seen with TCE exposure (e.g., involving IL-6) can play a role in cardiac dysfunction. The report explains logically why the Johnson et al. (2003) study was used to derive some reference points. Some recent publications confirm and reinforce the results obtained in the Johnson et al. (2003) study, so maybe they could be cited to make a stronger argument. They are listed as follows:

- In Rufer et al. (2010), low doses of TCE (8 ppb) caused high mortality, functional cardiac dysmorphology and, in chicks that survived hatching, significant frequency of muscular ventricular defects (VSDs) consistent with Johnson's findings. VSDs were observed after hatching, dismissing the hypothesis that they may be due to transitory effects of remodeling.

- TCE effects on the cardiac system were specific for a narrow window of development corresponding to myocardial expansion and endocardial cushion formation, consistent with previous findings from Drake et al,2006a and b; Mishima 2006; Boyer et al. 2000, and consistent with the definition of a teratogen.

- The types of defects and morphological changes (e.g cardiac hypertrophy and hypoplasia) were consistent with a mechanism of action involving disruption of calcium handling and cardiac contractility, observed by Caldwell et al, 2008, 2010 and Makwana et al., 2010 in rat and chick cardiomyocytes, respectively. Numerous literature data (reviewed in Lehnart et al., 2008; Lebeche et al, 2008; Yano et al., 2008; Gyorke et al., 2008) confirm the notion that alteration of calcium homeostasis is sufficient to induce alteration of contractility and in turn heart defects.

- A non-monotonic dose-response relationship was found that confirms several other reports (Caldwell et al., 2008; Drake et al., 2006; and earlier publications cited in Discussion section) suggesting the presence of more than one MOA due to presence of metabolites, enzymatic sensitivity, etc.

Other Editorial Comments for Charge Question #3.

- Typographical corrections - In the section on vestibular function – (headaches, dizziness, nausea) there is a typo on p 4-101 that should be corrected. LOAEL 1000 ppm human study (Kylin et al., 1967); 2700 ppm in rats (Tham et al 1984, Niklasson et al., 1993) and rabbits (Tham et al, 1983).

- In the kidney section, there needs to be added mention of the 18% increase in kidney weight (in male mice only) seen in the largely immunotoxicity study conducted by Peden-Adams (2008).

- Editorial Footnote #1 on page 146: "Elevation of NAG in urine is a sign of proteinuria, and proteinuria is both a sign and a cause of kidney malfunction (Zandi-Nejad et al., 2004). " Beta –N-acetylglucosaminidase (NAG) is an enzyme released by the proximal tubules. Usually total NAG is measured, however, this is comprised of NAG B, which reflects necrosis, and NAG A, which reflects milder forms of proximal tubule perturbation.

- Editorial note. The last sentence on p4-173 line 32, 33 needs to be reworded as it is unclear. Additionally, there is a double period on line 23, p4-199.

Recommendations

- If additional endpoints of renal dysfunction (e.g. diuresis, increased glucose excretion) were present in the reported studies, they should be included in the report. Often only one or two parameters of renal function and histopathology were presented. A better overall description of renal dysfunction should be presented if available (especially for animal studies).

- There should be a better description of the location of the renal lesion, including nephron segment, if known. For example, TCE and DCVC appeared to affect the proximal tubule at the level of the outer stripe of the medulla (S3 segment of proximal tubule). Is this the site of lesions seen with other TCE metabolites? Explaining the role (or lack of a role) of any other TCE metabolites in TCE nephrotoxicity could be strengthened by comparing the sites of the renal lesion.

- On page 4-338, please clarify the use of the phrase, "subpopulation levels", on lines 31 and 33.

- A statement should be added that the spectrum of TCE-induced immune dysfunctions (immunosuppression, autoimmunity, inappropriate and/or excessive inflammation) included in this EPA draft report has the potential to produce adverse effects that are seen well beyond lymphoid organs and involving several other physiological tissues and systems. The types of immune-inflammatory dysfunctions described in this report have been observed to affect function and risk of disease in the nervous system (e.g., loss of

hearing), the skin, the respiratory system, the liver, the kidney, the reproductive system (e.g., male sterility), and the cardiovascular system (e.g., heart disease, atherosclerosis).

- A statement should be added to emphasize the cell-mediated immune effects of TCE as some of this has been supported by the human epidemiology data and the issue is pertinent to risk of cancer.

4. Cancer Hazard Assessment

Using the approach outlined in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a), does EPA's hazard assessment of carcinogenicity logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions that TCE is carcinogenic to humans by all routes of exposure? Specifically, please address the epidemiologic evidence for associations between TCE and kidney cancer, lymphoma, and liver and biliary tract cancer; the extent to which the results of the meta-analyses contribute to the overall weight of evidence for TCE carcinogenicity; the laboratory animal data for rat kidney tumors, mouse liver tumors, and lymphatic cancers in rats and mice; and the toxicokinetic and other data supporting TCE carcinogenicity by all routes of exposure.

Response:

The Panel agreed that cancer hazard characterization hinges on the synthesis of the accumulated scientific evidence, especially the epidemiologic evidence supporting the carcinogenicity of TCE. Assessment of the causal association and weight of evidence supported the conclusion that TCE is carcinogenic to humans by all routes of exposure as outlined in the US EPA cancer guidelines. Results from animal bioassays and toxico-kinetic data provide further support to the EPA conclusion. The report logically, accurately, clearly, and objectively presented the methodological review of the epidemiologic evidence, highlighted the criteria for study inclusion in meta-analyses and the meta-analysis methods (as noted in charge question 2) and appropriately assessed the weight of the evidence to conclude that TCE is causally related to lymphoma, and kidney and liver cancer.

The report appropriately highlighted the causal criteria in support of the conclusion. The biologic plausibility and coherence of the epidemiologic findings were supported by the laboratory animal data, the toxicokinetic data, and epidemiologic data of other cancer sites and immune effects. The consistency of the findings was notable given the rarity of the cancers, differences in latency and potential for exposure misclassification as described in the study assessments highlighted in the hazard characterization. Multiple explanations would be needed to account for the associations between TCE and several cancers from studies with differing designs, strengths and weaknesses.

The summary risk estimates from the meta-analyses provided a clear indication of a cancer hazard from TCE. The pooled risk estimates from the meta-analyses for kidney cancer and liver cancer, although modest, were robust with no indication of publication bias or heterogeneity. Meta-analyses for both kidney cancer and lymphoma found higher increases in the risk estimates associated with higher TCE exposure than for any TCE exposure and no evidence of strong confounding, which further supported a causal association.

EPA concluded TCE is carcinogenic to humans by all routes of exposure. This conclusion was based on convincing evidence of a causal association between TCE exposure and kidney cancer, compelling evidence for lymphoma, and more limited evidence for liver cancer. The

epidemiologic data, in the aggregate, were quite strong. In addition, the epidemiologic data were supported by bioassays and toxicokinetic data. Although issues of concern could be raised about individual studies, the overall pattern and the results from the meta-analyses were quite compelling. Potential confounding from established risk factors for these cancers of concern could be directly assessed in some studies and indirectly evaluated by reviewing cancer excesses that did not occur in TCE exposed populations, e.g., the absence of an excess for lung cancer indicates confounding from smoking is not likely.

Some studies had low power to evaluate the TCE-cancer relationship, but the meta-analysis provides a tool to combine underpowered studies and assess the overall effect. Exposure assessment in epidemiologic studies is difficult in the best of circumstances. EPA appropriately focused on studies with the stronger exposure assessment efforts to minimize the effects of exposure misclassification. However, misclassification of exposure undoubtedly occurred. In the cohort studies the effect of exposure misclassification on estimates of relative risk will be largely non-differential because factors used in exposure assessment were recorded before occurrence of the disease. Thus, it will tend to depress estimates of relative risk and mute exposure-response gradients and is not an explanation for any observed excesses. Non-differential exposure misclassification would also occur in case-control studies. Differential misclassification is more of a concern in case-control studies. Differential misclassification can bias relative risks upward or downward, although the upward bias is usually raised in positive studies. However, no evidence is available to suggest that differential exposure bias occurs across all the case-control studies. Multiple explanations are needed to account for the associations between TCE and several cancers in studies with differing designs, geographic locations, and strengths and weaknesses. The summary estimates from the meta-analysis provided a clear indication of a cancer hazard from TCE. EPA concluded the association between TCE and lymphoma and liver cancer were more limited than that for kidney cancer. These conclusions about the epidemiologic data were supported by the statistically significant excesses for these tumors in the meta-analyses, no statistically significant heterogeneity, and consistency of findings after exclusion of individual studies in sensitivity analyses. The pooled risk estimates, although modest, were robust with no clear indication of publication bias or heterogeneity. The consistency of the findings was remarkable given the rarity of the cancers, differences in latency and potential for exposure misclassification, as described in the study assessments highlighted in the hazard characterization.

EPA concluded that the epidemiology data were convincing for a causal association between TCE and kidney cancer, compelling for lymphoma, and positive but more limited for liver cancer. The Panel did not have strong disagreement with this statement, although some felt that the data for liver cancer were as compelling as that for lymphoma. Liver cancer has a much lower incidence than kidney cancer or lymphoma in Western countries (where most of the epidemiologic studies were conducted) and this requires more reliance on the meta-analysis for a summary effect estimate with adequate power. The meta-analysis found that the association of TCE exposure with liver cancer was elevated and statistically significant. Further grouping liver cancer cases by the level of exposure resulted in numbers that were too small to adequately evaluate risks among persons with higher exposures. Nevertheless, we considered these results for liver cancer to be strong because there was no evidence of heterogeneity or publication bias

in the meta-analysis, and because the epidemiologic findings were supported by observations of liver cancer in animal models. Although potential confounding by other risk factors for liver cancer is possible, strong risk factors such as hepatitis are very rare in Western countries (where most of these studies were conducted), so this is unlikely to have caused such a degree of confounding. There were no studies to evaluate whether hepatitis might be a confounder in TCE studies, although this seemed unlikely.

The meta-analysis results were impressive for lymphoma, showing a significantly elevated relative risk for ever-exposure to TCE and an even higher effect estimate for high TCE exposure. However, it is important to note that there was weak evidence of publication bias in the lymphoma meta-analysis results, which means that studies showing no TCE effect or inverse associations may not have been published. In addition, there was significant heterogeneity in the meta-analysis results for lymphoma for ever-exposure to TCE, indicating that there is an unexplained factor causing heterogeneity that indicates it may be inappropriate to combine the estimates in a meta-analysis. This heterogeneity may reflect the complicated and changing definitions for lymphoma across studies and over time. It is also possible that effects from TCE may differ by type of lymphoma. The association with lymphoma was further supported by the larger relative risk in meta-analyses for the higher exposure categories compared to the overall relative risk. This was evidence for an exposure response gradient, even though no individual studies showed much evidence of this.

Recommendations:

- The immune effects as highlighted in the hazard assessment should be referred to in the conclusion especially in the criteria of biological plausibility and coherence because of the relationship between immune system dysfunction and cancer risk.

- Although the summary evaluation focused on the scientific evidence and meta-analysis for kidney, lymphoma and liver cancers, there is also some suggestive evidence for TCE as a risk factor for cancer at other sites including bladder, esophagus, prostate, cervix, breast and childhood leukemia. This evidence that also supports the conclusion should be mentioned in the summary evaluation (section 4.11.2.1).

- Add a paragraph describing the definition of lymphoma as used in IRIS. Change the terminology regarding the meta-analysis to 'non-Hodgkin lymphoma' instead of 'lymphoma', throughout the document. The term 'NHL' more accurately describes the intent of the analysis as well as the overwhelming majority of cases in the analysis, despite changing classification schemes. The focus of the meta-analysis on NHL and the exact classifications the meta-analysis includes where it may diverge from classical NHL (as in studies that included chronic lymphocytic leukemia) should be clearly explained in both Appendix C and in the Hazard Characterization document (section 4.6.1.2.2).

- To assist the reader, please include references in the summary section (section 4.11.2). For example, "The other 13 high-quality studies [note: besides Hardell and Hansen] reported elevated Relative Risk estimates with overall TCE exposure that were not

statistically significant." References for statements like this would be helpful. The Panel counted fewer than 13 studies in the meta-analysis after subtracting out Hardell and Hansen, and not all of these showed elevated risk estimates, so it would be helpful for the reader to know which 13 studies this statement refers to.

5. Role of Metabolism on TCE Toxicity

Does EPA's hazard assessment logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions regarding the role of metabolism in TCE carcinogenicity and non-cancer effects? Specifically, please address EPA's conclusions that the liver effects induced by TCE are predominantly mediated by oxidative metabolism, but not adequately accounted for by the metabolite trichloroacetic acid (TCA) alone and that the kidney effects induced by TCE are predominantly mediated by metabolites formed from the GSH-conjugation pathway.

Response

The Panel agreed that EPA's hazard assessment in the draft document has produced a systematic, thorough, objective and clear summary of information on the role of metabolism in TCE-induced toxicity with regards to both cancer and non-cancer health effects. The Panel also found that EPA has presented a comprehensive review of metabolite formation in animals and humans, and has provided a clear, logical assessment of the role these metabolites play in mediating its carcinogenic and non-cancer effects.

5a Mediation of TCE-Induced Liver Effects by Oxidative Metabolism

The Panel found that EPA's conclusion that oxidative metabolites of TCE are responsible for mediating the liver effects is sound and based on a wealth of supportive studies.

The document was a thorough review of the extensive literature on the role of oxidative metabolism in TCE toxicity to the liver. Direct evidence that oxidative metabolism was required for liver toxicity, such as studies which modulated TCE toxicity by modulating P450 activity, was somewhat limited. One noted exception is the study by Ramdhan et al. (2008), that reported CYP2E1-deficient mice produced considerably less oxidative metabolites and showed reduced hepatoxicity, although due to a small number of animals studied, effects were significant only at the highest TCE dose. Nonetheless, the collective evidence, especially from studies with two major oxidative metabolites of TCE - TCA and DCA, was very strong that in rodents, at doses where metabolism is not saturated, the majority of TCE was metabolized and that metabolites from the oxidative pathway predominated over those of the glutathione conjugation pathway. Mice are the most susceptible species with respect to TCE-induced liver effects and the majority of studies support the conclusion the oxidative metabolites are playing the major role.

5b Contribution of TCA to Adverse effects on the Liver

The Panel found the conclusion that "the adverse effects on the liver of one of the TCE metabolites, trichloroacetic acid, cannot adequately account for the liver effects of TCE" is sound and supported by several lines of experimental evidence.

TCA is the predominant oxidative metabolite of TCE and its effects are well known to be associated with liver toxicity and carcinogenicity. However, oxidative metabolism of TCE generates a number of molecules and the confidence in the ability to identify TCE's oxidative

metabolite(s) that may be responsible for hepatotoxicity and/or liver cancer in rodents or humans is much less than that for the overall role of oxidative metabolism. This uncertainty is due in part to the problems with quantitative assessment of DCA formation after TCE administration. There is sufficient evidence to implicate DCA in mediating carcinogenic effects of TCE that are not related to those produced by TCA. The EPA correctly stated that DCA was a minor metabolite of TCE in vivo, at least in rodents, and that some of the earlier reports on DCA dosimetry may have been erroneous due to the issues with the analytical methods. There are, however, several studies (Delinsky et al., 2005; Kim et al., 2009) which provide information on the blood levels of DCA after oral exposure to TCE in rats and mice. Such data, together with a large body of literature on TCA formation after treatment with TCE, should be carefully evaluated with regards to the estimation of the internal dose (or relative amounts) of each of these key metabolites.

The Panel found that EPA has taken several approaches to determine whether liver tumors induced by TCE can be accounted for by TCA formation alone. The first approach was to compare dose-response profiles for non-cancer liver toxicity endpoints from TCE and TCA based on TCA dose equivalents, an internal dose metric. In contrast to DCA, the quantitative data available for TCA and TCOH, together with PBPK models relying on their measurements, are among the most consistent and allow for the assessment of the oxidative metabolite flux from TCE. Analysis of liver weight changes (Fig 4-7, 4-8) suggested that while total TCE oxidative metabolism was strongly correlated with liver weight changes ($R^2 = 0.89$), the amount of TCA formed underestimated the degree of liver hypertrophy observed. The dose-response relationships for liver hypertrophy observed between TCE and TCA, based on TCA daily dose equivalents, were strikingly different in both slope of the dose-response and overall magnitude, suggesting that the mechanisms of hypertrophy, and/or the metabolites involved, were different. This analysis was compelling because TCA daily liver dose equivalents were used for comparison. The internal dose metrics, if accurately applied, should account for potential differences due to bioavailability and exposure route issues that have been previously raised for TCE and TCA. The Panel notes that the bioavailability of TCE, DCA and TCA in oral gavage studies was dependent, among many factors, on the type of the vehicle and the magnitude of the administered dose. It has been suggested [Sweeney et al., 2009; NRC review of the IRIS assessment of Tetrachloroethylene (Appendix B)] that the bioavailability of TCA (when administered directly) was highly non-linear with an increasing dose. Thus, the internal dose of each metabolite of interest, either through metabolism from TCE or following direct administration, was key for the comparison of health effects between the parent and its metabolites.

The second approach used in the draft document to support the conclusion that multiple metabolites were involved in liver tumors induced by TCE included comparisons of liver phenotypic markers (glycogen staining, c-jun staining) and tumor-derived genetic markers (incidence of H-ras mutations). This analysis was interesting, yet qualitative in nature. The use of phenotypic markers such as H&E staining, glycogen staining, antibody reactivity, tumor tincture, etc., must be interpreted with caution since the underlying biochemistry/molecular biology of these descriptive attributes is often not well understood and may be highly dependent on the state of progression of the tumors The criteria used in each study for phenotypic

classification (i.e., staining intensity, background staining) is not always clearly outlined in the original literature reports. The EPA has included adequate discussion noting the technical limitations for each of the studies, which increased the confidence that such evidence from a single study was not overly weighted in drawing conclusions about the role of TCA. While individual studies comparing phenotype/genotype of TCE-, TCA- and DCA- induced tumors have important limitations, the collective group of studies was consistent with the interpretation that TCE tumors displayed phenotypic and genotypic heterogeneity that was different than that of tumors induced by TCA alone. This was in agreement with the EPA conclusion that these data also did not support the hypothesis that TCA was a sole acting liver metabolite of TCE. However, since factors such as interactions among metabolites and tumor progression state may have unknown influences in the phenotype/genotypes observed, this type of qualitative evidence was not sufficient to invoke specific roles for other contributing metabolites, or to discount potential contributing roles of other metabolites.

The draft included little in terms of the comparative quantitative evaluation of the hepatocarcinogenic potency of TCE, TCA and DCA even though extensive information was available, especially in mice. A recent draft of the IRIS assessment of a highly related chemical, tetrachloroethylene (PERC), provided the evaluation of the consistencies between PERC and TCA with regards to the liver cancer endpoint (Appendix 4A of PERC IRIS draft document). TCA is a major metabolite of both TCE and PERC and it is debatable whether TCA toxicity can account for the majority (if not all) of the adverse liver effects of PERC.

Given the controversy of DCA as a contributing metabolite in liver effects induced by TCE and the importance of this issue as it relates to understanding TCA's role, it is somewhat surprising that there was relatively little analysis of the literature related to the use of DCA as a therapeutic agent in humans as an integrated part of this section of the review. Although these studies obviously involved high doses, they are relevant to the potential spectrum of effects observed in humans.

Recommendation:

- The EPA should examine studies that provide quantitative assessment of TCA and DCA formation after TCE exposure *in vivo* and draw conclusions with regards to the relative amount and kinetics of the oxidative metabolites of interest for liver toxicity.
- A careful evaluation of the concentration-time kinetics is needed to achieve certainty in the comparisons of liver effects and the conclusions drawn by the EPA which suggest that TCA-induced adverse liver effects do not explain those observed with TCE. Equally important is to fully consider the bioavailability of TCE itself with regards to the vehicle effects between studies.
- The body of the document could be further strengthened by reporting EPA's evaluation on the strength of the specific criteria used for phenotypic classification described in each study discussed, and noting where specific criteria were not reported. While most of this information was included in the appendix, the EPA may consider constructing a summary table for Section 4.5.6.
- Dose-response modeling, similar to that performed for PERC, may be considered by the EPA to provide science-based information on relative contribution, or lack thereof, of

TCA and/or DCA to the apical liver carcinogenesis effect of TCE. While data gaps exist and there are limitations in the comparisons between independent cancer bioassays, the document should clearly state what the limitations are should such analysis be deemed futile.

- The draft assessment may be strengthened by including information from human use of DCA in clinical practice.

5c Role of GSH-Conjugation Pathway on TCE-Induced Kidney Effects

The Panel concluded that EPA has provided a clear and comprehensive summary of the available evidence that metabolites derived from GSH conjugation of TCE are responsible for mediating kidney effects.

The Panel found the integration of the data from human epidemiological, animal and *in vitro* mechanistic studies produced a clear and transparent weight-of-evidence assessment supportive of TCE GSH conjugation metabolites' role in kidney toxicity and cancer. Whereas sufficient amounts of oxidative metabolites of TCE (i.e., TCOH) may be formed which could contribute to kidney effects, potentially through formic acid, the literature indicated the pathological effects on the kidney induced by oxidative metabolites were not consistent with those observed with TCE. In contrast, the pathological effects on the kidney induced by DCVC/DCVG were similar to TCE. Thus, a reasonable conclusion was that the glutathione conjugation pathway played a more important role in driving these effects. The primary challenge was to determine the true flux through the glutathione conjugation pathway.

Many uncertainties exist in PBPK model estimates for the GSH pathway. This issue is critical, since these uncertainties can result in orders of magnitude differences in flux between rodents and humans. The argument that mercapturates of the glutathione conjugates, as detoxication pathway products, are not quantitative markers of flux through the GSH pathway is rational and supported by in vivo human and rodent data. The level of urinary mercapturates, as deactivation products, is evidence that the pathway operates in humans, but does not necessarily reflect the amount of DCVC formed. Direct data on DCVG/DCVC formation, or its reactive metabolites, are the more appropriate measures of flux for this pathway. This was clearly and adequately discussed in the review.

The quantitative analysis of the species differences in GSH metabolism was somewhat narrow. Specifically, the issue of vast differences in human vs rodent metabolism of TCE to GSH conjugates hinged on the very limited experimental evidence. Only one human *in vivo* study was available that directly quantified DCVG in urine in a few subjects (Lash et al. 1998). The rodent in vivo data (Kim et al. 2009) was limited to only one isogenic (hybrid) mouse strain. Other important differences between these studies were that they utilized different exposure routes, doses, and analytical methods. The uncertainties associated with the potential several orders of magnitude difference in TCE metabolism through GSH pathway between species should be considered more carefully.

In addition, multiple *in vitro* studies have been published in the peer reviewed literature. For example, in vitro GSH conjugation data were used to develop prior distributions for GSH conjugation rates, something which was not done for previous PBPK models of TCE. Ample discussion was given to the data generated by the Lash laboratory, which was clearly the most extensive set of data relative to DCVG and DCVC levels in humans. These data indicated DCVG may be formed at levels similar to that of oxidative metabolites in humans. Based on these data, the conclusion that the GSH conjugation pathway plays an important role in kidney tumors/toxicity in both rodents and likely in humans is logical.

However the discussion of additional published in vitro studies that show disparately lower results for DCVG formation (beyond mercapturates) was not given a comparable level of attention. For example, the documents pointed out discrepancies between in vitro studies of DCVG formation conducted by the Green and Lash laboratories that report results differing by orders of magnitude. The studies from these labs reported very similar assay conditions using the same strain of rats, but differed in the analytical techniques used (HPLC-UV versus GC-MS). The analysis of these disparate results provided in the review was limited to nondescript statements that the differences may be "related to the different analytical methods employed such as detection of radiolabeled substrate vs. derivatized analytes" (section 3.3.2.7). Unfortunately, the authors of the original studies do not really provide technical explanations for the disparities either. Given such disparate results, the EPA has chosen to use the geometric mean of these two studies in estimating DCVG formation. This decision process and its impacts on the final rates for DCVG formation need to be more clearly spelled out in the discussion of these studies. The discrepancies in estimates of DCVG formation are among the most contentious issues associated with TCE risk analysis. Given the difficult task of drawing conclusions from such different results, the conservative approach the EPA has taken is defensible from a public safety policy perspective. From a strictly scientific perspective however, at a minimum, such large literature disparities call for a more complete discussion of the strengths and limitations of the analytical methodologies used than what is described in the review.

Recommendation:

- The issue of quantitative assessment of the metabolic flux of TCE through the GSH pathway vs. the oxidative metabolism pathway should be considered carefully since uncertainties exist with regard to the extent of formation of the dichlorovinyl metabolites of TCE between humans and rodents. EPA may need to provide appropriate reservations to the conclusions based on the limited data for GSH metabolites.
- The discussion of how each of the in vitro and in vivo data sets were used to estimate DCVG formation parameters for the PBPK model should be more transparent indicating strengths and weaknesses in the database.

6. Mode of Action

Using the approach outlined in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a), does EPA's hazard assessment logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions regarding the mode(s) of action [MOA(s)] of TCE carcinogenicity and non-cancer effects? Specifically, please address the conclusions that the weight of evidence supports a mutagenic MOA for TCE-induced kidney tumors; that a MOA for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation, possibly in combination with a mutagenic MOA, is inadequately supported by available data; that there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced liver carcinogenesis; that there are inadequate data to specify the key events and MOAs involved in other TCE-induced cancer and non-cancer effects; and that the available data are inadequate to conclude that any of the TCE-induced cancer and non-cancer effects in rodents are not relevant to humans.

Response

6a Hazard Assessment and Mode of Action

The Panel agreed that the IRIS TCE hazard assessment logically, accurately, clearly, and objectively represented and synthesized the available scientific evidence to support its conclusions regarding the mode(s) of action [MOA(s)] of TCE carcinogenicity and non-cancer effects. For each end point, the hazard assessment described the possible MOA and underlying mechanisms. In general, the assessment provided explanations for inconsistent data or lack of results. For example, Section 4.8.3.3.2 provided a comprehensive, detailed, and very useful discussion of potential reasons for inconsistencies in the body of literature on TCE exposure *in utero* and heart defects.

The Panel agreed that the MOA for TCE nephrotoxicity involves conversion of TCE to GSH derived metabolites followed by conversion of the glutathione conjugate (DCVG) to the cysteine conjugate (DCVC) and activation by β-lyase in the kidney to the ultimate nephrotoxic species. Thus, the EPA's hazard assessment logically, accurately, clearly, and objectively represents and synthesizes the available scientific evidence to support the conclusion regarding the MOA for TCE kidney non-cancer toxicity. However, as discussed in the response to charge question 3, the Panel noted that uncertainties remain with regards to quantity of metabolites formed in humans and rodents. The panel concluded that the narrative presentation of the data, along with the evaluation of the strengths and weaknesses of each study, was appropriate with supplemental information.

Recommendations:

- the impact of the inconsistencies in data on the quantity of GSH pathway metabolites formed in humans and rodents should be presented more transparently.
- In the body of the document, MOA information should be systematized and broken down into key events for each proposed MOA. The EPA may consider using a tabular format to

facilitate the ease of evaluation. Information on supporting/refuting (if any) evidence (with appropriate references indicated), human relevance (if available), and "strength" of each line of evidence/study should be included.

- EPA should consider tabular summaries by specific metabolites when studies used metabolite exposure rather than the parent compound.
- Data gaps should be clearly identified to help guide future research.
- Key conclusions supporting/refuting each key event should be presented in bullet form indicating where in the document a more detailed narrative/tables can be found.

6b MOA for TCE-Induced Kidney Tumors

The Panel agreed that the weight of evidence supported a mutagenic MOA for TCE-induced kidney tumors. However, the Panel concluded that the weight of evidence did not exclude the MOA for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation and including this MOA may more accurately reflect kidney tumor formation than a mutagenic mechanism alone. Furthermore, the combination of cytotoxicity, proliferation and DNA damage together may be a much stronger MOA than the individual components.

Recommendations:

- Modify the relevant text to reflect that the available data do, in fact, provide support for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation, possibly in combination with a mutagenic MOA, although not to the extent that support for a mutagenic MOA was provided.

6c Inadequate Support for PPARα agonism and its sequellae being key events in TCE-induced liver carcinogenesis

The Panel agreed that there was inadequate support for PPARα agonism and its sequellae being key events in TCE-induced human liver carcinogenesis. The Panel noted that PPARα agonists do not elicit peroxisomal proliferation in humans, a pathological change which is a hallmark effect of TCE and other peroxisome proliferators in rodents.

The Panel noted that a number of studies important for consideration of the relevance of PPARα mode of action to human liver carcinogenesis have been completed recently. These include, but are not limited to, studies in PPARα-null mice (Ito et al. 2007; Takashima et al. 2008; Eveillard et al. 2009), PPARα humanized transgenic mice (Morimura et al. 2006), and hepatocyte-specific constitutively-activated PPARα transgenic mice (Yang et al. 2007). The data from these animal models suggest that activation of PPARα is an important but not limiting factor for the development of mouse liver tumors and that additional molecular events may be involved.

The Panel noted the quantitative differences in the affinity of the various isoforms of PPARs to TCA, DCA and other model peroxisome proliferators are well established. Likewise, the quantitative differences in affinity between species are also known.

Recommendations:

- Graphical or tabular presentation of these data to strengthen the comparative analysis between metabolites and chemicals.
- Including some of the analyses which compare the receptor transactivation potency and the carcinogenic potential of TCA, DCA and other model peroxisome proliferators from Guyton et al (2009) to strengthen the arguments.

6d Inadequate Data to specify Key Events and MOAs involved in other TCE-Induced Cancer and Non-Cancer Effects

The Panel agreed that the data are inadequate to specify the key events and MOAs involved in other TCE-induced cancer (lung, lymphoma) and non-cancer effects (central nervous system, immune system, respiratory tract toxicity, reproductive effects, developmental effects)

6e Human Relevance of TCE-Induced Cancer and Non-Cancer Effects in Rodents

The Panel agreed that the data are inadequate to conclude that any of the TCE-induced cancer and non-cancer effects in rodents are not relevant to humans.

Recommendations:

- The impact of potential overestimation of the extent of the GSH pathway in humans in Section 4.4.7 (Kidney) must be transparent
- The MOA for carcinogenicity should be described as complex rather than unknown in Section 4.5.7.4. Mode of Action (MOA). With respect to conclusions regarding the liver, while the complete MOA in animals may not be clear at this time, complex is a more appropriate descriptor since it is likely that key events from several pathways may operate leading to acute, sub-chronic and chronic liver toxicity of TCE.

7. Susceptible Populations

Does EPA's hazard assessment logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions that the factors that could modulate susceptibility to TCE carcinogenicity and non-cancer effects include genetics, lifestage, background and co-exposures, and pre-existing conditions, but that only toxicokinetic variability in adults can be quantified given the available data?

Response

The Panel agreed that Section 4.10 of the Hazard Assessment provided a good review of potentially susceptible populations, and that the identified factors (genetics, lifestage, background, co-exposures and pre-existing conditions) may modulate susceptibility to TCE carcinogenicity and non-cancer effects. The review included adequate data to support factors that modulate exposure and pharmacokinetics in both animals and humans, but few data to demonstrate differing susceptibility to health effects from TCE exposure in either animals or humans. The Panel agreed with the conclusion that the existing data are inadequate from which to form a conclusion about whether the potentially modulating factors do or do not impact risk estimates for TCE and human health effects. The Panel agreed with the use of standard age-dependent adjustment factors in the protection of children.

***Recommendations*:**

- The Panel disagreed with the statement that "toxicokinetic variability in adults can be quantified given the existing data," as the main study characterizing toxicokinetic variability in adults was small (n<100) and was composed of subjects selected non-randomly. The Hazard Assessment document should note the limitations of the adult data for toxicokinetic modeling in terms of uncertainty and possible bias in section 4.10.3, and elsewhere in the document where these data are used for hazard characterization modeling.

- Section 4.10 of the Hazard Assessment should discuss explicitly the lack of data demonstrating modulation of health effects from TCE by the identified factors (genetics, lifestage, background, co-exposures, and pre-existing conditions), and the need for such data in risk assessment.

- EPA should make specific recommendations for studies that would fill the data gap for susceptible groups. For example, epidemiologic studies in which TCE exposure is well-characterized and in which internal comparisons can be made to determine whether there is effect modification, and animal studies comparing subgroups (e.g., based on genetics, obesity, multiple solvent exposures).

- Modulation of TCE exposure-related hypersensitivity dermatitis by genetic variation may be relevant for future study, given results of the study of hypersensitivity dermatitis in

Asian workers reported in Li et al. (2007) and increasing industrial chemical exposures in China.

- The wording in Section 4.10 was often not clear about whether it was describing results for a study that looked at effect modification of the TCE effect or not, as opposed to direct effects of age, gender, etc. Also, the draft document needs to state explicitly where effects of TCE within one subgroup were stated, whether the other subgroup was also examined in the same study.

- The Panel recommended that exposure to solvent mixtures should be added as a potential susceptibility factor (co-exposures) to Section 4.10, since exposure to more than one chemical to the same target organ likely increases risk.

- Section 4.10.2.4.1 (page 4-585) should be more accurately titled 'Obesity', rather than 'Obesity and metabolic syndrome'. As presently written, Section 4.10.2.4.1 gives no clear message as to how obesity affected the kinetics of TCE, and the section should be revised to provide clarification.

8. Non-Cancer Dose-Response Assessment

EPA's dose-response assessment includes the development of a chronic inhalation Reference Concentration (RfC) and chronic oral Reference Dose (RfD) for non-cancer effects. Please address the following methods and results from EPA's non-cancer dose-response assessment in terms of the extent to which they are clearly and transparently described and technically/scientifically adequate to support EPA's draft RfC and RfD:

a. The screening, evaluation, and selection of candidate critical studies and effects;
b. The points of departure, including those derived from benchmark dose modeling (e.g., selection of dose-response models, benchmark response levels);
c. The selected PBPK-based dose metrics for inter-species, intra-species, and route-to-route extrapolation, including the use of body weight to the ¾ power scaling for some dose metrics;
d. The selected uncertainty factors;
e. The equivalent doses and concentrations for sensitive humans developed from PBPK modeling to replace standard uncertainty factors for inter- and intra-species toxicokinetics, including selection of the 99th percentile for overall uncertainty and variability to represent the toxicokinetically-sensitive individual;
f. The qualitative and quantitative characterization of uncertainty and variability;
g. The selection of NTP (1988) [toxic nephropathy], NCI (1976) [toxic nephrosis], Woolhiser et al. (2006) [increased kidney weights], Keil et al. (2009) [decreased thymus weights and increased anti-dsDNA and anti-ssDNA antibodies], Peden-Adams et al. (2006 [developmental immunotoxicity], and Johnson et al. (2003) [fetal heart malformations] as the critical studies and effects for non-cancer dose-response assessment;
h. The selection of the draft RfC and RfD on the basis of multiple critical effects for which candidate reference values are in a narrow range at the low end of the full range of candidate critical effects, rather than on the basis of the single most sensitive critical effect.

Response

8a The screening, evaluation, and selection of candidate critical studies and effects

The Panel agreed that the screening, evaluation, and selection of candidate critical studies and effects were generally adequate to support EPA's draft RfC and RfD. The Panel noted that a very large number of studies were considered and included in the tables, and agreed that it was

appropriate to evaluate all studies showing dose-response for neurological, kidney, liver, immunologic, respiratory system, reproductive, and developmental effects, and body weight change. The Panel's comments on sub-question (a) related primarily to making the information presented in the document more clear and transparent to the reader, rather than to the screening, evaluation, and selection process itself.

The Panel believed that it was important that the reader easily be able to find the details of the studies included in the Chapter 5 tables.

For instance, four different studies with different durations were cited as "Crofton and Zhao (1997)" in Table 4-23, and it was not clear which duration was the basis for the cRfD in Table 5-1. In other cases, it was not stated whether the cRfD or cRfC was based on males or females when both were included in the study, or which strain was the basis when multiple strains were used. For example, from Table 5-2 and the text on p. 5-15 to 5-16, it was not clear which strain, gender, or exposure duration was used for the RfC for increased liver weight based on Kjellstrand et al. (1983b) (discussed in Chapter 4 and Appendix E). Another example for which cross-referencing the different sections of the document would be helpful is the information on the doses in the drinking water study of Keil et al. (2009). In the description of the study on p. 4-395, the doses were given as drinking water concentrations (ppb), but in Table 5-3, the LOAELs for this study were given in mg/kg/day, and the conversion from ppb in drinking water to mg/kg/day is found in Appendix E (p. E-34). A final example of where cross-referencing would be helpful relates to the studies of Carney et al. (2006) and Schwetz et al. (1975). These studies were listed in Table 5-4 (Reproductive Toxicity) because the key effect, decreased maternal body weight gain in a developmental study, was considered a "reproductive" effect. However, these studies were discussed under developmental toxicity in Chapter 4, making it difficult to locate them while reading the section on reproductive toxicity in Chapter 5.

Finally, it was stated on p. 5-1, point (1) that studies with "quantitative dose-response data" were considered. Some of the studies which were considered as the basis for RfCs and RfDs used only one dose of TCE and a control group (for example, Barrett et al., 1992). If a control group and a single treated group were considered adequate "quantitative dose-response data," this should be stated.

Recommendations:

- Chapter 5 should include a list of all non-cancer health effects and studies discussed in Chapter 4, noting those which were considered candidate critical effects and studies.
- Tables 5.1-5.5 should provide cross-references to the table or page in Chapter 4 and/or to the Appendices (such as Appendix E for hepatic studies) where the listed study was discussed, and should include more details (e.g. gender, strain, duration) of the studies selected as the basis for cRfDs and cRfCs when these details were needed to prevent ambiguity.
- Consistent dose units should be used in discussing the same study in different places in the document.

8b The points of departure, including those derived from benchmark dose modeling (e.g., selection of dose-response models, benchmark response levels)

The Panel agreed that the derivation of the points of departure (PODs) was generally technically/scientifically adequate to support EPA's draft RfC and RfD. The Panel noted that the graphics in Appendix F provided a good presentation of the BMD analyses.

The Panel noted that, although BMD modeling was generally an appropriate approach for POD determination, the results of BMD modeling were very uncertain with some datasets. For example the log-logistic BMD analysis for toxic nephropathy in female Marshall rats in the NTP (1988) study, shown in Figure F-10, may greatly overestimate the risks at low doses. This modeling involved extrapolation from a high LOAEL at which a high percentage of the animals were affected.

Recommendation:

- Chapter 5 should include the information on POD derivation from Table F-13 of Appendix F, including approach, selection criterion and decision points.

8c The selected PBPK-based dose metrics for inter-species, intra-species, and route-to-route extrapolation, including the use of body weight to the ¾ power scaling for some dose metrics

The Panel agreed that the use of PBPK-based dose metrics for inter-species, intra-species, and route-to-route extrapolation modeling were, for the most part, technically and scientifically adequate to support EPA's draft RfC and RfD.

However, it was noted by the Panel that the RfDs and RfCs for kidney endpoints were highly sensitive to the rate of renal bioactivation of DCVC (ABioactDCVCBW34) in human versus rodents. Specifically, it was noted that p-cRfDs/RfCs based on this dose-metric were several hundred-fold lower than RfDs/RfCs for the same endpoints based on applied dose with standard uncertainty factors, while p-cRfDs/RfCs for endpoints based on other dose metrics were much closer to RfDs/RfCs based on applied dose and standard uncertainty factors.

In addition to the strong dependence of the p-cRfDs and p-cRfCs on the rate of renal bioactivation of DCVC, the Panel noted that the uncertainties about the in vitro and in vivo data used to estimate this dose metric were much greater than for other dose metrics. For example, there were very large discrepancies in the rates of human glutathione conjugation reported by Lash et al. (1999a) and Green et al. (1997a).

The Panel understood that the rationale for scaling the dose metric to body weight$^{3/4}$, in conjunction with the interspecies extrapolation, is that the PBPK model predicted the dose rate to the target tissue rather than the internal concentration of TCE. However, this distinction and the associated rationale would likely not be readily apparent to most readers of the document as currently written. Confusion might arise because, for other contaminants, PBPK models were used to estimate serum levels or other metrics of internal concentration, rather than delivered doses, and in such case, scaling of body weight$^{3/4}$ would not be used.

The discussion of "empirical dosimetry" vs. "concentration equivalence dosimetry"as presented in the draft document would likely not be readily understandable to many readers. Furthermore, since body weight$^{3/4}$ scaling was used for all of the dose metrics discussed in sections 5.1.3.1.1-5.1.3.1.5, it may not be necessary to include the extensive discussion of the two dosimetry approaches in each of these sections.

Recommendations:

- The uncertainty about the rate of human glutathione conjugation found in Lash et al. (1999a) versus Green et al. (1997a) should be highlighted in the current assessment.
- The basis for the renal bioactivation dose metric should be more clearly and transparently presented and discussed in Chapter 3 and other appropriate sections. If this dose metric was derived indirectly from data on other metabolic pathways leading to and/or competing with bioactivation, this should be more clearly discussed.
- The rationale for scaling the dose metric to body weight$^{3/4}$, in conjunction with the interspecies extrapolation based on PBPK modeling, should be presented in a clearer and more transparent way (e.g. on pp. 5-33 – 5-36).
- The discussion of "empirical dosimetry" vs. "concentration equivalence dosimetry" should be made clearer and more transparent (pp. 5-33 – 5-36).

Editorial comments:

p. 5-33, line 25. Does "delivered dose" mean "administered dose"? If so, the term "administered dose" would be clearer.

p. 5-37, line 17. Should "kidney tumors" be changed to "kidney toxicity", since this section discusses non-cancer effects?

8d Uncertainty factors

The Panel agreed that, in general, the selection of uncertainty factors was clearly and transparently described and technically/scientifically adequate to support EPA's draft RfC and RfD. The uncertainty factors were consistently applied in Tables 5-8 to 5-13. However it was noted that the uncertainty factors were appropriately applied only if the BMD-PBPK 99th percentile (HEC_{99} and HED_{99}) dose metrics were correctly derived.

The Panel recognized that EPA guidance defines the duration of subchronic rodent studies as 4 weeks to 90 days, and chronic rodent studies as 90 days to 2 years, and noted that some of the subchronic studies considered as the basis for risk assessment were of duration as short as 4 weeks (e.g. Isaacson, 1990). Also, some studies of duration only slightly greater than 90 days (e.g. 18 weeks for Kulig et al., 1987) were classified as chronic, as appropriate under the EPA definition of chronic as longer than 90 days. However, exposures for 18 weeks may not always accurately predict effects for lifetime duration, since 18 weeks is only a small percentage of a two year (104 week) rodent lifespan (less than 18%).

Recommendations:

- The definitions of chronic and subchronic studies should be provided in the document and a citation given.
- The discussion of the subchronic to chronic uncertainty factor on p. 5-6 should be clarified as far as durations of studies considered suitable as the basis of a chronic risk assessment.
- The draft document should include discussion of whether studies in the lower end of the range defined as subchronic (e.g. 4 weeks) are of sufficient duration to be used as the basis for a chronic (lifetime) risk assessment.
- Studies only slightly longer than the minimum needed to be considered chronic should be noted as such, and the use of an uncertainty factor to account for less than lifetime exposure (of less than the full uncertainty factor of 10) could be considered for studies of such durations, especially for endpoints thought to progress in incidence or severity with time.

Editorial comment: On p. 5-10, line 9, Barrett et al., 1992, was referred to as an "acute study". On p.4-91, Table 4-21, it was shown that Barrett et al., 1991, was acute and Barrett et al., 1992, was subchronic (10 weeks). This should be corrected.

8e The equivalent doses and concentrations for sensitive humans developed from PBPK modeling to replace standard uncertainty factors for inter- and intra-species toxicokinetics, including selection of the 99th percentile for overall uncertainty and variability to represent the toxicokinetically-sensitive individual

The Panel generally agreed that this information is clearly and transparently described and technically/scientifically adequate to support EPA's draft RfC and RfD. It was noted that the 99th percentile estimates may be very sensitive to modeling assumptions, such as the choice of prior distribution and the shape of the distribution for population variability in the toxicokinetic parameters. The Panel concluded that approach used, including the selections of idPODs and the extrapolations from rodent to human followed by consideration of the 99th percentile human estimates, was acceptable to address the sensitive population. It was also concluded that the approach used to simulate a large range of exposure doses in order to obtain the distribution for the relationship between human exposure and internal dose (page 5-68) was appropriate.

Recommendations:

- The Panel noted variability/uncertainty for the toxicokinetically-sensitive individual could be quantified in future work by considering distributions in addition to the distribution of the 99th percentile, such as the 95th percentile.
- A quantile regression looking simultaneously at several quantiles could be developed in the future and presented in future refinements of this assessment.

Editorial Comment: On p. 5-2, point (7), the use of the 99th percentile HEC and HED estimates was discussed. The reason for choosing 99th percentile instead of 95th percentile was explained

later in the chapter (p. 5-45). A reference to this discussion (p. 5-48) here would be helpful for clarification, since the 95th percentile was more commonly used in other risk assessments.

Additional issue related to sub-questions (c), (d), and (e**)** discussed by the Panel:

The question arose as to whether the general approach used in the draft document to develop p-RfDs and p-RfCs was appropriately protective, as opposed to being overly conservative. Specifically, the Panel noted that the PODs identified through BMD analysis were based on most sensitive species, strain, and sex, and that the idPODs based on lower bound estimates of the 1% or 5% response in animals were used as a central dose estimate in humans. It was also noted that uncertainty factors for interspecies and intra-human pharmacodynamic variability were applied to the 99th percentile estimates (i.e. the doses for the 1% most pharmacokinetically sensitive humans) of the internal dose (HEC_{99} and HED_{99}).

The Panel endorsed the use of BMD modeling instead of an approach based on an uncertainty factor for LOAEL-to-NOAEL extrapolation, and the use of PBPK modeling instead of default uncertainty factors for inter- and intra-species pharmacokinetic differences, when these approaches were supported by the data. The Panel recognized that these approaches were not intended to introduce greater conservatism, but rather to incorporate data to replace default assumptions when appropriate.

There was consensus among the Panel members that the general approach described above was consistent with accepted EPA methodology for RfD/RfC development. It was specifically noted that the uncertainty factors for interspecies and intra-human pharmacodynamic variability were intended to account for variability as well as uncertainty, and that some p-RfDs/p-RfCs based on PBPK modeling were higher than RfDs/RfCs for the same endpoints based on the default methodology. The Panel recommended that HEC_{50} and HED_{50} values be included in Tables 5-8 to 5-13 for informational purposes.

Finally, as discussed further under sub-question (h), the Panel concluded that the consistency of RfDs and RfCs, although based on dose metrics of varying levels of certainty, gave confidence in the PBPK approach, as follows:

-Uncertain dose metric: DCVC activation - used for renal endpoints.
-Relatively certain dose metrics: Total metabolism - used for decreased thymus weight, anti-ss and ds DNA antibodies; Total oxidative metabolism – used for cardiac malformations.
-Applied dose (For applied, dose, a dose metric based on PBPK modeling was not used): Developmental immunotoxicity.

8f The qualitative and quantitative characterization of uncertainty and variability;

The Panel generally agreed that the uncertainties related to the RfC and RfD were clearly and transparently described and technically/scientifically adequate to support EPA's draft RfC and RfD.

It was noted that in the PBPK model, the uncertainty and variability were quantified with the posterior distributions, as appropriate for any Bayesian framework, while in the more general dose-response framework, the uncertainty is characterized with uncertainty factors which account for the main sources of variability and uncertainty. One Panel member commented that it was inconsistent to use a Bayesian approach in the PBPK modeling but not in the dose-response analysis, which uses numeric uncertainty factors, rather than distributions, which represent variability and uncertainty as a fixed effect.

The Panel recognized that the use of uncertainty factors in the TCE assessment followed the currently accepted EPA approach.

Recommendations:

- The quantitative uncertainty analysis of PBPK model-based dose metrics for LOAEL or NOAEL based PODs (Section 5.1.4.2) should be revised to clarify the objective of this 2-D type analysis, as well as the methodology used.
- In future work, EPA could develop an approach using distribution to characterize uncertainty in a Bayesian framework.

8g The selection of NTP (1988) [toxic nephropathy], NCI (1976) [toxic nephrosis], Woolhiser et al. (2006) [increased kidney weights], Keil et al. (2009) [decreased thymus weights and increased anti-dsDNA and anti-ssDNA antibodies], Peden-Adams et al. (2006 [developmental immunotoxicity], and Johnson et al. (2003) [fetal heart malformations] as the critical studies and effects for non-cancer dose-response assessment

The Panel concluded that the choices of Keil et al. (2009) [decreased thymus weights and increased anti-dsDNA and anti-ssDNA antibodies], Peden-Adams et al. (2006) [developmental immunotoxicity], and Johnson et al. (2003) [fetal heart malformations] as critical studies and effects were technically/scientifically adequate to support EPA's draft RfC and RfD. The Panel noted that questions related to the use of cardiac malformations from Johnson et al. (2003) as a critical endpoint were adequately addressed in the response to Charge Question 3. It was noted that BMD modeling for the data from Johnson et al. (2003) was highly sensitive to model choice. It was also noted that, although a tremendous amount of information was available on liver toxicity, hepatic effects were not a critical endpoint because they were less sensitive than other endpoints.

The Panel expressed concerns about the use of NTP (1988) [toxic nephropathy], NCI (1976) [toxic nephrosis], and Woolhiser et al. (2006) [increased kidney weights] as critical studies and effects. For all three of these studies, uncertainties exist for the PBPK modeling based on renal bioactivation of DCVC, as discussed in sub-question (c) above.

Additional issues related to choice of toxic nephropathy in female Marshall rats from NTP (1988) as a critical effect and study include excessive mortality due to dosing errors and

possibly other causes, and a high level of uncertainty in the extrapolation to the BMD due to the use of very high doses and a high incidence (>60%) of toxic nephropathy at both dose levels used. It was also noted that the incidence of this effect was lower in this study in other strains of rats and in male Marshall rats, suggesting that the sensitivity for this effect was highest in the female Marshall rats.

It should be noted that the uncertainties noted by the Panel about the quantitative risk assessment based on toxic nephropathy in NTP (1988) did not indicate that there was uncertainty that TCE caused renal toxicity in this study. The Panel noted that renal cytomegaly, which was not selected as a critical effect, occurred at a very high frequency in both sexes of all four strains used in this study, with 90-100% incidence in almost all dosed groups, and toxic nephropathy also occurred in all treated groups. In contrast, neither renal cytomegaly nor toxic nephropathy was seen in any of 396 control animals in study, which included groups of 50 males and females of the four different rat strains.

Additional issues related to the choice of toxic nephrosis in mice from NCI (1976) were that BMD analysis was not supported because the effect occurred in nearly 100% of animals in both dose groups, and that a high level of uncertainty was associated with extrapolation from the LOAEL at which nearly 100% animals were affected. It was noted by the Panel that toxic nephrosis did not occur in any control animals of either sex in this study.

Thus, although the numerical values for the RfD and RfC based on the renal endpoints were highly uncertain, TCE could clearly cause renal toxicity in both sexes of the four strains of rats tested, as well as in both sexes of mice, when administered in sufficient doses.

8h The selection of the draft RfC and RfD on the basis of multiple critical effects for which candidate reference values are in a narrow range at the low end of the full range of candidate critical effects, rather than on the basis of the single most sensitive critical effect.

The Panel supported the selection of a draft RfC and a draft RfD based on multiple candidate reference values in a narrow range which was at the low end of the full range of candidate reference values developed, rather than basing these values on the single most sensitive critical endpoint. This approach was supported by the Panel because it was a very robust approach that increases confidence in the final RfC and RfD.

<u>Reference Concentration</u>

As noted in the draft assessment, the proposed RfC, 0.001 ppm (5 ug/m^3), was within a factor of 3 of the p-cRfCs for the six critical endpoints selected. The Panel agreed with the use of PBPK modeling for route-to-route extrapolation for the five p-cRfCs which were based on oral studies.

EPA stated in the draft document (p. 5-83) that there was high confidence in the three p-cRfCs based on renal endpoints [increased kidney weight (Woolhiser et al., 2006), toxic nephrosis (NCI, 1976), and toxic nephropathy, (NTP,1988)] because of the clearly adverse

nature of the effects, the fact that two of them were based on chronic studies, and high confidence in its estimate of the dose metric which was clearly related to toxicity, while there was somewhat less confidence in the three p-cRfCs based on other endpoints [decreased thymus weight and anti-DNA antibodies (Keil et al., 2009) and cardiac malformation (Johnson et al., 2003)]. As stated in the response to (g), TCE can clearly cause significant renal toxicity when administered in sufficient doses. Thus, the Panel agreed that kidney toxicity was indisputably a key effect of TCE from a hazard identification perspective. However, as discussed above, the Panel concluded that the three p-cRfCs for renal endpoints were based on an uncertain dose metric, especially in regard to the relative rate of formation of the toxic metabolite in humans and animals. Although there was somewhat less confidence in the immune and cardiac malformation endpoints from a hazard identification perspective, for reasons discussed extensively in other sections of this response, there was sufficient confidence in them to consider them critical endpoints to support the RfC. While the confidence in these three endpoints was less than for the kidney endpoints as far as hazard identification, the three p-cRfCs for these endpoints were based on relatively certain dose metrics.

Although there was much greater pharmacokinetic uncertainty for the RfCs based on the three studies with renal endpoints [(Woolhiser et al., NCI (1976), and NTP (1988)], they provided additional support for the RfC.

The Panel noted that the same final RfC, 0.001 ppm, was supported by the p-cRfCs based on both the three principal studies (0.0003 ppm, 0.0004 ppm, and 0.003 ppm) and the supporting (kidney) studies (0.0006 ppm, 0.001 ppm, and 0.002 ppm), and concluded that the use of p-cRfCs for multiple critical effects to derive the final recommended RfC reduced uncertainty and better characterizes variability. It was noted that, in general, this approach may create more work for the risk assessors and the users of the risk assessment than use of the single most sensitive endpoint. However, it was recognized that, even if the RfC were to be based on the single most sensitive endpoint, it would be necessary to develop p-cRfCs for multiple endpoints in order to rigorously determine which study and endpoint provides the most sensitive RfC. It was also noted that a single RfC value was provided to users of the risk assessment.

Reference Dose

As discussed in the draft document, the proposed RfD, 0.0004 mg/kg/day, was within 25% of the p-cRfDs for the four critical endpoints selected (toxic nephropathy (NTP, 1988), decreased thymus weight [(Keil et al, 2009), developmental immunotoxicity (Peden-Adams et al., 2006), and cardiac malformations (Johnson et al., 2003)]. All four p-cRfDs were based on oral exposure, and three of them were based on drinking water exposure, a route relevant to environmental exposures. EPA stated in the draft document (p. 5-83) that there was high confidence in the p-cRfD based on a renal endpoint (toxic nephropathy, (NTP, 1988)) because of the clearly adverse nature of the effects in a chronic study and the high confidence in the estimate of the dose metric which was clearly related to toxicity, while there was somewhat less confidence in the three p-cRfCs based on other endpoints [decreased thymus weight (Keil et al., 2009), developmental immunotoxicity (Peden-Adams et al., 2006), and cardiac malformations (Johnson et al., 2003)]. As stated in the response to (g), TCE could clearly cause significant renal toxicity when administered in sufficient doses. Thus, as in the RfC discussion above, the Panel

agreed that kidney toxicity was indisputably a key effect of TCE from a hazard identification perspective. However, as discussed above, the Panel concluded that the p-cRfD for the kidney endpoint was based on an uncertain dose metric in regard to the relative rate of formation of the toxic metabolite in humans and animals. Although there was somewhat less confidence in the immune and cardiac malformation endpoints from a hazard identification perspective, for reasons discussed extensively in other sections of this response, there was sufficient confidence in them to consider them critical endpoints to support the RfC. While the confidence in these three endpoints was less than for the kidney endpoints as far as hazard identification, the three p-cRfCs for these endpoints were based on relatively certain dose metrics.

Although there was greater pharmacokinetic uncertainty for the p-cRfD based on the renal endpoint (NTP, 1988), it provided additional support for the final RfD.

The Panel noted that the same final RfD, 0.0004 mg/kg/day was supported by the p-cRfCs based on both the three principal studies (0.0004 mg/kg/day, 0.0005 mg/kg/day, and 0.0005 mg/kg/day) and the supporting (kidney) study (0.0003 mg/kg/day), and concluded that the use of p-cRfDs for multiple critical effects to derive the final recommended RfD reduced uncertainty and better characterizes variability. As discussed above for the RfC, it was noted that, in general, this approach may create more work for the risk assessors and the users of the risk assessment than use of the single most sensitive endpoint. However, it was recognized that, even if the RfD were to be based on the single most sensitive endpoint, it would be necessary to develop p-cRfCs for multiple endpoints in order to rigorously determine which study and endpoint would give the most sensitive RfD. It was also noted that a single RfD value was provided to users of the risk assessment.

Recommendations:

- The two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) should be considered the principal studies supporting the RfC.
- The endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2009) and the cardiac malformations from Johnson et al. (2003) should be considered as the principal studies supporting the RfD.

Editorial Comment:

- Table 5-23, NCI (1976), last bullet. 0.9 ug/m^3 should be corrected to 9 ug/m^3.
- p. 5-24, lines 31-32. Change to “within 2-fold of each other” (1.1-1.9 mg/kg/day).

9. Cancer Dose-Response Assessment

In accordance with the approach outlined in the U.S. EPA Cancer Guidelines and Supplemental Guidance (U.S. EPA, 2005a; U.S. EPA, 2005b), EPA's dose-response assessment includes the development of an inhalation unit risk and oral unit risk for the carcinogenic potency of TCE. Please address the following methods, results, and conclusions from EPA's cancer dose-response assessment in terms of the extent to which they are clearly and transparently described and technically/scientifically adequate to support EPA's draft inhalation and oral unit risks:

a. the estimation of unit risks for renal cell carcinoma from the Charbotel et al. (2006) case-control study;
b. the adjustments of renal cell carcinoma unit risks to account for the added risk of other cancers using the meta-analysis results and Raaschou-Nielsen et al. (2003);
c. the estimation of human unit risks from rodent bioassays;
d. in accordance with the approach in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a) and the conclusions as to MOA (above), the use of linear extrapolation from the point of departure (POD) for the cancer dose-response assessment of TCE;
e. the applications of PBPK modeling, including the selection of dose metrics and the use of PBPK model predictions for inter-species, intra-species, and route-to-route extrapolation based on internal dose, and their preference over default approaches based on applied dose;
f. the qualitative and quantitative characterization of uncertainty and variability;
g. the conclusion that the unit risk estimates for TCE based on human epidemiologic data and those based on rodent bioassay data are consistent overall; and,
h. **the preference for the unit risk estimates for TCE based on human epidemiologic data over those based on rodent bioassay data**.

9a Estimation of Unit Risks for Renal Cell Carcinoma

The Panel agreed that the analysis of the Charbotel et al. (2006) data was well described and scientifically appropriate and that the study should be used to estimate unit risks. The Panel did, however, agree that some more discussion was needed on cutting oils and whether or not it was necessary to adjust for exposure to cutting oils when computing an odds ratio or relative risk relating TCE exposure to kidney cancer. As noted in the document (p. 5-136), Charbotel et al. (2006) found a marginally significant relationship between cutting and petroleum oils and RCC (p-value < 0.1) though the relationship disappeared after adjustment for other variables. Given that there was some suggestion of a relationship, the Panel recommended that the EPA take a closer look at the literature to determine if there were other studies which suggested that exposure to cutting oils was a risk factor for kidney cancer.

Recommendations:

- The Panel believed that the EPA should provide a more detailed discussion of the limitations of their analysis. In particular, the model described on p. 5-131 made some very restrictive assumptions: linear dose-response and exposure was measured without error. In addition, the life table analysis applied the same estimated RR to each age interval; another restrictive assumption. While the Panel understood that these assumptions were necessary due to limited data, there was inadequate discussion of how violations of these assumptions may affect the results.

- Finally, in constructing the life table, the EPA used background kidney cancer rates in the US though the Charbotel et al. (2006) data were based on a French cohort. Hence, a comparison of background cancer rates in France and the U.S. would be helpful in supporting their conclusions.

9b Adjustment of Renal Cell Carcinoma Unit Risks

The Panel agreed that the analysis and presentation should be accepted in its current form.

9c Estimation of Human Unit Risks from Rodent Bioassays

EPA also calculated cancer unit risk estimates based on chronic bioassays on rats and mice. Five inhalation bioassays and 7 oral bioassays were selected for dose-response analyses. Dose-response modeling using the linearized multistage model was performed using applied doses as well as PBPK model-based internal doses. Bioassays for which time-to-tumor data were available were analyzed using a Multistage Weibull model. A cancer potency estimate for different tumor types combined were derived from bioassays in which there was more than one type of tumor response in the same sex and species. Unit risk estimates based on PBPK model-estimated internal doses were then extrapolated to human population unit risk estimates using the human PBPK model. Based on these results, the most sensitive bioassay (i.e. the one with the greatest unit risk estimate) was considered as a candidate unit risk estimates for TCE.

Recommendations:

- The Panel agreed that the analysis and results were appropriate but recommended that the EPA provided some more details about their implementation and potential biases. For instance, in bioassays in which mortality occurred before time to first tumor, the authors simply adjusted their denominators to equal the number alive at time to first tumor. This approach assumed that drop-out prior to time to first tumor was unrelated to future risk of a tumor which could result in biased estimates.
- In addition, more information was needed on the priors used in their Bayesian analysis of combined risk across tumor types.

9d Use of Linear Extrapolation for Cancer Dose-Response Assessment

The Panel agreed that the analysis was consistent with current cancer guidelines. There was sufficient evidence to conclude that a mutagenic MOA was operative for TCE-induced kidney tumors, so linear extrapolation was used to derive unit risk estimates for this site. For all other tumor types, linear extrapolation was used as the default approach, in accordance with EPA's cancer guidelines. Hence, the Panel recommended accepting the analysis and presentation of the results in its present form.

9e Application of PBPK Modeling

The Panel agreed that the PBPK models provided valuable information to the risk assessment and agreed that the internal dose should be preferred over applied dose as it was the only way one could, at the mechanistic level, combine information about pharmacokinetics and pharmacodynamics.

9f Qualitative and Quantitative Characterization of Uncertainty and Variability

The Panel agreed that consideration of uncertainty and variability was adequate. The Panel believed that the characterization of uncertainty and variability in the PBPK models was exceptionally strong. Use of AIC to select the best fit model was an adequate way to address model uncertainty. However, the authors' use of a 0.05 significance level for goodness of fit tests was inappropriate; typically, larger type-I error rates are used in such tests (e.g., values between 0.1 and 0.2) since one usually does not want to reject the null hypothesis that the model fits the data.

9g Conclusion on the Consistency of Unit Risk Estimates Based on Human Epidemiologic Data and Rodent Bioassay Data

The Panel agreed with this conclusion. For inhalation, the most sensitive rodent bioassay responses based on the preferred dose metrics ranged from 2.6×10^{-3} per ppm to 8.3×10^{-2} per ppm across the sex/species combinations. For oral exposure, the most sensitive bioassay responses based on the preferred dose metrics ranged from 2.3×10^{-3} per mg/kg/d to 2.5×10^{-1} per mg/kg/d across the sex/species combination. For both routes of exposure, the most sensitive sex/species response was male rat kidney cancer based on the preferred dose metric. When the human epidemiologic data were considered, a cancer inhalation unit risk estimate of 2.2×10^{-2} per ppm and oral unit risk estimate of 5×10^{-2} per mg/kg/d were obtained, which are both within the ranges reported in the aforementioned animal studies.

9h Preference for the Unit Risk Estimates based on Human Epidemiologic Data

The Panel agreed that human data, when available, should be preferred over rodent data when estimating unit risk, since within-species uncertainty was easier to address than between-species uncertainty.

10. Age-Dependent Adjustment Factors

Based on the conclusions that the weight of evidence supports a mutagenic MOA for TCE-induced kidney cancer and that the MOAs for TCE-induced liver cancer and lymphomas are not known, the Age-Dependent Adjustment Factors (ADAFs) are only applied to the kidney cancer component of the unit risk estimates. Please address the extent to which the recommended approach to applying the ADAFs in this situation is clearly, transparently, and accurately described.

Response

The Panel concluded that EPA has done an excellent job of describing and presenting the ADAF computations for both oral and inhalation situations. Application of ADAFs in the TCE analysis consistently followed recommendations in U.S. EPA Cancer Guidelines (U.S. EPA, 2005a) and Supplemental Guidance (U.S. EPA, 2005b). All of the steps were clearly presented for inhalation exposure. However, the discussion for the oral exposure route was shortened and referred back to the inhalation section, making understanding of the example less easy to follow.

EPA supplemental guidance recommends adjustment for children based on the presumption that children <16 years of age are intrinsically more susceptible than adults to mutagenic carcinogens because of biochemical and physiological factors related to the development of many organs and tissues during this time period; the rationale for the application of an ADAF is not based on the assumption that children have greater exposure on a per body weight basis than adults.

The Panel recognized that EPA wished to maximize utility in its IRIS database for TCE and other chemicals for which ADAFs were applied by providing slope factors and unit risk factors that allow users to compute risks for situation-specific drinking water intake values and for exposures to different age groups. Drinking water concentrations for specified lifetime cancer risk levels (10^{-4}, 10^{-5}, 10^{-6}) are routinely included in IRIS assessments in which ADAFs are not applied; this information is very helpful to public health professionals who use the IRIS database to evaluate situations of water contamination. For IRIS assessments in which ADAFs are applied, as in TCE, it would be useful to users to include this information, using representative drinking water intakes for various age groups. Other drinking water estimates may be used if determined to be more applicable.

The Panel was somewhat concerned that the use of ADAFs was in conflict with the assumptions that underlie the life-table analysis described in Section 5.2.2.1.2 and Appendix H. As indicated on p. 5-131, lines 25-28, the life-table method used to calculate lifetime extra risks from the Charbotel et al. (2006) study assumed that relative risk (RR) was independent of age; as seen in Table H-1, the same estimate of RR was used in each age interval of the life-table to compute the exposed RCC hazard rate (column L). However, ADAFs were applied under the assumption that children were more susceptible to the mutagenic effects which implied that RRs were age-dependent. The Panel recommended that EPA clarify whether this conflict in assumptions truly exists and if so, what impact it might have on risk estimation and how it may

be resolved in the future. For example, it might make more sense to apply ADAFs during the life-table analysis instead of at the end of the analysis, following estimation of the unit risk.

Recommendations:

- The Panel recommended that the statement on page 5-151, lines 14-18, be expanded to better explain why age-dependent adjustment factors were used for <16 years of age, but not for the elderly, and why EPA did not directly produce age dependent unit risks per mg/kg/d.

- Include all details presented for the inhalation sample calculations as was done for the oral exposure sample calculations.

- IRIS assessments in which ADAFs are applied, such as TCE, should include estimated drinking water concentrations for specified lifetime cancer risk levels (10^{-4}, 10^{-5}, 10^{-6}), using representative drinking water intakes for various age groups, while noting that other drinking water estimates may be used if preferred.

- Include in the documentation a discussion of the perceived conflict between the use of ADAFs and the assumptions underlying the life table analysis of the Charbotel et al. (2006) data.

11. Additional key studies

Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review and should therefore be considered in the assessment of the noncancer and cancer health effects of TCE.

Response

The Panel has identified additional studies to be considered in the assessment:

11a Fetal Cardiac Effects

Some recent publications confirm and reinforce the results obtained in the Johnson et al. (2003) study, so maybe they could be cited to make a stronger argument. They are listed as follows:

Caldwell, PT; Thorne, PA; Johnson, PD et al. (2008) Trichloroethylene disrupts cardiac gene expression and calcium homeostasis in rat myocytes. Toxicol Sci 104: 135-143.

Caldwell, PT; Manziello, A; Howard, J et al. (2010) Gene expression profiling in the fetal cardiac tissue after folate and low-dose trichloroethylene exposure. Birth Defects Research (Part A): Clinical and Molecular Teratology 88: 111-127.

Györke S, Terentyev D. (2008) Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. Cardiovasc Res. 77(2):245-55. Epub 2007 Oct 15. Review. PubMed PMID: 18006456.

Lehnart SE, Mongillo M, Bellinger A,et al.(2008) Leaky Ca2+ release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. J Clin Invest. 118(6):2230-45. PubMed PMID: 18483626; PubMed Central PMCID: PMC2381750.

Lebeche D, Davidoff AJ, Hajjar RJ. (2008) Interplay between impaired calcium regulation and insulin signaling abnormalities in diabetic cardiomyopathy. Nat Clin Pract Cardiovasc Med. 5(11):715-24. Epub 2008 Sep 23. Review. PubMed PMID: 18813212.

Makwana,O; King, NM; Ahles, L et al. (2010) Exposure to low-dose trichloroethylene alters shear stress gene expression and function in the developing chick heart. Cardiovasc Toxicol. 26 Feb, DOI 10.1007/s12012-010-9066-y.

Pace, BM; Lawrence, DA; Behr, MJ; et al. (2005) Neonatal lead exposure changes quality of sperm and number of macrophages in testes of BALB/c mice. Toxicology 210: 247-256.

Rufer, ES; Hacker, T; Flentke, GR; et al. (2010) Altered cardiac function and ventricular septal defect in avian embryos exposed to low-dose trichloroethylene. Toxicological Sciences 113: 444-452.

Yano M, Yamamoto T, Kobayashi S. et al. (2008) Defective Ca2+ cycling as a key pathogenic mechanism of heart failure. Circ J. 72 Suppl A:A22-30. Epub Sep 4. Review. PubMed PMID: 18772523.

11b Kidney Effects

Jacob, S; Héry, M ; Protois, JC ; et al. (2007) New insight into solvent-related end-stage renal disease : occupations, products and types of solvents at risk. Occup Environ Med 64: 843-848.

12. Research Needs

Please discuss research likely to substantially increase confidence in the database for *future* assessments of TCE.

Response

12a PBPK Model

The Panel concluded the analysis presented in the TCE Review Document defined how EPA expects to use PBPK models to integrate what is known about animal and human biology with TCE mode of action information and available animal and human study data to improve the transparency and accuracy of chemical risk assessments. This is a substantial piece of research and the EPA is to be applauded for this effort. The Panel discussed additional research, which could further improve the TCE risk assessment as well as influence the broader use of PBPD models in risk assessment.

The current model does not account for the temporal variability of the inputs and outputs in humans. Future development of the trichloroethylene PBPK model requires accommodation in the model for inter-individual temporal variability in the population. This is particularly important for modeling both sub-chronic and chronic exposures. If anything, the model should be most accurate in modeling the effects of human exposure over an extended period. Support for adding an inter-individual temporal component to the model can be found in a number of places in the report. For example on page 3-108 (lines 14-16) the text reads: ‘’However, data from Chiu et al. (2007) indicated substantial interoccasion variability, as the same individual exposed to the same concentration on different occasions sometimes had substantial differences in urinary excretion.’’ In this paper Chiu et al. (2007), found that there was variability in urinary excretion from the same individual exposed to the same concentration on different occasions. Also, Fisher et al. (1998) (see Table 3-45, page 3-111) documents an occasion in which a female was exposed to both 50 and 100 ppm. Assuming the same subject-specific estimates across the two occasions at different doses resulted in over-prediction at the higher exposure.

To substantially improve the PBPK model for trichloroethylene, EPA should perform a global sensitivity analysis. A formal Bayesian sensitivity analysis is one approach available, but even a more traditional approach to model sensitivity would provide useful information. In addition, the impact of changing priors and/or incorporating correlations among parameters should be examined. Because key dose metrics include upper tails from the predicted posterior distribution, future work should evaluate the sensitivity of the predictions to distributional assumptions for the random effects, for example by replacing uniform priors with normal or lognormal priors or by modifying the bounds on the priors. In future studies, the EPA should perform at least a limited analysis of sensitivity of results to model form (especially sensitivity to different assumed GSH pathways).

However, the hierarchical approach formulated in this report also made important assumptions about the relationship between the PBPK model parameters across the different species. These assumptions should be used consistently throughout the model development and not just in the case where there is limited prior information about a particular species.

Recommendations:

- Continue to look for data to support further refinement of priors, especially improving non-informative priors to informative priors and wide priors to narrower priors.

- Develop more efficient sophisticated model algorithms/environments to improve the simulation and reduce run time.

- Incorporate inter-individual temporal variability in future enhancements of the PBPK model for TCE.

- Perform a sensitivity analysis that ranges from the traditional assessment of the impact of parameter changes on final model predictions to an examination of the effect of changing prior distributions.

12b Immune System Effects

Recommendations:

- In future studies, it would be worthwhile to know more about the interaction between nutritional status, including metabolic disorders such as diabetes and risk of TCE-induced immunotoxicity.

12c Male Reproductive System

Recommendations:

- In section 4.8.1.3.2, it may be useful to note that male potency/sterility issues can be associated with inflammatory dysfunction in the testes produced by some environmental pollutants (usually associated testicular macrophage dysfunction) (see Pace et al., 2005). Since inflammatory dysfunction is associated with TCE exposure, this is an additional possible mechanism that may be associated with adverse outcome for male potency.

- For in utero exposure studies in rodents using lower doses of TCE and metabolites, where effects (carcinogenic and non-carcinogenic) can be observed trans-generationally, attention should be directed to epigenetic changes as possible MOA for TCE-mediated effects on the reproductive systems.

12d Susceptibility Factors

Recommendations:

- There is lack of data demonstrating modulation of health effects from TCE by the identified factors (genetics, lifestage, background, co-exposures, and pre-existing conditions). Such data is needed in risk assessment.

- Modulation of TCE exposure-related hypersensitivity dermatitis by genetic variation may be relevant for future study, given results of the study of hypersensitivity dermatitis in Asian workers reported in Li et al. (2007) and increasing industrial chemical exposures in China.

12e Derivation of RfD and RfC

Recommendations:

- The uncertainty about the rate of human glutathione conjugation found in Lash et al. (1999a) versus Green et al. (1997a) should be highlighted in the current assessment and addressed by sensitivity analysis in future refinements of this assessment**.**

- The variability/uncertainty for the toxicokinetically-sensitive individual could be quantified in future work by considering distributions in addition to the distribution of the 99th percentile, such as the 95th percentile. A quantile regression looking simultaneously at several quantiles could be developed in the future and presented in future refinements of this assessment.

- In future work, EPA could develop an approach using distribution to characterize uncertainty in a Bayesian framework.

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Appendix A: EPA's Charge Questions

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of TCE that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

In 2000, a monograph comprising 16 articles on the "State-of-the-Science" on TCE health risks, co-sponsored by EPA, other federal agencies, and the Halogenated Solvents Industry Alliance, was published in Environmental Health Perspectives[1]. EPA synthesized the information from these studies to develop an external review draft *Trichloroethylene Health Risk Assessment: Synthesis and Characterization*[2], released in August 2001. This 2001 draft was subject to peer review by an independent panel of the EPA Science Advisory Board (SAB). In December 2002, the SAB published its peer review report in *Review of Draft Trichloroethylene Health Risk Assessment: Synthesis and Characterization: An EPA Science Advisory Board Report*[3]. In addition, the public submitted more than 800 pages of comments to EPA during a 120-day public comment period. In February 2004, EPA held a public symposium on new TCE science at which recently published research was presented by a number of scientists.[4] Due to continuing scientific issues as well as emerging significant new science, EPA cosponsored with the Department of Defense, Department of Energy, and the National Aeronautics and Space Administration a consultation on TCE science issues with an expert panel convened by the National Academy of Sciences (NAS) Board on Environmental Studies and Toxicology. EPA developed four issue papers, presented to the NAS panel, highlighting important scientific issues related to TCE[5]. EPA scientists subsequently published a mini-monograph on these TCE science issues in Environmental Health Perspectives.[6] In 2006, the NRC released its report *Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues*[7].

The current external review draft TCE human health risk assessment is based on a comprehensive review of the available scientific literature on the human health effects of TCE, consideration of the input and advice from all the above sources, and adherence to the general

[1] *Environmental Health Perspectives.* Vol 108, Suppl 2, May 2000.
[2] EPA/600/P-01/002A, available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23249.
[3] Available at <http://www.epa.gov/sab/pdf/ehc03002.pdf>
[4] Symposium presentations and a transcript are available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=75934.
[5] Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=117502.
[6] *Environmental Health Perspectives.* Volume 114, Number 9, September 2006.
[7] Available at http://www.nap.edu/catalog.php?record_id=11707.

guidelines for risk assessment set forth by the NRC in 1983[8] and numerous guidelines and technical reports published by EPA (see Chapter 1 of the assessment). Specifically, this IRIS assessment provides an overview of sources of exposure to TCE, reviews the data on the toxicokinetics of TCE and its metabolites, describes the development of an updated physiologically based pharmacokinetic (PBPK) model of TCE and metabolites, characterizes the hazard posed by TCE exposure for carcinogenicity and non-cancer health effects based on the available scientific evidence, and presents a quantitative risk assessment for TCE health effects, including the derivations of a chronic inhalation Reference Concentration (RfC) and chronic oral Reference Dose (RfD) for non-cancer effects and an inhalation unit risk and oral unit risk for carcinogenic effects.

Charge Questions

Below is a set of charge questions that address scientific issues in the assessment of TCE. Please provide detailed explanations for responses to the charge questions, and focus any recommendations on improving the accuracy, objectivity, transparency, and utility of EPA's current analyses and conclusions.

PBPK Modeling

1. Is EPA's updated PBPK model for TCE and its metabolites (also reported in Evans et al., 2009, and Chiu et al., 2009) clearly and transparently described and technically and scientifically adequate for supporting EPA's hazard characterization and dose-response assessment? Specifically, please address the PBPK model structure; Bayesian statistical approach; parameter calibration; model predictions of the available in vivo data; and characterization of PBPK model dose metric predictions, including those for the GSH conjugation pathway.

Meta-analysis of cancer epidemiology

2. NRC (2006) recommended that EPA develop updated meta-analyses of the epidemiologic data on TCE exposure and cancer, and provided advice as to how EPA should conduct such analyses. Is EPA's updated meta-analysis of the epidemiologic data on TCE exposure and kidney cancer, lymphoma, and liver cancer clearly and transparently described and technically and scientifically adequate for supporting EPA's hazard characterization and dose-response assessment? Specifically, please address the standards of epidemiologic study design and analysis as they were applied to select studies for inclusion in the meta-analysis; the rationales for study relative risk estimate selections; the meta-analysis methods; and the characterization of the conclusions of the meta-analyses.
   Note: The scope of this charge question only includes the meta-analysis methods and results and not the overall weight of evidence for TCE carcinogenicity, which is addressed as part of a subsequent charge question.

[8] NRC (1983). *Risk Assessment in the federal government: managing the process.* Washington DC: National Academy Press.

Hazard Assessment

3. Does EPA's hazard assessment of non-cancer human health effects of TCE logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions that TCE poses a potential human health hazard for non-cancer toxicity to the central nervous system; the kidney; the liver; the immune system; the male reproductive system; and the developing fetus, including the role of TCE in inducing fetal cardiac defects?

4. Using the approach outlined in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a), does EPA's hazard assessment of carcinogenicity logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions that TCE is carcinogenic to humans by all routes of exposure? Specifically, please address the epidemiologic evidence for associations between TCE and kidney cancer, lymphoma, and liver and biliary tract cancer; the extent to which the results of the meta-analyses contribute to the overall weight of evidence for TCE carcinogenicity; the laboratory animal data for rat kidney tumors, mouse liver tumors, and lymphatic cancers in rats and mice; and the toxicokinetic and other data supporting TCE carcinogenicity by all routes of exposure.

5. Does EPA's hazard assessment logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions regarding the role of metabolism in TCE carcinogenicity and non-cancer effects? Specifically, please address EPA's conclusions that the liver effects induced by TCE are predominantly mediated by oxidative metabolism, but not adequately accounted for by the metabolite trichloroacetic acid (TCA) alone and that the kidney effects induced by TCE are predominantly mediated by metabolites formed from the GSH-conjugation pathway.

6. Using the approach outlined in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a), does EPA's hazard assessment logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions regarding the mode(s) of action [MOA(s)] of TCE carcinogenicity and non-cancer effects? Specifically, please address the conclusions that the weight of evidence supports a mutagenic MOA for TCE-induced kidney tumors; that a MOA for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation, possibly in combination with a mutagenic MOA, is inadequately supported by available data; that there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced liver carcinogenesis; that there are inadequate data to specify the key events and MOAs involved in other TCE-induced cancer and non-cancer effects; and that the available data are inadequate to conclude that any of the TCE-induced cancer and non-cancer effects in rodents are not relevant to humans

7. Does EPA's hazard assessment logically, accurately, clearly, and objectively represent and synthesize the available scientific evidence to support its conclusions that the factors that could modulate susceptibility to TCE carcinogenicity and non-cancer effects include

genetics, lifestage, background and co-exposures, and pre-existing conditions, but that only toxicokinetic variability in adults can be quantified given the available data?

Dose-Response Assessment

8. EPA's dose-response assessment includes the development of a chronic inhalation Reference Concentration (RfC) and chronic oral Reference Dose (RfD) for non-cancer effects. Please address the following methods and results from EPA's non-cancer dose-response assessment in terms of the extent to which they are clearly and transparently described and technically/scientifically adequate to support EPA's draft RfC and RfD:
    - **a.** The screening, evaluation, and selection of candidate critical studies and effects;
    - **b.** The points of departure, including those derived from benchmark dose modeling (e.g., selection of dose-response models, benchmark response levels);
    - **c.** The selected PBPK-based dose metrics for inter-species, intra-species, and route-to-route extrapolation, including the use of body weight to the ¾ power scaling for some dose metrics;
    - **d.** The selected uncertainty factors;
    - **e.** The equivalent doses and concentrations for sensitive humans developed from PBPK modeling to replace standard uncertainty factors for inter- and intra-species toxicokinetics, including selection of the 99^{th} percentile for overall uncertainty and variability to represent the toxicokinetically-sensitive individual;
    - **f.** The qualitative and quantitative characterization of uncertainty and variability;
    - **g.** The selection of NTP (1988) [toxic nephropathy], NCI (1976) [toxic nephrosis], Woolhiser et al. (2006) [increased kidney weights], Keil et al. (2009) [decreased thymus weights and increased anti-dsDNA and anti-ssDNA antibodies], Peden-Adams et al. (2006 [developmental immunotoxicity], and Johnson et al. (2003) [fetal heart malformations] as the critical studies and effects for non-cancer dose-response assessment;
    - **h.** The selection of the draft RfC and RfD on the basis of multiple critical effects for which candidate reference values are in a narrow range at the low end of the full range of candidate critical effects, rather than on the basis of the single most sensitive critical effect.

9. In accordance with the approach outlined in the U.S. EPA Cancer Guidelines and Supplemental Guidance (U.S. EPA, 2005a; U.S. EPA, 2005b), EPA's dose-response assessment includes the development of an inhalation unit risk and oral unit risk for the carcinogenic potency of TCE. Please address the following methods, results, and conclusions from EPA's cancer dose-response assessment in terms of the extent to which they are clearly and transparently described and technically/scientifically adequate to support EPA's draft inhalation and oral unit risks:
    - **a.** the estimation of unit risks for renal cell carcinoma from the Charbotel et al. (2006) case-control study;
    - **b.** the adjustments of renal cell carcinoma unit risks to account for the added risk of other cancers using the meta-analysis results and Raaschou-Nielsen et al. (2003);
    - **c.** the estimation of human unit risks from rodent bioassays;

d. in accordance with the approach in the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a) and the conclusions as to MOA (above), the use of linear extrapolation from the point of departure (POD) for the cancer dose-response assessment of TCE;

e. the applications of PBPK modeling, including the selection of dose metrics and the use of PBPK model predictions for inter-species, intra-species, and route-to-route extrapolation based on internal dose, and their preference over default approaches based on applied dose;

f. the qualitative and quantitative characterization of uncertainty and variability;

g. the conclusion that the unit risk estimates for TCE based on human epidemiologic data and those based on rodent bioassay data are consistent overall; and,

h. the preference for the unit risk estimates for TCE based on human epidemiologic data over those based on rodent bioassay data.

10. Based on the conclusions that the weight of evidence supports a mutagenic MOA for TCE-induced kidney cancer and that the MOAs for TCE-induced liver cancer and lymphomas are not known, the Age-Dependent Adjustment Factors (ADAFs) are only applied to the kidney cancer component of the unit risk estimates. Please address the extent to which the recommended approach to applying the ADAFs in this situation is clearly, transparently, and accurately described.

Additional key studies

11. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review and should therefore be considered in the assessment of the noncancer and cancer health effects of TCE.

Research Needs

12. Please discuss research likely to substantially increase confidence in the database for *future* assessments of TCE.