Preliminary Subgroup Responses to Charge Questions for the Toxicological Review of Trichloroethylene (TCE) (October 2009)

Subgroup for Charge Question 1: Drs. Emond (lead discussant), Bartell, Fuentes, Johanson, Pennell and Portier

Charge Question 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 [Section 3.5, Appendix A].

Response: The Panel extracted the following points from the Charge Question #1 and provided a discussion of each.

Is the PBPK model structure clearly and transparently described?

According to the TCE Review Document (page 3-64) the version of the PBPK model published by Hack et al. in 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. This modified model seems to accurately predict the internal dose in the target tissue. *The Panel agrees that using a PBPK model does improve the quality of the predictions for risk assessment and anticipates that the current model will reduce uncertainties that resulted from the use of previous PBPK models.*

The Panel noted that, for the most part, the PBPK model is well presented in the TCE Review Document but there is still room for improvement. For example, the conceptual representation of the PBPK model given in Figure 3-7 [page 3-69] though useful in understanding the changes made to the Hack (2006) model, does 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 is structured. However, the description of the PBPK model is 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 3) the fits and the graph 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 can 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.

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

Recommendation: *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).*

Recommendation: *Clarify the strategy behind the model structure and describe the biological relevance of each model equation.*

The Panel felt 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 remains a significant amount of variability between animals that does not seem to be accounted for in the final model. According to the report this variability is assumed captured in the prior distributions for model parameters. Because the rough 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 infeasible.

Recommendation: *Document model assumptions and discuss the consequences of potential violations of these assumptions. (e.g. impacts on bias and accuracy) .*

Recommendation: *Provide a more detailed justification for how between animal variability is accounted for in the model.*

Is the Bayesian statistical approach clearly and transparently described?

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 is 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 suffers from a lack of details and more details are needed to provide complete transparency. Several model parameters entered the Bayesian estimation method with wide and uniform prior distributions. The large number of such parameters makes the MCMC chains longer resulting in a long times to convergence and wide posterior distributions. The Panel noted high variability in the posterior distributions of many model inputs and the state parameters. However the posterior distributions for many internal dose state parameters are much less variable.

Recommendation: *Provide 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.*

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 would this correlation be accounted for in human-specific model parameter estimates?

Recommendation: *Provide some information on correlations among posterior medians for species-specific parameters.*

Recommendation: *Provide more information on the model ordinary differential equations and on the likelihood function used in the Bayesian estimation.*

Is parameter calibration clearly and transparently described?

Parameter calibration as described in the TCE Review Document was accomplished via a hierarchical fitting 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 generally endorses 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.*

Is model fit assessment and dose model projections clearly and transparently described?

There are a very large number of parameters in the PBPK model the Panel was asked to review which made critical review of the whole model and in particular identifying the key issues around model fit a big challenge.

A review of Figures 3-9, 3-10, A-3 and A-4, suggests that the updated model has adequate fit. Very useful was Table 3-45 and 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 TCE Review Document authors focused on agreement of the interquartile ranges. One Panel member noted that on Figure 3-9 (page 3-107) Bothersome in Figure 3.9 (page 3-107) is that the vertical axes change from the Hack model fit to the Updated model fit. This adds a challenge to assessing model fit since the models are 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].

Recommendation: *Move some graphical presentations from the linked graphics documents into the body of the report or into Appendix A.*

As a measure of model goodness of fit, the report 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.

Recommendation: *Include more discussion on model fit and in particular indicate areas where the model fit well and areas where it did not fit well. Tie this discussion somehow to Table 3.41.*

Recommendation: *Include graphs that show predicted versus observed values for all data points used in the analysis (one graph per endpoint).*

The Panel pointed out other issues related to the evaluation of the posterior distributions. Some of the posteriors were flatter than their priors, which is an unexpected result. In addition, in Figure 3-36, (section 3.5.6.2), pages 3-88 to 3-90 3, the Panel observed that prior and posterior distributions of model parameters were almost identical and only in a few cases were the distributions different. To help readers of the TCE Review Document draw their own conclusions, the Panel suggested a number of additions to the document:

Recommendation: *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).*

Recommendation: *Identify those parameters whose prior and posterior distributions are very different 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.*

Recommendation: *Clarify which parameters are related to variability and which address parameter uncertainty. Separate the discussion of the two types of parameters.*

The Panel noted that the EPA had available a large number of studies 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, 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 improves the confidence of users in the final PBPK model and helps point to areas where the model may still be inadequate.

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 input and state parameter values (especially GSH-related rates) impact changes in output dose metrics, and to examine how input, state and output parameters are correlated. Provide graphical presentations of the sensitivity to the key parameters.*

Research needs.

The analysis presented in the TCE Review Document defines how EPA expects to use PBPK models to integrate what we know 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 feels that with little additional effort, the TCE Review Document can be improved and as a result the findings of this analysis will be clearer and will find greater acceptance in the broader scientific community. As suggested by the Panel's recommendations, effort should be directed at describing and justifying various aspects of the model and model fitting process.

In the current model, the temporal variability of the inputs and outputs within humans is not accounted for. 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 Chui 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.

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

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)

Recommendation: *Perform a sweries of sensitivity analyses that ranges from the traditional assessment of the impact of parameter changes on final model predictions over an examination of the effect of changing prior distributions*

However, the hierarchical approach formulated in this report makes 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.

The Panel suggested the following activities that they feel would contribute to the improvement of the Trichloroethylene PBPK model.

- Continue to look for data to support further refinement of priors, especially moving 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.

Reference List

Hack, C. E., Chiu, W. A., Jay Zhao, Q., and Clewell, H. J. (2006). Bayesian population analysis of a harmonized physiologically based pharmacokinetic model of trichloroethylene and its metabolites. *Regulatory Toxicology and Pharmacology* **46**(1), 63-83.

Subgroup for Charge Question 2: Drs. Vena (lead discussant), Bartell, Blair, and Hoel

Charge Question 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 [Section 4.4.2.5], lymphoma [Section 4.6.1.2.2], and liver cancer [Section 4.5.2] 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 [Section 4.1, Appendix B]; the rationales for study relative risk estimate selections; the meta-analysis methods; and the characterization of the conclusions of the meta-analyses [Sections 4.4.2.5, 4.5.2, 4.6.1.2.2 and Appendix C].

Response: The Panel agreed that:

- The meta-analyses of the epidemiologic data on TCE exposure and kidney cancer [Section 4.4.2.5], lymphoma [Section 4.6.1.2.2], and liver cancer [Section 4.5.2] followed the NRC recommendations for conducting a Meta-analysis. The EPA approach was clearly and transparently described and technically and scientifically appropriate for supporting EPA's hazard characterization and dose-response assessment.
- EPA performed a thorough literature review and developed clear and appropriate criteria for selection of studies to be included in the meta-analyses.
- Studies included in the meta-analyses were required to have individual level TCE exposure estimates in the study.
- The report has a strong discussion on potential confounding. The lack of effect of TCE for lung cancer in individual studies provided convincing evidence that confounding by smoking is unlikely.
- 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 report characterized the strengths and weaknesses of meta-analyses. It was clear which studies were included in the meta-analysis and why. Study exclusions were justified and listed.
- Meta-analysis was performed on three cancers. The text was not clear why only these three were selected for to 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 would have wanted to see other cancers to address confounding, e.g. Smoking confounding, no studies to have excess of lung cancer. A meta- analysis of lung cancer showing no association with TCE would drive home the point regarding confounding. Smoking could not cause excesses of kidney cancer, liver cancer or lymphoma without also causing an excess of lung cancer.

- The panel was pleased with use of random effects models and appropriate testing for heterogeneity, sensitivity and publication bias.
- It was helpful to see the detailed process of going through the literature, review of all literature relevant to various cancer sites, and documenting the rationale for selection of studies for inclusion in the meta-analyses. Selection criteria were described and justified. The analyses considered both incidence and mortality outcome measures.
- Conservative approaches were used in the meta-analysis. Effect sizes included in the meta-analyses were selected appropriately using the most conservative selection criteria.
- Findings of several community studies were intriguing, but the choice was to leave these out of 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 decision to keep those out was appropriate.
- EPA appropriately discussed the changing grouping 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 get at studies with the best outcome definitions, rather than pick at studies where the hematopoietic cancers were grouped. (e.g. myeloid and lymphoid neoplasms together). They 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, we feel 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 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).
- The panel agreed that the EPA conclusions from the meta-analyses that TCE increases the risk for the three cancers studied. Our agreement with their conclusion is based on the strict and appropriate inclusion criteria, the methods of conducting the meta-analysis 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. It might also be possible to provide this information without a formal meta-analysis.
- Provide measures of heterogeneity such as the I2 statistic for each meta-analysis. Although this information is provided and accurately explained in Appendix C, it is 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 I2 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 (add reference) or Zhang and Yu, (JAMA 1998; 280:1690-1691) 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.

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. EPA followed these principles in their meta-analyses for lymphoma, and cancers of the kidney and liver.EPA clearly developed a comprehensive listing of candidate studies for the meta-analyses. They characterized the strengths and weaknesses of each study, and clearly presented this information in the documents. The agency described procedures for selection of studies for the meta-analyses. 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 is 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-level 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 like result in classification of some unexposed individuals as exposure which would increase exposure misclassification and bias estimates of relative risk downward. 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 individual studies are fully considered and these, along with the strengths and weaknesses of the meta-analyses, are appropriately considered in the evaluation and interpretation of the results in relation to hazard characterization.

EPA clearly described possible misclassification of exposure and disease for the studies included in the meta-analyses. They appropriately noted that most these exposure assessment limitations would diminish relative risks and mute exposure-response gradients.

EPA indicates that in only one study were the interviewers blinded with regard to case/control status. Although it is desirable attempt blinding for case-control studies, but 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.

EPA clearly described the statistical techniques used in the meta-analyses. Both random and fixed-effect models were used in the meta-analyses. This is useful to assess the accuracy of the underlying assumptions regarding study variation. The Panel agreed with the EPA 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. This was based on an assessment of the literature that suggested that these were the cancer sites most likely to be associated with TCE exposure. Although this is a reasonable determination, it might be useful to have information on some other cancers to provide evidence regarding possible confounding. For example, kidney cancer is 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 is very strong evidence that workers exposed to TCE do not smoke more than the unexposed, or comparison, population.

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 provides a full discussion of these issues.

Bias and confounding are concerns in epidemiologic studies. 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 points out that many potential confounders, e.g., obesity, diabetes, tobacco, and hypertension in kidney cancer, are unlikely to be associated with the level of TCE exposure and, thus, are unlikely to confound. If these factors did confound, we would expect to see other cancers affected. Other occupational exposures are 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 is weak or non-existent, so they are 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 bias is obvious that would occur across studies of different designs, in different countries, and with different exposure metrics to falsely produce an association with TCE .The Panel does not think confounding or bias are likely explanations for the findings from the epidemiologic studies and meta-analyses.

Subgroup for Charge Question 3: Drs. Dietert (lead discussant), Keil, McMillan, Rankin, Selmin and Weaver

Charge Question 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 [Section 4.3]; the kidney [Section 4.4]; the liver [Section 4.5]; the immune system [Section 4.6]; the male reproductive system [Sections 4.8.1.1.3, 4.8.1.2, and 4.8.1.3.2]; and the developing fetus, including the role of TCE in inducing fetal cardiac defects [Section 4.8.3]?

Response:

The Panel agrees 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.

Additionally the Panel has the following system-specific comments in addressing Charge Question #3:

Section 4.3 – Central Nervous System

- Overall, the SAB supports the conclusions reached by the EPA concerning the effects of TCE on the nervous system.

- TCE-associated auditory impairment is discussed in this section (4.3.2.3.). Auditory impairment is commonly seen with various autoimmune conditions and inflammation-based diseases. Because these are among the immune dysfunctions observed with TCE exposure, this relationship should be noted in the discussion of auditory impairment.

- Vestibular function – (headaches, dizziness, nausea) (there is a typo on p4-101). 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).

Section 4.4 - The Kidney

- Overall the EPA's hazard assessment of the non-cancer adverse health effects of TCE logically, accurately, clearly and objectively represented and synthesized the available scientific evidence to support its conclusion that TCE poses a potential human health hazard for the kidney. A similar excellent presentation was made for the TCE metabolites. In particular, the role of GSH-derived metabolites of TCE in mediating cytotoxic effects in the kidney is well described.
- If additional endpoints of renal dysfunction (e.g. diuresis, increased glucose excretion) are present in the reported studies, they should be included in the report. Often only one or two parameters of renal function and histopathology are presented. A better overall description of renal dysfunction should be presented if available (esp. for animal studies).
- Another point is the need to better describe the location of the renal lesion, including nephron segment if known. For example, TCE and DCVC appear 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.

- In regard to the effects of TCE in the kidney, EPA has (again) provided a thorough but clear description of these effects. One issue of concern here is the quantitative aspect of the GSH pathway metabolites. Dr. Wolfgang Dekant, in his public comment, suggested that the data of Lash et al., 1999a overestimates the amount of DCVG produced in humans and animals by using the "Reed method". The data by Lash et al. suggest that mice should be more susceptible to TCE nephrotoxicity, since mice make more DCVG than rats. However, rats are more susceptible to TCE nephrotoxicity than mice. The data of Green et al., 1997a, which measures DCVG production by 14C TCE and radiochemical detection followed by mass spec identification of the metabolites, have lower production DCVG production levels than Lash, but the order of DCVG production demonstrate that rats should be more susceptible to TCE nephrotoxicity than mice, which is what is observed. Thus, the values of DCVG produced in the Green et al. study may better reflect the level of DCVG produced. In addition, in Dr. Lash's report, a clear dose-response relationship for production of DCVG was not observed, which could support that the "Reed method" may overestimate DCVG production via the production of non-specific derivatives identified as DCVG. Thus, interpretation of DCVG levels from the Lash et al., 1999a paper should be made with caution and averaging the data from the two studies probably overestimates DCVG production in humans.
- 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. Occup Environ Med 2007;64:843–848). These authors also did not discuss how they addressed exposure to solvent mixtures as they attempted to focus on specific agents.
- 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 double period line 23, p4-199.

Section 4.5 – The Liver

- As with the kidney, the SAB supports the EPA's conclusions regarding the effects of TCE in the liver. This issue has received significant attention due to the relationship between non-cancer and cancer effects, and the EPA covers this information in a straightforward fashion.
- The only criticism here is the (perhaps unavoidable) repetitive nature of their coverage, as these issues appear elsewhere in the document. Less repetition and better integration of these sections would improve the readability of the document.

Section 4.6 – The Immune System

- Overall, the SAB agrees with the EPA's conclusions regarding the immunotoxicity of TCE including the prioritization given for the developing immune system as a sensitive target of adverse health outcomes. The evidence supports the broad spectrum of TCE-induced adverse immune outcomes which include: immunosuppression, elevated risk of autoimmunity and dysregulation of inflammation. Additionally, the SAB agrees with the EPA's conclusions regarding the exposure levels that can produce developmental immunotoxicity as well as immunotoxicity following the exposure of adults.
- It should be indicated that the spectrum of TCE-induced immune dysfunctions (immunosuppression, autoimmunity, inappropriate and/or excessive inflammation) included in this EPA report has the potential to produce adverse effects that are seen well beyond lymphoid organs and involving several other physiological tissues and systems. For example, these types of immune dysfunctions have been observed to affect function and risk of disease in the nervous system, the skin, the respiratory system, the liver, the kidney and the cardiovascular system.
- It is useful to emphasize the cell mediated immune effects of TCE as some of this has been supported by the human epidemiology data.
- Additionally, it is useful to emphasize the children's exposure data which are consistent with immunotoxicity reported in the animal developmental models.
- It should be mentioned that while TCE exposure can produce a range of immune dysfunction, 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.

- On page 4-338, please clarify the use of the phrase, "subpopulation levels", on lines 31 and 33.
- In future studies, it would be worthwhile to know more about the interaction between nutrition and risk of TCE-induced immunotoxicity.

Sections 4.8.1.1.3, 4.8.1.2, and 4.8.1.3.2 - The Male Reproductive System

- Overall in its review of these sections pertaining to the reproductive system and particularly that of males, the SAB panel members agree with the conclusions of the EPA and found that the studies in this area are comprehensively and correctly described and compared. Both the effects of TCE exposure to produce adverse reproductive outcomes are well characterized. The report described clearly, accurately, and objectively both human and animal studies on TCE effects regarding male and female reproductive systems. Consistency between data obtained from human and rodent studies increased confidence in the interpretation of the results offered by the report in regard to non-carcinogenic and carcinogenic effects of TCE and possible MOAs in the reproductive systems. Summaries on studies and conclusions were nicely tabulated and helped with clarity and transparency. We join in the recommendation put forward in the report that further attention should be directed into the assessment of outcomes from current studies (LeJune, NRC 2009) and have the following recommendation for future areas of research (for charge #12):

- 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, Toxicology, 210(2-3):247-56). 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.

Sections 4.8.1.1.3, 4.8.1.2, and 4.8.1.3.2 - The Developing Fetus, Including the Role of TCE in Inducing Fetal Cardiac Defects

- Overall the panel supports the conclusions of the EPA regarding the effects of TCE on the developing fetus including the role of TCE in inducing cardiac defects. The panel found no bias for the criteria for selecting studies. On the contrary, the EPA made a major effort to consider all available studies and to examine reasons for discrepancies that might exist among studies.

- Specifically, the EPA's conclusions regarding the effects of TCE on the developing immune system (also considered under Section 4.6) are thoughtful, complete and appropriate in consideration of the information. The panel supports not only the conclusions regarding the nature of the adverse developmental immune outcomes but also the analysis of the exposure levels of TCE producing these adverse outcomes. The panel agrees with the prioritization of the adverse outcome.
- It may be useful to mention the role of cytokine dysregulation, particularly that seen with TCE exposure (e.g., involving IL-6), in cardiac dysfunction.
- The report explains logically why the Johnson et al. study was used to derive some reference points. Some recent publications confirm and reinforce the results obtained in the Johnson et al study, so maybe they could be cited to make a stronger argument. They are listed as follows:

Summarized below are the results from recent publications (PDF files were sent to the EPA staff contact).

- In Rufer et al., 2010 (is there a typo mentioning Rufer etal., 2008?) low doses of TCE (8ppb) 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 (Kimmel and De Sesso).

- 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., 2003 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.

Subgroup for Charge Question 4: Drs. Vena (lead discussant), Blair, De Roos and Hoel

Charge Question 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 [Section 4.11.2].

Response:
The Panel agreed that:

- The 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 supports 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 toxicokinetic data provide further support to the EPA conclusion. The report logically, accurately, clearly, and objectively presents the methodological review of the epidemiologic evidence, highlights the criteria for study inclusion in meta-analyses and the meta -analysis methods (as noted in charge question 2) and appropriately assesses the weight of the evidence to conclude that TCE is causally related to lymphoma, and kidney and liver cancer .
- The report appropriately highlights the causal criteria in support of the conclusion. The biologic plausibility and coherence of the epidemiologic findings are supported by the laboratory animal data, the toxicokinetic data, and epidemiologic data of other cancer sites and immune effects. The consistency of the findings is 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 provide 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. Both meta-analyses for kidney cancer and lymphoma found higher increases in the risk estimates associated with higher TCE exposure than for any TCE exposure, which further supports a causal association.

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 (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 (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. We count 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.

EPA concludes TCE is carcinogenic to humans by all routes of exposure. They base this on convincing evidence of a causal association between TCE and kidney cancer, compelling evidence for lymphoma, and more limited evidence for liver cancer. The epidemiologic data, in the aggregate, are quite strong. In addition, the epidemiologic data are supported by bioassays and toxicokinetic data. Although issues of concern can be raised about individual studies, the overall pattern and the results from the meta-analyses are 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 do 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 occurs. 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 depress estimates of relative risk and mute exposure-response gradients. 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 provide a clear indication of a cancer hazard from TCE. EPA concludes the association between TCE and lymphoma and liver cancer is more limited than that for kidney cancer. These conclusions about the epidemiologic data are 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 is 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 concludes that the epidemiology data are convincing for a causal association between TCE and kidney cancer, compelling for lymphoma, and positive but more limited for liver cancer. The Panel does not have strong disagreement with this statement, although some felt that the data for liver cancer as compelling as that for lymphoma. Liver cancer is a rare disease than kidney cancer or lymphoma 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 dividing 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 consider 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 epidemiology findings are supported by observations of liver cancer in animal models. Although potential confounding by other risk factors for liver cancer is possible, risk factors such as hepatitis are very rare in developed countries (where most of these studies were conducted), so this is unlikely to have caused such a degree of confounding. Hepatitis may be more of a concern for confounding in the study conducted in Taiwan, where hepatitis is more prevalent.

The meta-analysis results are 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 is further supported by the larger relative risk in meta-analyses for the higher exposure categories compared to the overall relative risk. This is evidence for an exposure response gradient, even though no individual studies showed much evidence of this.

Subgroup for Charge Question 5: Drs. Rusyn (lead discussant), Manautou, McMillan and Thrall

Charge Question 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 [Section 4.5.6] and that the kidney effects induced by TCE are predominantly mediated by metabolites formed from the GSH-conjugation pathway [Section 4.4.6].

Response:

- The Panel agrees that EPA's hazard assessment in the draft IRIS 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.

- 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 Board recommends that in the hazard assessment, EPA provides a more balanced description of the TCE's adverse health effects on the kidney and the liver since the role of the liver as a target tissue should not be underestimated.

- A conclusion that the adverse effects on the liver of one of the TCE metabolites, trichloroacetic acid, can not adequately account for the liver effects of TCE is supported by several lines of evidence. It is commendable that the hazard assessment section of the IRIS draft attempts to provide quantitative, rather than qualitative comparisons between the effects of trichloroacetic and dichloroacetic acid metabolites. The Board recommends that EPA considers a dose-response modeling approach to provide science-based information on the relative contribution of each metabolite, where data is available, to the liver effects of TCE. Should such analysis is deemed futile due to data gaps and/or difficulty in extrapolating between independent bioassays, EPA is encouraged to provide a detailed rationale and justification.

- EPA has provided clear and comprehensive summary of the available evidence that metabolites derived from GSH conjugation of TCE are responsible for mediating kidney effects. The integration of the data from human epidemiological studies, animal studies and in vitro mechanistic studies produces a clear and transparent weight-of-evidence assessment supportive of TCE GSH conjugation metabolites' role in kidney toxicity and cancer. It is recommended that the issue of quantitative assessment of the metabolic flux of TCE through the GSH pathway vs. the oxidative metabolism pathway is considered carefully since uncertainties exist with regard to the extent of formation of the dichlorovinyl metabolites of TCE between humans and rodents.

Subgroup for Charge Question 6: Drs. Weaver (lead discussant), Dietert, Keil, Manautou, Rankin, Rusyn and Selmin

Charge Question 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 [Section 4.4.7.1]; 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 [Section 4.4.7.2]; that there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced liver carcinogenesis [Section 4.5.7.2]; 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 [Section 4.3.10 (Neuro); Section 4.4.7 (Kidney); Section 4.5.7 (Liver), Section 4.7.4 (Lung), Section 4.8.1.3.3.2 (Reproductive), Section 4.8.3.3.2.1 (Fetal cardiac malformations)].

Response:
The Panel agreed that:

- the IRIS TCE hazard assessment logically, accurately, clearly, and objectively represents and synthesizes 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 provides 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 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. Similarly, as discussed in the response to charge question 8, the impact of the inconsistencies in these data should be presented more transparently.

- the narrative presentation of the data, along with the evaluation of the strengths and weaknesses of each study, is appropriate as supplemental information; however, in the body of the document, this 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. The EPA should consider tabular summaries by specific metabolites when studies used metabolite exposure rather than the parent compound. Likewise, data gaps should be clearly identified to help guide future research. Similarly, 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.

EPA's second charge in Question 6 asked the panel to address the conclusion "that the weight of evidence supports a mutagenic MOA for TCE-induced kidney tumors [Section 4.4.7.1]":

The Panel agreed that:

- the weight of evidence supports a mutagenic MOA for TCE-induced kidney tumors

EPA's third charge in Question 6 asked the panel to address the conclusion "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 [Section 4.4.7.2]"

The Panel agreed that:

- weight of evidence does 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.

EPA's fourth charge in Question 6 asked the panel to address the conclusion that "there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced liver carcinogenesis [Section 4.5.7.2];

The Panel agreed that:

- there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced human liver carcinogenesis

- the EPA's hazard assessment states that, in humans, "Primary hepatocellular carcinoma and cholangiocarcinoma (intrahepatic and extrahepatic bile ducts) are the most common primary hepatic neoplasms (El-Serag, 2007; Blehacz and Gores, 2008)." (4.5.2. Liver Cancer in Humans). The panel notes that these type of tumors appear to be independent of a PPARα dependent MOA. In support of this, induction of peroxisome proliferation in human liver carcinogenesis is not a common feature of exposure to PPARα agonists. The panel recommends additional discussion of the fact that common forms of liver cancer seen in humans are not seen in rodent models of TCE liver cancer where hepatocellular carcinomas are seen primarily in a PPARα dependent-manner.

- 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 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. Graphical or tabular presentation of these data would strengthen the comparative analysis between metabolites and chemicals. While the Guyton et al (2009) paper is cited in the document in several places, including some of the analyses from this publication which compare the receptor transactivation potency and the

carcinogenic potential of TCA, DCA and other model peroxisome proliferators will strengthen the arguments.

The fifth charge in Question 6 asked the panel to address the conclusion that "there are inadequate data to specify the 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 and non-cancer effects

The sixth charge in Question 6 asked the panel to address the conclusion 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 [Section 4.3.10 (Neuro); Section 4.4.7 (Kidney); Section 4.5.7 (Liver), Section 4.7.4 (Lung), Section 4.8.1.3.3.2 (Reproductive), Section 4.8.3.3.2.1 (Fetal cardiac malformations)]"

The panel agreed 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;
- in Section 4.4.7 (Kidney) the extent of the GSH pathway in humans may be overestimated and the impact of this must be transparent;
- in Section 4.5.7.4. Mode of Action (MOA) Conclusions regarding the liver: The MOA for carcinogenicity should be described as complex rather than unknown. 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;
- in Section 4.7.4 (Lung), a stronger discussion on the MOA for lung non-cancer and cancer effects should be included and the data for chloral hydrate should be given more emphasis.

Subgroup for Charge Question 7: Drs. De Roos, Bartell, Vena and Weaver

Charge Question 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 [Section 4.10]?

Response:

The Panel agrees 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 among humans to health effects from TCE exposure. The panel agrees with the conclusion that the potentially modulating factors do not have enough evidence to conclude that they do or do not impact risk estimates for TCE and human health effects.

- 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. We recommend that this statement be deleted from the Hazard Assessment report.

- The wording in Section 4.10 is often not clear about whether it is 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, it's often not clear, where effects of TCE within one subgroup are stated, whether the other subgroup was also examined or not.

- Section 4.10 of the Hazard Assessment should discuss explicitly the lack of such data and the need for such data in risk assessment.

- The EPA should make specific recommendations for studies that would fill this 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., genetics, obesity, multiple solvent exposures).

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

- Clarify the role of obesity in TCE metabolism and retention, as Section 4.10 refers to both higher concentration of TCE in adipose tissue of children compared to adults due to lower percentage body fat (page 4-570) and also increased body burden of TCE with obesity (page 4-585). While these two statements are not

exactly conflicting, the role of obesity in TCE metabolism and retention should be further clarified.

- The comments on early-life stages are understated. Adding literature and discussion on the obesity epidemic in children in terms of its impact on TCE metabolism/retention is recommended. The panel agreed with the use of standard age-dependent adjustment factors in the protection of children.

- Genetic susceptibility (page 4-584) is an evolving area that may become much more important for TCE and should be given more emphasis in Section 4.10. Hypersensitivity dermatitis in Asian workers reported in Li et al. EHP 2007 (HLA-B*1301 as a Biomarker for Genetic Susceptibility to Hypersensitivity Dermatitis Induced by Trichloroethylene among Workers in China) may manifest as serious disease and appears to be much more common among Asians than Caucasians. Given increasing industrial chemical exposures in China, this may be a significant concern from occupational TCE exposure in the future.

Subgroup for Charge Question 8: Drs. Post (lead discussant), Emond, Fuentes, Johanson, Portier and Weaver

Charge Question 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 [Section 5.1]. 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;

Response: The Panel agreed that the screening, evaluation, and selection of candidate critical studies and effects were generally technically/scientifically 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) relate 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 felt that it is important that the reader easily be able to find the details of the studies included in the Chapter 5 tables. In order to improve clarity, it is suggested that Chapter 5 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 also 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 are needed to prevent ambiguity. Also, consistent dose units should be used in discussing the same study in different places in the document.

For instance, four different studies with different durations are cited as "Crofton and Zhao (1997)" in Table 4-23, and it is not clear which duration is the basis for the cRfD in Table 5-1. In other cases, it is not stated whether the cRfD or cRfC is based on males or females when both were included in the study, or which strain is the basis when multiple strains were used. For example, from Table 5-2 and the text on p. 5-15 to 5-16, it is 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 Kiel et al. (2009). In the description of the study on p. 4-395, the doses are given as drinking water concentrations (ppb), but in Table 5-3, the LOAELs for this study are 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 are 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 are 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 is 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 are considered adequate "quantitative dose-response data," this should be stated.

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

Response: 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. In order to improve clarity and transparency, it was suggested that Chapter 5 include the information on POD derivation from Table F-13 of Appendix F, including approach, selection criterion and decision points. The Panel noted that the graphics in Appendix F provide a good presentation of the BMD analyses.

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

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;

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

In addition to the strong dependence of the p-RfDs and p-RfCs 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 are much greater than for other dose metrics. For example, there are very large discrepancies in the rates of human glutathione conjugation reported by Lash et al. (1999a) and Green et al. (1997a). This uncertainty should be highlighted in the current assessment, and should be addressed by sensitivity analysis in future refinements of this assessment.

Additionally, the Panel noted that 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 Panel recommended that the rationale for scaling the dose metric to body weight3/4, 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 Panel understands that the rationale for this adjustment is that the PBPK model predicts the dose rate to the target tissue rather than the internal concentration of TCE, but 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 are used to estimate serum levels or

other metrics of internal concentration, rather than delivered doses, and in such case, scaling of body weight¾ scaling would not be used.

Additionally, the discussion of "empirical dosimetry" vs. "concentration equivalence dosimetry" should be made clearer and more transparent (pp. 5-33 – 5-36). The discussion as presented in the draft document would likely not be readily understandable to many readers. Furthermore, since body weight3/4 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.

Specific 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?

d. The selected uncertainty factors;

Response: 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 derived 99th percentile (HEC99 and HED99) dose metrics are correctly derived.

It is recommended that the discussion of the subchronic to chronic uncertainty factor on p. 5-6 be clarified as far as durations of studies considered suitable as the basis of a chronic risk assessment. The Panel recognized that EPA guidance defines the duration of subchronic rodent studies 4 weeks to 90 days, and chronic rodent studies as 90 days to 2 years, and it is recommended that this information be stated in the document and a citation given.

The Panel 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). Although this duration does fall within the definition of subchronic used by EPA, it should be considered whether 4 weeks is sufficient be used as the basis for a chronic (lifetime) risk assessment.

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) lifespan (less than 18%). 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 of10) might be considered for studies of such durations, especially for endpoints thought to progress in incidence or severity with time.

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

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;

Response: 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 are probably very sensitive to the choice of prior distribution. The Panel concluded that approach used, including the selections of idPODs and the extrapolations for rodent to human followed by consideration of the 99th percentile human estimates, is 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) is appropriate.

It was noted by the Panel that 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.

Specific Comment: On p. 5-2, point (7), the use of the 99th percentile HEC and HED estimates is discussed. The reason for choosing 99th percentile instead of 95th percentile is 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 is 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 is appropriately protective, as opposed to being overly conservative. Specifically, the Panel noted that the PODs identified through BMD analysis are 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 are used as a central dose estimate in humans. It was also noted that uncertainty factors for interspecies and intra-human pharmacodynamic variability are applied the 99th percentile estimates (i.e. the doses for the 1% most pharmacokinetically sensitive humans) of the internal dose (HEC99 and HED99).

The Panel endorsed the use of BMD modeling instead of an approach based on an uncertainty factor for NOAEL-to-LOAEL extrapolation, and the use of PBPK modeling instead of default uncertainty factors for inter- and intra-species pharmacokinetic differences, when these approaches are supported by the data. The Panel recognized that these approaches are 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 is consistent with accepted EPA methodology for RfD/RfC development. It was specifically noted that the uncertainty factors for interspecies and intra-human pharmacodynamic variability are intended to account for variability as well as uncertainty, and that some p-RfDs/p-RfCs based on PBPK modeling are higher than RfDs/RfCs for the same endpoints based on the default methodology. The Panel recommended that HEC50 and HED50 values in 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, gives 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 is not used): Developmental immunotoxicity.

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

Response: The Panel generally agreed that the uncertainties related to RfC and RfD they are clearly and transparently described and technically/scientifically adequate to support EPA's draft RfC and RfD. The Panel recommended that the quantitative uncertainty analysis of PBPK model-based dose metrics for LOAEL or NOAEL based PODs (Section 5.1.4.2) be revised to clarify the objective of this 2-D type analysis, as well as the methodology used.

It was noted that in the PBPK model, the uncertainty and variability are 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 member recognized that the use of uncertainty factors in the TCE assessment followed the currently accepted EPA approach, but recommended that in future work, EPA could develop an approach using distribution to characterize uncertainty in a Bayesian framework.

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;

Response: 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 are 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) is highly sensitive to model choice. It was also noted that, although a tremendous amount of information is available on liver toxicity, hepatic effects were not a critical endpoint because they are less sensitive than other endpoints

The Panel expressed concerns about use of NTP (1988) [toxic nephropathy], NCI (1976) [toxic nephrosis], 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 is 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) do not indicate that there is 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) include the fact 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 is 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 are highly uncertain, TCE can 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.

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: The Panel supported the selection of a draft RfC and a draft RfC based on multiple candidate references values in a narrow range which is 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 is supported by the Panel because it is a very robust approach that increases confidence the final RfC and RfD.

As noted in the draft assessment, the proposed RfC, 0.001 ppm (5 ug/m3), is within a factor of 3 of the p-RfCs for the six critical endpoints selected. The Panel agrees with the use of PBPK modeling for route-to-route extrapolation for the five p-RfCs which are based on oral studies.

EPA states in the draft document (p. 5-83) that there is high confidence in the three p-RfCs 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 are based on chronic studies, and high confidence in its estimate of the dose metric which is clearly related to toxicity, while there is somewhat less confidence in the three p-RfCs 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 agrees that kidney toxicity is indisputably a key effect of TCE from a hazard identification perspective. However, as discussed above, the Panel concluded that the three p-RfCs for renal endpoints are 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 is 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 is sufficient confidence in them to consider them critical endpoints to support the RfC. While the confidence in these three endpoints is less than for the kidney endpoints as far as hazard identification, the three p-RfCs for these endpoints are based on relatively certain dose metrics.

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

The Panel noted that the same final RfC, 0.001 ppm, is supported by the p-RfCs 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-RfCs for multiple critical effects to derive the final recommended RfC reduces 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-RfCs for multiple endpoints in order to rigorously determine which study and endpoint provides the most sensitive RfC. It is also noted that a single RfC value is provided to users of the risk assessment.

As discussed in the draft document, the proposed RfD, 0.0004 mg/kg/day, is within 25% of the p-RfDs 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-RfDs are based on oral exposure, and three of them are based on drinking water exposure, a route relevant to environmental exposures. EPA states in the draft document (p. 5-83) that there is high confidence in the p-RfD 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 is clearly related to toxicity, while there is somewhat less confidence in the three p-RfCs 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 can clearly cause significant renal toxicity when administered in sufficient doses. Thus, as in the RfC discussion above, the Panel agrees that kidney toxicity is indisputably a key effect of TCE from a hazard identification perspective. However, as discussed above, the Panel concluded that the p-RfD for the kidney endpoint is based on an uncertain dose metric in regard to the relative rate of formation of the toxic metabolite in humans and animals. Although there is 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 is sufficient confidence in them to consider them critical endpoints to support the RfC. While the confidence in these three endpoints is less than for the kidney endpoints as far as hazard identification, the three p-RfCs for these endpoints are based on relatively certain dose metrics.

Based on the above, the Panel 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. Although there is greater pharmacokinetic uncertainty for the p-RfD based on the renal endpoint (NTP, 1988), it provides additional support for the final RfD.

The Panel noted that the same final RfD, 0.0004 mg/kg/day is supported by the p-RfCs 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-RfDs for multiple critical effects to derive the final recommended RfD reduces 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-RfCs for multiple endpoints in order to rigorously determine which study and endpoint would give the most sensitive RfD. It is also noted that a single and RfD value is provided to users of the risk assessment.

Specific Comment: Table 5-23, NCI (1976), last bullet. 0.9 ug/m3 should be corrected to 9 ug/m3.

Additional Specific Comment:
p. 5-24, lines 31-32. Change to “within 2-fold of each other” (1.1-1.9 mg/kg/day).

Subgroup for Charge Question 9: Drs. Pennell (lead discussant), Emond and Johanson

Charge Question 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 [Section 5.2]. 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;

Response: The panel agreed that the analysis of the Charbotel et al. data was well described and scientifically appropriate and that the study should be used to estimate unit risks. We did, however, agree that some more discussion is needed on cutting oils and 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. As noted in the document (p. 5-136), Charbotel et al. 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 is some suggestion of a relationship, we recommend that the 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 or bladder cancer.

We also feel that the EPA should provide a more detailed discussion of the limitations of their analysis. In particular, the model described on p. 5-131 makes some very restrictive assumptions: linear dose-response, RR independent of age, and exposure is measured without error. While we understand that these assumptions were necessary due to limited data, we don't think that there was an adequate 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. data are 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.

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. (2006);

Response: The panel agreed that the analysis and presentation should be accepted in its current form.

c. the estimation of human unit risks from rodent bioassays;

Response: The panel agreed that the analysis and results are appropriate but recommend that the EPA provide 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 assumes that drop-out prior to time to first tumor is unrelated to future risk of a tumor which could result in biased estimates. In addition, more information is needed on the priors used in their Bayesian analysis of combined risk across tumor types.

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;

Response: The panel agreed that the analysis was consistent with current guidelines for non-threshold toxins; hence, we recommend accepting the analysis and presentation of the results in its present form.

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;

Response: 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 is the only way we can, at the mechanistic level, combine information about pharmacokinetics and pharmacodynamics.

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

Response: The panel agreed that their consideration of uncertainty and variability was adequate. We felt 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 is 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.

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,

The panel agreed to accept this conclusion.

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

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.

Subgroup for Charge Question 10: Drs. Portier (lead discussant), Pennell and Post

Charge Question 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 [Section 5.2.3.3].

Response: The Panel felt 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 follows recommendations in U.S. EPA Cancer Guidelines (U.S. EPA, 2005a) and Supplemental Guidance (U.S. EPA, 2005b). All of the steps are clearly presented for inhalation exposure. However, the discussion for the oral exposure route was shortened and refers back to the inhalation section, making understanding of the example less easy to follow. The Panel recommended including of all details presented for the inhalation sample calculations in the oral exposure sample calculations as well.

The use of ADAFs in estimating total risk for TCE does not seem to have a major impact on the final estimates (in the example, the total cancer unit risk increased 17.5%). This is because only one tumor type received the adjustment. It was recognized that in other situations where multiple tumor types are eligible for adjustment, the impact of using the ADAFs would be greater.

One Panel member questioned the validity of the assumption of equal susceptibility for individuals ≥ 16 years of age. The use of age-dependent adjustment factors seems in conflict with the assumption that relative risk (RR) is independent of age that supports use of the linear model (RR = 1+slope x dose [p. 5-131, lines 25-28]) to calculate lifetime extra risks [0.001205 per ppm x year] in the Charbotel et al (2006) study.

The Panel recommended that the statement on page 5-151, lines 14-18 [copied below], be expanded to better explain why age-dependent adjustment factors are used for < 16 years of age but not for the elderly and why EPA does not directly produce age dependent unit risks per mg/kg/d. EPA supplemental guidance (reference???) recommends adjustment for children based on findings that children <16 years of age are intrinsically more susceptible to mutagenic carcinogens than adults because many organs and tissues are developing during this time period and not necessarily due to an assumption of greater exposure on a per body wt basis.

> "Because the TCE intake is not constant across age groups, one does not calculate a lifetime unit risk estimate in terms of risk per mg/kg/d adjusted for increased early life susceptibility. One could calculate a unit risk estimate for TCE in drinking water in terms of μg/L from the results in Table 5-42, but this is not something that is commonly reported, and it is dependent on the water ingestion rates used."

One Panel member recommended that EPA compute and include total lifetime risk values for Office of Water standard water consumption levels using the ADAF approach and representative drinking water intakes for various age groups, while noting that other drinking water estimates may be used if preferred. Including these estimates into the IRIS database for TCE and other chemicals would be helpful to users of these assessments.

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.

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