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TOXICOLOGICAL REVIEW

OF

TRICHLOROETHYLENE

APPENDIX I

(CAS No. 79-01-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2011

I. EPA RESPONSE TO MAJOR PEER REVIEW AND PUBLIC COMMENTS

I.1. PBPK MODELING (SAB REPORT SECTION 1): COMMENTS AND EPA RESPONSE

I.1.1. SAB Overall Comments:

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

I.1.2. Major SAB Recommendations and EPA Response:

I.1.2.1. PBPK Model Structure (SAB Report Section 1a)

- 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).

EPA response: EPA accepts this recommendation and has provided revised model structure diagrams in Appendix A, Section A.4.1.

- Clarify the strategy behind the model structure and describe the biological relevance of each model equation.

EPA response: EPA accepts this recommendation and has clarified the model structure and equations, and their biological relevance, in Appendix A, Section A.4.1.

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

EPA response: EPA accepts this recommendation and has expanded the discussion of limitations of the model to include added discussion of model assumptions and the consequences of potential violations in Section 3.5.7.4.

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

EPA response: EPA accepts this recommendation and has expanded the discussion of how between animal variability is addressed in the model in Section 3.5.5.2.

I.1.2.2. Bayesian Statistical Approach (SAB Report Section 1b)

- Present better descriptions and/or details on the choice of prior distributions, the Bayesian fitting methodology and fit of the posterior distribution for each model parameter.

EPA response: EPA accepts this recommendation and has added a description of the choice of prior distribution functions in Section 3.5.5.2; presented a description of the overall Bayesian posterior distribution function used in the parameter fitting in Section A.4.4; and added graphical presentation to Section A.5.1 of the posterior distributions, in comparison with the prior distribution, for each model parameter. In addition, the use of the terms "population" and "group" have been clarified throughout Chapter 3 and Appendix A.

- Provide some information on correlations around posterior medians for species-specific parameters.

EPA response: EPA accepts this recommendation and provided tables of correlation coefficients in Appendix A, Section A.5.1.

- Supply more information on the model ordinary differential equations and on the likelihood function used in the Bayesian estimation.

EPA response: EPA accepts this recommendation and has supplied more information on the model ordinary differential equations in Appendix A, Section A.4.1, and more information on the likelihood function in Appendix A, Section A.4.3.4.

I.1.2.3. Parameter Calibration (SAB Report Section 1c)

- 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.

EPA response: EPA accepts this recommendation and revised Table A-4 to clarify the scaling assumptions consistently used throughout the parameter calibration process, and revised Section 3.5.5.3 to clarify the description of the assumptions underlying the hierarchical approach.

I.1.2.4. Model Fit Assessment and Dose-Metric Projections (SAB Report Section 1d)

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

EPA response: EPA accepts this recommendation and has moved (in a more condensed form) graphical presentations of the PBPK model predictions as compared to the in vivo data to the body of Appendix A.

- Incorporate more discussion on model fit and in particular indicate areas where the model fits well and areas where it did not fit well. Tie this discussion somehow to Table 3-41.

EPA response: EPA accepts this recommendation and has incorporated more discussion of model fit in Section 3.5.6.3 indicating areas where the model fits well and areas where it did not fit well. This discussion is tied to the Table previously labeled "3-41," as recommended. In addition, the interpretation of the residual error GSD is more closely tied to this revised discussion.

- Include graphs that show predicted vs. observed values for all data points used in the analysis (one graph per endpoint).

EPA response: EPA accepts this recommendation and has added graphics showing predicted vs. observed values for all data points used in the analysis (one graph per endpoint) to Section 3.5.6.3. The width of the residual error GSDs are also included on these graphs for comparison. In addition, this is tied to the revised discussion on model fit and the Table previously labeled "3-41."

- 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).

EPA response: EPA accepts this recommendation and has added a table to Section 3.5.6.2 of model parameters listed in reverse order by the width of their posterior variability, indicated by the width of 95% CI.

- Identify those parameters with very different prior and posterior distributions and discuss why this might be a reasonable result of the parameter calibration process. An alternative would be to provide a table where parameters are ranked based on the percent change of the posterior from the prior.

EPA response: EPA accepts this recommendation and has included a table in Section 3.5.6.2 that indicates the fold-change between the prior and posterior medians. This table is already sorted by reverse order of the width of the posterior variability (see previous recommendation). In order to identify those parameter with more different priors and posteriors, the fold-change was bolded if the change was greater than threefold. It is noted in the revised text for

Section 3.5.6.2 that those parameters with shifts >3-fold had prior CIs greater (sometimes substantially) than 100-fold, so that such shifts are reasonable in that context.

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

EPA response: EPA accepts this recommendation and has replaced the tables in Section 3.5.6.2 that previously showed combined uncertainty and variability with tables that separately summarize parameter uncertainty and variability. This separation of uncertainty and variability has the added benefit of removing the appearance that posterior parameter distributions appear flatter than prior distributions, since posterior parameter uncertainty should always be less than or equal to prior parameter uncertainty. In addition, the text of Section 3.5.6.2 has been revised to discuss separately estimates of the central tendency of the population from estimates of population variability.

I.1.2.5. Lack of Adequate Sensitivity Analysis (SAB Report Section 1e)

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

EPA response: EPA accepts this recommendation and has conducted a local sensitivity analysis starting from the final fitted PBPK model, and assessing how small changes (5% increase or decrease) in model parameter estimates impact predictions. Two types of model predictions are analyzed. First, in Section 3.5.6.4, the sensitivity of predictions of calibration data is assessed, including a graphical presentation of the number of data points that are sensitive to each parameter. Second, in Section 3.5.7.2, the sensitivity of prediction of dose-metrics is assessed, including a graphical presentation of the sensitivity coefficient for each parameter and dose-metric. The results of these local sensitivity analyses confirms that the calibration data inform the value of most model parameters, with the remaining parameters either informed by substantial prior information or having little sensitivity with respect to dose metric predictions.

I.1.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with the extent and degree of variability of GSH conjugation in humans predicted by the PBPK model.

EPA response: In accordance with SAB recommendations (see response below in Section I.5.2.3), EPA has revised the discussions in Sections 3.3 and 3.5 to reflect the uncertainty in GSH conjugation predictions in humans.

- Some public commenters disagreed with the extent of population variability predicted by the PBPK model for some parameters.

EPA response: The External Review Draft reported posterior distributions as lumped uncertainty and variability. For the parameters raised as a concern in the comments, the high apparent variability is actually predominantly uncertainty, so the extent of population variability is not exceedingly high. In accordance with SAB recommendations (see response above in Section I.1.2.4), EPA has revised the description of posterior parameters to separate uncertainty and variability, providing additional clarity to the posterior predictions.

- Some public commenters recommended that EPA perform a sensitivity analysis on the PBPK model.

EPA response: In accordance with SAB recommendations (see response above in Section I.1.2.5), EPA has conducted a local sensitivity analysis of the PBPK model.

- Some public commenters recommended that EPA incorporate additional data in its PBPK model.

EPA response: In accordance with SAB recommendations (see response below in Section I.5.2.2), EPA incorporated additional data on TCA bioavailability in the TCA submodel of the PBPK model. Other data cited by the commenters were evaluated in Appendix A for the purposes of additional validation, but were not directly incorporated in the PBPK model.

I.2. META-ANALYSES OF CANCER EPIDEMIOLOGY (SAB REPORT SECTION 2): COMMENTS AND EPA RESPONSE

I.2.1. SAB Overall Comments:

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

I.2.2. Major SAB Recommendations and EPA Response:

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

EPA Response: EPA accepts this recommendation and has added text to Section 4.1 and Appendix C.

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

EPA Response: EPA accepts this recommendation and has included a meta-analysis for lung cancer in Appendix C. Additionally, in the discussion in Chapter 4 of the possible role of smoking in confounding the association between TCE exposure and kidney cancer, EPA compares the RR estimates for lung and kidney cancers in five smoking cohorts and discusses the expected contribution by smoking to kidney cancer in Raaschou-Nielsen et al. (2003), which was estimated as 1–6%, far smaller than the 20–40% excess reported in this study. Meta-analyses were not conducted for other cancer types for which there may have been suggestive associations because there was inadequate reporting in the cohort studies, and for childhood leukemia, there were too few studies of sufficient quality.

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

EPA Response: EPA accepts this recommendation and has provided measures of heterogeneity in the primary document. EPA has also corrected this sentence in Section 4.4.2.5; it now reads "there was no observable heterogeneity for any of the meta-analyses of the 15 studies and no indication of publication bias."

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

EPA Response: The papers cited by the SAB describe methods for correcting ORs in studies of common outcomes. Each of the cancer types for which EPA did meta-analyses has a background incidence <10% and is thus considered a rare disease, so no correction should be necessary. In the case of rare diseases, only high ORs might notably overestimate RRs. In the TCE studies, only Hardell et al. (1994) reported an OR high enough to be of potential concern, a Mantel-Haenszel-adjusted OR of 7.2 for NHL. According to Zhang and Yu (1998), the Mantel-Haenszel adjustment is a suitable way to estimate the RR; in fact, in the example they provide, the Mantel-Haenszel adjustment outperforms the adjustment they are proposing. Furthermore, according to McNutt et al. (2003), the Zhang and Yu method is incorrect when applied to an adjusted OR and will produce a biased estimate when confounding is present. Additionally, the model-based methods for estimating a RR from a case-control study described by Greenland

(2004) are only applicable when one has the raw data. Thus, neither of the papers cited by the SAB provides a satisfactory way to convert the Hardell et al. (1994) OR. However, any overestimation that might occur by treating the Hardell et al. (1994) OR as an RR estimate is negligible in the overall analysis. Removing the study all together only decreases the RRm from 1.23 to 1.21, and the latter result is still statistically significant (p = 0.004).

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

EPA Response: EPA accepts this recommendation and has revised the terminology throughout the document.

I.2.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters requested a glossary of epidemiology terms be included in the document.

EPA response: EPA did not implement this recommendation, as definitions of epidemiologic terms can be easily found from authoritative sources on the internet.

- Some public commenters suggested that EPA examine the TCE subregistry for information about the association between TCE and cancer.

EPA response: EPA did not implement this recommendation with respect to cancer, as the ATSDR TCE subregistry provides only limited information on cancer outcomes, as analyses are for total cancers and less informative than for cancer types. EPA did consider, however, observations on neurotoxicity and other noncancer outcomes.

- Some public commenters disagreed with the meta-analysis conclusions from the External Review Draft, noting heterogeneity of findings, lack of consistent exposure-response, and other methodological problems. These comments noted that while EPA's meta-analysis methods and summaries are generally consistent with recent published summaries of this literature, the commenters did not agree with EPA's interpretation. These comments asserted that it is more accurate to report the epidemiologic evidence as "mixed" rather than "consistent" or "robust."

  Other public commenters agreed with the meta-analysis conclusions from the External Review Draft, noting that epidemiologic studies are usually biased towards the null, making it harder to detect a true causal relationship.

EPA response: In accordance with the SAB review, EPA maintains its meta-analysis conclusions. EPA agrees with the public commenters that the characterization of the general association between overall TCE exposure and cancer is "modest"; this was one of the points explicitly brought out in the discussion in Section 4.11.2.1.2 concerning the strength of the association. EPA also carefully considered the questions raised by the public commenters

regarding consistency of the results and regarding alternative explanations for these findings. This consideration is discussed in detail in Section 4.11.2.1. A strength of the meta-analytic approach is its ability to assess heterogeneity among studies, which is of particular importance in situations in which the overall RR estimate is modest and in situations in which results from individual studies may be quite imprecise because of sample size limitations. In reviewing the available data, including the results of the meta-analyses, EPA noted that chance was not supported as an explanation for the findings, nor was there support for confounding by other known or suspected risk factors as an explanation for the results.

I.3. NONCANCER HAZARD ASSESSMENT (SAB REPORT SECTION 3): COMMENTS AND EPA RESPONSE

I.3.1. SAB Overall Comments:

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

I.3.2. Major SAB Recommendations and EPA Response:

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

EPA Response: EPA accepts this recommendation, and has added the information to all studies where such data are available.

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

EPA Response: EPA accepts this recommendation, and has added the information to all studies where such data are available.

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

EPA Response: EPA accepts this recommendation, and has clarified the use of "subpopulations."

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

EPA Response: EPA accepts this recommendation, and has added statements to Sections 4.6 and 4.6.3.1 that immume-related and inflammatory effects, particularly cell-mediated immunity, may influence a broader range of disease, including cancer.

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

EPA Response: See previous response.

I.3.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's draft conclusion that TCE poses a human health hazard for developmental cardiac effects due to limitations in the available data.

EPA response: In accordance with the SAB review, EPA acknowledges the limitations of the available data, but maintains its conclusion that TCE poses a human health hazard for developmental cardiac effects.

- Some public commenters disagreed with EPA's draft conclusion TCE poses a human health hazard for immunotoxicity because additional confirmatory studies are needed.

EPA response: In accordance with the SAB review, EPA concludes that adequate data are available to conclude that TCE poses a human health hazard for immunotoxicity.

I.4. CARCINOGENIC WEIGHT OF EVIDENCE (SAB REPORT SECTION 4): COMMENTS AND EPA RESPONSE

I.4.1. SAB Overall Comments:

The Panel agreed with EPA's conclusion that TCE is "carcinogenic to humans" by all routes of exposure. This is based on convincing evidence of a causal association between TCE exposure and kidney cancer, compelling evidence for lymphoma, and more limited evidence for

liver cancer as presented in the draft document. The epidemiologic data, in the aggregate, were quite strong. The summary risk estimates from the meta-analyses provided a clear indication of a cancer hazard from TCE. In addition, both animal data and toxicokinetic information provide biological plausibility and support the epidemiologic data.

I.4.2. Major SAB Recommendations and EPA Response:

- 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.

EPA Response: EPA accepts this recommendation, and has added a statement to Section 4.11.2.1.6 that immune-related effects should also be considered in the biologic plausibility of TCE carcinogenicity.

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

EPA Response: EPA accepts this recommendation, and has added a statement mentioning the suggestive evidence of association between TCE and other types of cancer in Section 4.11.2.1.10.

- 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 CLL) should be clearly explained in both Appendix C and in the Hazard Characterization document (Section 4.6.1.2.2).

EPA Response: EPA accepts this recommendation and has added text describing the definition of NHL as used in the assessment, in addition to revising the terminology and indicating divergent case definitions in both Appendix C and Section 4.6.1.2.2.

- To assist the reader, please include references in the summary section (Section 4.11.2). For example, "The other 13 high-quality studies [note: besides Hardell and Hansen] reported elevated RR estimates with overall TCE exposure that were not statistically significant." References for statements like this would be helpful. The Panel counted fewer than 13 studies in the meta-analysis after subtracting out Hardell and Hansen, and not all of these showed elevated risk estimates, so it would be helpful for the reader to know which 13 studies this statement refers to.

EPA Response: EPA accepts this recommendation and has added references to conclusions in Section 4.11.2.1.

I.4.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's draft conclusion in the External Review Draft that TCE is "carcinogenic to humans," judging the epidemiologic evidence to be inadequate due to limitations of the body of evidence. Limitations cited by these comments include exposure assessment limitations, potential unmeasured confounding, potential selection biases, and inconsistent findings across groups of studies. Comments also cited limitations in the experimental animal data, such as conflicting or negative experimental animal data for kidney and immune tumors. These comments suggested that a classification of "likely to be carcinogenic in humans" or "suggestive evidence of carcinogenicity" would be more appropriate. Some of these comments cited the NAS/NRC *Contaminated Water Supplies at Camp Lejeune: Assessing Potential Health Effects* (NRC, 2009) as support.

  Other public commenters supported EPA's draft conclusion in the External Review Draft that TCE is "carcinogenic to humans."

EPA response: In accordance with the SAB review, EPA concludes that TCE is "Carcinogenic to humans." EPA also notes that the NRC (2009) Camp Lejeune report was discussed during the SAB review meetings. The meeting minutes from the June 24, 2010 teleconference call state that "Panelists discussed the extent to which the EPA draft risk assessment document should discuss or compare its findings and conclusions to those of the 2009 NAS Report on Camp Lejeune. It was generally agreed that it was not necessary to compare EPA conclusions to all of the other reviews, particularly in view of the different criteria applied across reviews, different studies used across assessments and different scopes of each review and the fact that the current draft risk assessments carries out a meta-analysis that was not considered in the 2009 NAS review" (SAB, 2010a). In the meeting minutes from the December 15, 2010 SAB quality review teleconference call, the Panel chair stated that "the material reviewed by the Panel was different from what the NAS had reviewed" (SAB, 2010a, b).

I.5. ROLE OF METABOLISM (SAB REPORT SECTION 5): COMMENTS AND EPA RESPONSE

I.5.1. SAB Overall Comments:

The Panel agreed with EPA's conclusion that oxidative metabolites of TCE were likely responsible for mediating the liver effects. The Panel recommended that EPA examine studies that provided quantitative assessment of TCA and DCA formation after TCE exposure. Dose-response modeling, similar to that performed for tetrachloroethylene, may be considered by EPA

to provide scientifically-based information on relative contribution, or lack thereof, of TCA and/or DCA to the liver carcinogenesis effect of TCE.

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

I.5.2. Major SAB Recommendations and EPA Response:

I.5.2.1. Mediation of TCE-Induced Liver Effects by Oxidative Metabolism (SAB Report Section 5a)

- No major recommendations in this section.

I.5.2.2. Contribution of TCA to Adverse effects on the Liver (SAB Report Section 5b)

- The EPA should examine studies that provide quantitative assessment of TCA and DCA formation after TCE exposure in vivo and draw conclusions with regards to the relative amount and kinetics of the oxidative metabolites of interest for liver toxicity.

EPA response: Most studies of TCA following TCE exposure have already been incorporated into the PBPK model-based analyses, and previous studies of DCA following TCE exposure are limited by the rapid clearance of DCA at low concentrations and analytical artifacts in DCA detection. Section 4.5.6.1 has been revised to include discussion of the studies by Delinksy et al. (2005) and Kim et al. (2009), which use more reliable analytic methods to quantify DCA formation after TCE exposure *in vivo*.

- A careful evaluation of the concentration-time kinetics is needed to achieve certainty in the comparisons of liver effects and the conclusions drawn by the EPA, which suggest that TCA-induced adverse liver effects do not explain those observed with TCE. Equally important is to fully consider the bioavailability of TCE itself with regards to the vehicle effects between studies.

EPA response: EPA assumes that the first part of this comment refers to the issue of TCA bioavailability, which is mentioned in the narrative text preceding this recommendations. EPA has incorporated into Section 4.5.6.2.1 an updated analysis of TCA bioavailability and its impact on conclusions regarding the role of TCA in TCE-induced hepatomegaly (Chiu, 2011). With respect to TCE vehicle effects, there are not enough kinetic data using different vehicles to quantitatively address vehicle effects. However, it is noted that if they are important, they may

be a significant contributor to the residual variability in the combined analysis of TCE-induced hepatomegaly.

- The body of the document could be further strengthened by reporting EPA's evaluation on the strength of the specific criteria used for phenotypic classification described in each study discussed, and noting where specific criteria were not reported. While most of this information was included in the appendix, the EPA may consider constructing a summary table for Section 4.5.6.

EPA response: Section 4.5.6.3.3.1 has been revised to note that no specific criteria are usually given as to what constitutes "basophilic" or "eosinophilic," with the one exception of Carter et al. (2003) noted. For immunochemical staining, it is noted that some studies use negative controls as a comparison.

- Dose-response modeling, similar to that performed for PERC, may be considered by the EPA to provide science-based information on relative contribution, or lack thereof, of TCA and/or DCA to the apical liver carcinogenesis effect of TCE. While data gaps exist and there are limitations in the comparisons between independent cancer bioassays, the document should clearly state what the limitations are should such analysis be deemed futile.

EPA response: EPA agrees that a quantitative analysis of the relative contributions of TCA and/or DCA to TCE-induced liver carcinogenesis would be useful if feasible. However, as noted in the revised Section 4.5.6.3.2.5, such an analysis is precluded by the high degree of heterogeneity both within and across the databases for TCE and its oxidative metabolites. The revised section gives notes substantial variability across bioassays in characteristics such as study duration, control group incidences, and apparent carcinogenic potency, thus precluding either quantitative analysis.

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

EPA response: Human data on use of DCA in clinical practice is summarized in EPA's Toxicological Review of Dichloroacetic Acid (2003b), and reference has been made to this document in Section 4.5.6. In particular, it is noted that data on DCA in humans are scarce and complicated by the fact that available studies have predominantly focused on individuals who have a pre-existing (usually severe) disease.

I.5.2.3. Role of GSH-Conjugation Pathway on TCE-Induced Kidney Effects (SAB Report Section 5c)

- The issue of quantitative assessment of the metabolic flux of TCE through the GSH pathway vs. the oxidative metabolism pathway should be considered carefully since uncertainties exist with regard to the extent of formation of the dichlorovinyl metabolites

of TCE between humans and rodents. EPA may need to provide appropriate reservations to the conclusions based on the limited data for GSH metabolites.

- The discussion of how each of the in vitro and in vivo data sets were used to estimate DCVG formation parameters for the PBPK model should be more transparent indicating strengths and weaknesses in the database.

EPA responses: EPA accepts these two related recommendations. EPA has revised Section 3.3.3.2 to articulate additional reservations as to its conclusions regarding the except of formation of dichlorovinyl metabolites of TCE between rodents and humans, and to be more transparent regarding the strengths and weaknesses in vitro and in vivo datasets. In addition, cross-references to this discussion have been added in the context of the PBPK model parameters and predictions to Sections 3.5.4.3, 3.5.5.1, 3.5.6.3.3, 3.5.7.3.1, 3.5.7.3.2, 3.5.7.4, and 3.5.7.5.

I.5.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's conclusion that DCA may play a role in TCE-induced liver effects. These commenters also recommended that EPA take into account the bioavailability of TCA in its evaluation of liver effects.

EPA response: In accordance with SAB recommendations, EPA has re-examined the data on the contributions of TCA and/or DCA to TCE-induced liver effects, including incorporation of data on TCA bioavailability, in Section 4.4. However, EPA's conclusion remains that TCA cannot adequately account for account the liver effects of TCE.

- Some public commenters disagreed with EPA's conclusion that GSH-conjugation-derived metabolites play the primary role in TCE-induced nephrotoxicity and nephrocarcinogenicity.

EPA response: EPA maintains its conclusions, and notes that both the SAB review and the NRC (2006) report support the conclusion that the GSH pathway is primarily responsible for TCE-induced kidney effects.

I.6. MODE OF ACTION (SAB REPORT SECTION 6): COMMENTS AND EPA RESPONSE

I.6.1. SAB Overall Comments:

The Panel agreed that the weight of evidence supports a mutagenic mode of action for TCE-induced kidney tumors. However, the Panel concluded that the weight of evidence also supported an mode of action involving cytotoxicity and compensatory cell proliferation and including these may more accurately reflect kidney tumor formation than does a mutagenic mechanism alone. The combination of cytotoxicity, proliferation and DNA damage together may be a much stronger mode of action than any individual components.

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

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

I.6.2. Major SAB Recommendations and EPA Response:

I.6.2.1. Hazard Assessment and Mode of Action (SAB Report Section 6a)

- The impact of the inconsistencies in data on the quantity of GSH pathway metabolites formed in humans and rodents should be presented more transparently.

EPA Response: EPA accepts this recommendation, and has added discussion and/or mention of the quantitative uncertainties with respect to GSH conjugation wherever relevant throughout the document.

- In the body of the document, mode-of-action information should be systematized and broken down into key events for each proposed mode of action. The EPA may consider using a tabular format to facilitate the ease of evaluation. Information on supporting/refuting (if any) evidence (with appropriate references indicated), human relevance (if available), and "strength" of each line of evidence/study should be included.

EPA Response: EPA accepts this recommendation, and has added tables summarizing the proposed modes of action and conclusions for kidney and liver carcinogenesis.

- EPA should consider tabular summaries by specific metabolites when studies used metabolite exposure rather than the parent compound.

EPA Response: EPA considered this recommendation, but decided against adding the tables for the metabolites because in most cases (TCA, DCA, and CH), those studies are described and tabulated in detail in other toxicological reviews.

- Data gaps should be clearly identified to help guide future research.

EPA Response: EPA considered this recommendation, and decided to focus on data gaps related to dose-response, as these will have the greatest impact on any future revision to the Toxicological Review. These research needs are now included as a separate section at the end of Chapter 5.

- 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 Response: EPA accepts this recommendation, and has included key conclusions in the summary mode-of-action tables described above for kidney and liver carcinogenesis.

I.6.2.2. Mode of Action for TCE-Induced Kidney Tumors (SAB Report Section 6b)

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

EPA Response: EPA accepts this recommendation and has included additional discussion along the lines suggested to the section on kidney tumor mode of action.

I.6.2.3. Inadequate Support for PPARα Agonism and its Sequellae Being Key Events in TCE-Induced Liver Carcinogenesis (SAB Report Section 6c)

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

EPA Response: EPA accepts these recommendations, and has added the tabular presentation of quantitative differences among PPARα agonists and the quantitative analyses comparing carcinogenic potential and the receptor transactivation potency or other short-term markers of PPARα activation from Guyton et al. (2009) to strengthen the comparative analysis and arguments.

I.6.2.4. Inadequate Data to specify Key Events and Modes of Action Involved in Other TCE-Induced Cancer and Noncancer Effects (SAB Report Section 6d)

- No major recommendations in this section.

I.6.2.5. Human Relevance of TCE-Induced Cancer and Noncancer Effects in Rodents (SAB Report Section 6e)

- The impact of potential overestimation of the extent of the GSH pathway in humans in Section 4.4.7 (Kidney) must be transparent

EPA Response: EPA accepts this recommendation, and has added discussion and/or mention of the quantitative uncertainties with respect to GSH conjugation wherever relevant throughout the document.

- The mode of action for carcinogenicity should be described as complex rather than unknown in Section 4.5.7.4. Mode of Action. With respect to conclusions regarding the liver, while the complete mode of action 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, subchronic, and chronic liver toxicity of TCE.

EPA Response: EPA accepts this recommendation, and has rephrased the liver mode of action conclusions in Section 4.5.7.4 along the lines suggested.

I.6.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with EPA's conclusion that a mutagenic mode of action is operative for TCE-induced kidney tumors, recommending instead that a mode of action involving cytotoxicity is involved.

EPA response: EPA maintains its conclusion, in accordance with the SAB review (see Section I.6.1, above), that a mutagenic mode of action is operative for TCE-induced kidney tumors. However, in accordance with the SAB recommendations (see Section I.6.2.2, above) and in partial response to this public comment, EPA has added additional discussion of the data supporting a mode of action involving cytotoxicity.

- Some public commenters disagreed with EPA's conclusion that there is inadequate support for PPARα agonism and its sequellae being key events in TCE-induced hepatocarcinogenesis. Other public commenters agreed with EPA's conclusions.

EPA response: In accordance with the SAB recommendations (see Section I.6.2.3, above), EPA has provided additional analysis to support its conclusions.

- Some public commenters disagreed with EPA's conclusion that a cytotoxic mode of action was inadequately supported for TCE-induced lung tumors, citing analogies to other chemicals and other indirect data.

EPA response: EPA has added discussion of data from other compounds hypothesized to have the same mode of action for inducing mouse lung tumors. However, in accordance with the SAB review, EPA still concludes that there are inadequate data to specify the key events and modes of action involved in TCE-induced lung cancer and noncancer effects.

I.7. SUSCEPTIBLE POPULATIONS (SAB REPORT SECTION 7): COMMENTS AND EPA RESPONSE

I.7.1. SAB Overall Comment:

The Panel found that EPA's hazard assessment provided a good review of potentially susceptible populations, and identified factors (genetics, lifestage, background, co-exposures, and pre-existing conditions) that may modulate susceptibility to TCE carcinogenicity and noncancer effects. However, the Panel disagreed with EPA's conclusion that toxicokinetic

variability can be adequately quantified using existing data. The Panel recommended that exposure to solvent mixtures should be considered for potential co-exposures, since exposure to more than one chemical with the same target organ likely increases risk.

I.7.2. Major SAB Recommendations and EPA Response:

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

EPA response: EPA accepts this recommendation and has added a statement in Section 4.10.3 noting the limitations of the toxicokinetic database.

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

EPA response: A statement has been added to the introduction of Section 4.10 noting the lack of data on susceptible populations and the need for such data. A statement on the need for additional data to address uncertainties regarding susceptible populations has been added to Section 4.10.3. The title of Section 4.10.3 has been amended to now read "Uncertainty of Database and Research Needs for Susceptible Populations."

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

EPA response: Where appropriate, statements on the need for additional research to fill data gaps regarding susceptible populations have been added where appropriate throughout Section 4.10.

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

EPA response: The need for future research on the relationship between genetic variation and generalized hypersensitivity skin diseases is now highlighted in Section 4.10.3.

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

EPA response: Additional clarification was added throughout Section 4.10 where necessary to address when the results were unrelated to TCE exposure or related to TCE exposure. Additional information was also added regarding the comparison group.

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

EPA response: A new Section 4.10.2.6 has been added on mixtures. This text is broader than solvent mixtures, as there are available studies that address exposure to TCE together with non-solvents.

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

EPA response: As recommended, Section 4.10.2.4.1 has been retitled as "Obesity," and the text has been amended to more clearly present the data on toxicokinetics of TCE as it relates to obesity.

I.7.3. Summary of Major Public Comments and EPA Responses:

- Some comments noted that there is widespread exposure to TCE, including potentially vulnerable subpopulations.

EPA response: No response needed.

- Some comments questioned why EPA was not basing its assessment on in utero exposures.

EPA response: For noncancer effects, studies with in utero exposures were considered and, in one case used, for the basis of the RfC or RfD. No data on in utero exposures and cancer effects were located that were adequate for dose-response analysis.

I.8. NONCANCER DOSE-RESPONSE ASSESSMENT (SAB REPORT SECTION 8): COMMENTS AND EPA RESPONSE

I.8.1. SAB Overall Comments

I.8.1.1. Selection of Critical Studies and Effects

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

The Panel recommended that the two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) be considered the principal studies supporting the RfC. The Panel also recommended that the endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2008) and the cardiac malformations from Johnson et al. (2003) be considered as the principal studies supporting the RfD.

I.8.1.2. Derivation of RfD and RfC

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

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

I.8.1.3. UFs

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

I.8.2. Major SAB Recommendations and EPA Response:

I.8.2.1. The Screening, Evaluation, and Selection of Candidate Critical Studies and Effects (SAB Report Section 8a)

- Chapter 5 should include a list of all noncancer health effects and studies discussed in Chapter 4, noting those that were considered candidate critical effects and studies.

EPA Response: EPA considered this recommendation and concluded that a list of *all* of the noncancer health effects and studies discussed in Chapter 4 would be overly long and redundant. As an alternative, first, EPA has ensured that each section of Chapter 4 includes tables of the relevant noncancer health effects and studies discussed, with studies and effects in **bold** designating those considered in Chapter 5. Second, EPA has added to Chapter 5 tables with the experimental details (e.g., which species, doses, effects) of the candidate studies for each endpoint type, with cross-references back to the tables in Chapter 4 that contain all of the studies for each type of effect. Therefore, there is now a transparent trace-back from the PODs used in Chapter 5 (tables in the external review draft), to the experimental details from which the POD

was derived (new tables in Chapter 5), to the larger set of studies considered for each effect type (tables in Chapter 4).

- Tables 5-1–5-5 should provide cross-references to the table or page in Chapter 4 and/or to the Appendices (such as Appendix E for hepatic studies) where the listed study was discussed, and should include more details (e.g., gender, strain, duration) of the studies selected as the basis for cRfDs and cRfCs when these details were needed to prevent ambiguity.

EPA Response: EPA accepts this recommendation and has addressed it as part of its response to the previous recommendation for a table in Chapter 5 listing all of the studies.

- Consistent dose units should be used in discussing the same study in different places in the document.

EPA Response: EPA accepts this recommendation and has checked the dose units used as it developed the new tables for Chapter 5.

I.8.2.2. The PODs, Including those Derived from BMD Modeling (e.g., Selection of Dose-Response Models, BMR Levels) (SAB Report Section 8b)

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

EPA Response: After reviewing Chapter 5, EPA did not implement this suggestion. Chapter 5 describes the modeling approaches and selection criteria and important decisions in sufficient detail, and on page 5-3, the reader is directed to Appendix F for further details. The succeeding pages of Chapter 5 describe studies and effects by effect domain quite extensively, and the tables and footnotes contain sufficient detail on BMRs, PODs, and reasons for study selection. We think that it is appropriate to provide the mass of numerical modeling details in Appendix F, and that the modeling decisions are well described therein. Integrating this material into Chapter 5 would greatly increase the length of Chapter 5 and make it unwieldy for the reader.

I.8.2.3. 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 (SAB Report Section 8c)

- The uncertainty about the rate of human GSH conjugation found in Lash et al. (1999a) vs. Green et al. (1997a) should be highlighted in the current assessment.

EPA Response: EPA accepts this recommendation and has added discussion and/or mention of the quantitative uncertainties with respect to GSH conjugation wherever relevant throughout the document.

- 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.

EPA Response: EPA accepts this recommendation and has revised Section 3.5.7.3.1 to more clearly discuss the basis of the renal bioactivation dose-metric. In other sections of the document where the dose-metric is discussed, reference is made to Section 3.5.7.3.1.

- The rationale for scaling the dose-metric to body weight$^{3/4}$, in conjunction with the interspecies extrapolation based on PBPK modeling, should be presented in a clearer and more transparent way (e.g., on pp. 5-33–5-36).

EPA Response: EPA accepts this recommendation and has revised the discussion of this rationale substantially.

- The discussion of "empirical dosimetry" vs. "concentration equivalence dosimetry" should be made clearer and more transparent (pp. 5-33–5-36).

EPA Response: As noted by the SAB in the narrative preceding this recommendation, it is not necessary to include an extensive discussion of the two dosimetry approaches in these sections. EPA accepts this recommendation and has replaced this discussion with a clearer and more transparent rationale for the body weight$^{3/4}$ scaling.

I.8.2.4. UFs (SAB Report Section 8d)

- The definitions of chronic and subchronic studies should be provided in the document and a citation given.

EPA Response: EPA accepts this recommendation and has added a footnote with this information on page 5-6 in the paragraph describing UFs for subchronic-to-chronic extrapolation.

- The discussion of the subchronic to chronic UF on p. 5-6 should be clarified as far as durations of studies considered suitable as the basis of a chronic risk assessment.

EPA Response: There is no hard and fast rule in this area. Longer studies are generally preferred as the basis for a chronic risk assessment; however, in any given case, the basis of an RfC or RfD, or whether one is derived at all, will depend on the studies available and an assessment of their relevance for extrapolation to longer durations.

- The draft document should include discussion of whether studies in the lower end of the range defined as subchronic (e.g., 4 weeks) are of sufficient duration to be used as the basis for a chronic (lifetime) risk assessment.

EPA Response: EPA notes that studies of this duration have been evaluated for other risk assessments. For any study and endpoint that is used as a basis for a POD in this and previous

assessments, EPA has explained its applicability in the light of alternative studies of the same endpoint having longer and shorter duration and alternative studies and endpoints within the same domain having various durations.

- Studies only slightly longer than the minimum needed to be considered chronic should be noted as such, and the use of an UF to account for less than lifetime exposure (of less than the full UF of 10) could be considered for studies of such durations, especially for endpoints thought to progress in incidence or severity with time.

EPA Response: If there is evidence suggesting there might be further progression with increased exposure duration, a subchronic-to-chronic UF of 3 might be considered for a nominally chronic study. The example given by the panel could merit special justification of an UF of 3 if there were evidence that the response continued to increase with exposure durations longer than 18 weeks. No such evidence was found. For the study of Kulig et al. (1987), severity didn't progress beyond week 9 for the two-choice response, and in the 1,000 ppm exposure group, it didn't progress much in those first 9 weeks; thus, it is not anticipated that the 500 ppm response, which was flat over the 18 weeks, would become significant over an extended duration of exposure.

I.8.2.5. The Equivalent Doses and Concentrations for Sensitive Humans Developed from PBPK Modeling to Replace Standard Ufs for Inter- and Intra-Species Toxicokinetics, Including Selection of the 99th Percentile for Overall Uncertainty and Variability to Represent the Toxicokinetically-Sensitive Individual (SAB Report Section 8e)

- The Panel noted variability/uncertainty for the toxicokinetically-sensitive individual could be quantified in future work by considering distributions in addition to the distribution of the 99th percentile, such as the 95th percentile.

EPA Response: EPA agrees that this could be the subject of future work.

- A quantile regression looking simultaneously at several quantiles could be developed in the future and presented in future refinements of this assessment.

EPA Response: EPA agrees that this could be developed in the future and presented in future refinements of this assessment.

I.8.2.6. The Qualitative and Quantitative Characterization of Uncertainty and Variability (SAB Report Section 8f)

- The quantitative uncertainty analysis of PBPK model-based dose-metrics for LOAEL or NOAEL based PODs (Section 5.1.4.2) should be revised to clarify the objective of this 2-D type analysis, as well as the methodology used.

EPA Response: EPA accepts this recommendation and has revised the discussion, clarifying its objective and methodology.

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

EPA Response: EPA agrees that such an approach could be developed in future work.

I.8.2.7. 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. (2008) [Developmental Immunotoxicity], and Johnson et al. (2003) [Fetal Heart Malformations] as the Critical Studies and Effects for Noncancer Dose-Response Assessment (SAB Report Section 8g)

EPA Response: See recommendation in Section I.8.2.8, below.

I.8.2.8. 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 (SAB Report Section 8h)

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

EPA Response: EPA accepts this recommendation and has revised Chapter 5 accordingly.

- The endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2008) and the cardiac malformations from Johnson et al. (2003) should be considered as the principal studies supporting the RfD.

EPA Response: EPA accepts this recommendation and has revised Chapter 5 accordingly.

I.8.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters disagreed with the choices of critical studies for dose-response analyses of noncancer endpoints.

EPA response: In accordance with SAB recommendations (see Section I.8.2.8), EPA has selected the immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) as the principal studies supporting the RfC, and the immune effects from Keil et al. (2009) and Peden-Adams et al. (2008) and the cardiac malformations from Johnson et al. (2003) as the principal studies supporting the RfD.

- Some public commenters recommended that EPA not rely on PBPK model-based estimates of DCVC bioactivation in conducting dose-response analysis for kidney endpoints.

EPA response: In accordance with SAB recommendations (see Section I.8.2.3), EPA has noted the uncertainties in the PBPK model-based DCVC bioactivation dose-metrics and considers the kidney effects as supporting, rather than as principal bases for, the RfC and RfD.

- Some public commenters recommended that EPA provide a more concise and consolidated characterization of the RfC and RfD determination, particularly in the context of kidney effects.

EPA response: A concise and consolidated characterization of the RfC and RfD determination appears in Sections 5.1.5.2 and 5.1.5.3. EPA has added discussion of the uncertainties related in kidney effects to these summary characterizations.

- Some public commenters recommended that EPA provide more discussion of the proportionality between applied and internal dose and its impact on the quantitative analysis.

EPA response: The impact of the proportionality of applied and internal dose, as well as its impact both dose-response analysis, is discussed in Section 5.1.3.3 and shown graphically in Appendix F.

- Some public commenters viewed the use of PBPK modeling as "double counting" variability, based on the idea that the observed dose-response is in part due to pharmacokinetic variability.

EPA response: In accordance with the SAB review, the methodology that EPA used is consistent with existing practice in the derivation of RfDs and RfCs. The methodology used is also consistent with previous applications of PBPK modeling in noncancer risk assessment. The comments highlight some uncertainties and ambiguities inherent in the RfD/RfC methodology, but disaggregating the multiple contributions to dose-response assessment—including effects of TK variation, TD variation, experimental error, stochasticity, and other factors in both the experimental animal and human population—requires development of new approaches that are beyond the scope of the assessment. While some published literature have addressed some of these issues, further research and development are needed, as no alternative approach has been generally accepted at the current time. EPA agrees with the SAB (see Section I.8.2.6, above) that a future research need is the development of an approach using distributions to characterize uncertainty in a Bayesian framework. Such an approach, when developed, could also help address the commenters' concerns.

I.9. CANCER DOSE-RESPONSE ASSESSMENT (INHALATION UNIT RISK AND ORAL UNIT RISK) (SAB REPORT SECTION 9): COMMENTS AND EPA RESPONSE

I.9.1. SAB Overall Comment:

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

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

I.9.2. Major SAB Recommendations and EPA Response:

I.9.2.1. Estimation of Unit Risks for RCC (SAB Report Section 9a)

- The Panel believed that more discussion was needed on whether or not it is necessary to adjust for exposure to cutting oils when computing an OR or RR relating TCE exposure to kidney cancer. The Panel recommended that EPA take a closer look at the literature to determine if there are other studies that suggest that exposure to cutting oils is a risk factor for kidney cancer.

EPA Response: EPA accepts this recommendation and has discussed other studies examining cutting fluids (Section 4.4.2.3). These studies suggest that potential confounding by cutting fluids is of minimal concern, and thus, including these exposures in the logistic regression may over-adjust because of the correlation with TCE exposure. Nonetheless, EPA has included, as a sensitivity analysis, the derivation of a unit risk estimate based on the Charbotel et al. (2006)

RCC ORs further adjusted for cutting fluids and petroleum oils, and this estimate is essentially the same as the original estimate (Section 5.2.2.1.3).

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

EPA Response: EPA accepts the recommendation and has added text pertaining to these assumptions. Note, too, that the uncertainties in the unit risk estimate, including uncertainties about the exposure assessment, are discussed in some detail in the uncertainty section (Section 5.2.2.1.3).

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

EPA Response: EPA accepts this recommendation, and has added additional information to Section 5.2.2.1.2. In particular, this section now notes that the usual assumption is that RR transfers across populations independent of background rates. In addition, this section now contains information comparing background kidney cancer rates in France and the United States.

I.9.2.2. Adjustment of RCC Unit Risks (SAB Report Section 9b)

- No major recommendations in this section.

I.9.2.3. Estimation of Human Unit Risks from Rodent Bioassays (SAB Report Section 9c)

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

EPA Response: EPA accepts this recommendation and has added a paragraph discussing the potential biases of this approach, along with citations to relevant literature, to Section G.1.1.

- In addition, more information was needed on the priors used in their Bayesian analysis of combined risk across tumor types.

EPA Response: EPA accepts this recommendation and has added this information to Section G.8.1.2.

I.9.2.4. Use of Linear Extrapolation for Cancer Dose-Response Assessment (SAB Report Section 9d)

- No major recommendations in this section.

I.9.2.5. Application of PBPK Modeling (SAB Report Section 9e)

- No major recommendations in this section.

I.9.2.6. Qualitative and Quantitative Characterization of Uncertainty and Variability (SAB Report Section 9f)

- No major recommendations in this section.

I.9.2.7. Conclusion on the Consistency of Unit Risk Estimates Based on Human Epidemiologic Data and Rodent Bioassay Data (SAB Report Section 9g)

- No major recommendations in this section.

I.9.2.8. Preference for the Unit Risk Estimates based on Human Epidemiologic Data (SAB Report Section 9h)

- No major recommendations in this section.

I.9.3. Summary of Major Public Comments and EPA Responses:

- Some public commenters stated that the time courses of kidney cancer, liver and biliary cancer, and NHL do not support the hypothesis that TCE poses a great risk of cancer in the human population. These comments recommended that EPA perform a "validation" exercise to determine if the draft cancer classification and quantitative risk estimates are consistent with the observable facts concerning human cancer rates and other known risk factors for the tumor types listed.

EPA response: The analysis suggested by this comment is beyond the scope of the Toxicological Review. Moreover, such an analysis would require data that do not currently exist, including detailed historical population estimates not only of TCE exposure, but also of all other exposures and risk factors associated with each cancer, as well as quantitative estimates as to how each risk factor modulates the risk of cancer. It is noted, however, that limited

"validation" was performed by comparing qualitative and quantitative inferences based on epidemiologic data to those based on animal bioassay data. Further quantitative "validation" may be possible in the future if epidemiologic studies with quantitative exposure information are conducted.

- Some public commenters disagreed with the use of epidemiologic data as the primary basis for the cancer dose-response analysis.

EPA response: EPA maintains its conclusion in accordance with the SAB review (see Section I.9.1, above), that the epidemiological data are appropriate to use for estimating cancer risks. In response to recommendations by the SAB, EPA has provided more detailed discussions as to the limitations of the analysis.

- Some public commenters disagreed with the use of linear low-dose extrapolation for estimating cancer risks at levels below the POD, recommending instead the use of nonlinear extrapolation.

EPA response: EPA maintains its conclusion in accordance with the SAB review (see Section I.9.1, above), that the linear low-dose extrapolation is appropriate to use given the available data.

I.10. ADAFs (SAB REPORT SECTION 10): COMMENTS AND EPA RESPONSE

I.10.1. SAB Overall Comment:

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

I.10.2. Major SAB Recommendations and EPA Response:

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

EPA Response: EPA accepts these recommendations. Section 5.2.3.3 notes that due to lack of appropriate data, no ADAFs are used for other life-stages, such as the elderly. ADAF-adjusted unit risks per ppm and per mg/kg-day are now presented in each sample calculation table in Sections 5.2.3.3.1 and 5.2.3.3.2.

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

EPA Response: EPA accepts this recommendation and has revised Sections 5.2.3.3.1 and 5.2.3.3.2 to include all of the details for each sample calculation.

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

EPA Response: EPA accepts this recommendation and has added drinking water concentrations for specified lifetime cancer risks under the assumptions used in the drinking water example calculation to Section 5.1.3.3.2. Similarly, EPA has added air concentrations for specified lifetime cancer risks under the assumptions used in the inhalation example calculation to Section 5.1.3.3.1.

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

EPA Response: EPA accepts this recommendation and has added a discussion addressing the use of the ADAFs and the assumptions underlying the life table analysis.

I.10.3. Summary of Major Public Comments and EPA Responses:

- None

I.11. ADDITIONAL KEY STUDIES (SAB REPORT SECTION 11) AND EDITORIAL COMMENTS: COMMENTS AND EPA RESPONSE

- The Panel has identified additional studies to be considered in the assessment, as well as a number of editorial comments.

EPA Response: EPA has incorporated the additional studies in the appropriate sections, and addressed the editorial comments.