

U.S. EPA
Science Advisory Board
TCE Review Panel

Washington, D.C.

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Charge Question # 1

**Dr S. Bartell, Dr C. Emond,
Dr. M. Fuentes, Dr .G. Johanson,
Dr. M. Pennell, Dr K Portier**

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Question changes was:

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*].

The PBPK model structure

- PBPK model can strongly predict the internal dose in the target tissue. **EPA has made a significant progress in this direction.** Using PBPK model can clearly improve the quality of the prediction for risk assessment and then reduce the uncertainty compare to 2000 model.
- PBPK model well presented (figure 3-7)
- The figure is not helpful in understanding the model structure but is very useful to see the changes made the Hack model.
- The details provided in section A4 fully explain the model used.
- **Need better description of final model**, diagram that places the parameters into the states and pathways of the figure.
- it would be nice to **follow also their strategy and biological relevant on what they based their equations** for the TCE model.
- **Variability between animal individuals ignored**
- **Variability within humans (over time) ignored**
- Did not allow for the pharmacokinetic parameters **to change over time.**

Bayesian statistical approach

- Applaud the Bayesian framework for estimation and characterization of model and parameters uncertainty.
- Bayesian approach is fine, text description is good, **More transparency in the Bayesian analysis is needed**. Explaining role of different parameters.
- **List model parameters in reverse order** by the width of their posterior variability (width of the IQR or width of 95% CI). Allows reviewer to know which parameters are least specified.
- **Unclear how to interpret the residual error** (Table 3-41), especially since graph presentations are lacking. E.g. residual error is GSD 2.7 for venous blood TCE. Sounds very high, says what?
- **Structural unclarity: many parameters vary widely**, yet target dose varies less. **Degree of correlation not clear to me**, nor if/how rodent parameter correlations were transferred to the human model.
- Many wide and uniform priors makes MCMC chains long and posteriors wider. **In the future try to find exp data supporting priors**.
- **Sensitivity analyses**, sens of
 - “simple” PBPK model to parameter values (especially GSH-related)
 - Bayesian model to different assumptions about prior

Bayesian statistical approach

- I generally liked the idea of using the posteriors of mice to establish the rat priors and the rat posteriors to set the human priors. However, this type of hierarchical relationship between the models is making an important assumption about the relationship between the PBPK model parameters, between the different species which should be used consistently throughout their models, not just in the case where there is limited prior information about a particular species.
- I felt the methodology was treated as a black box. More information on the likelihood and ode's would have been useful.
- Some of the posteriors were flatter than the priors.
 - This is an unexpected result in a Bayesian analysis though it could happen if the prior was poorly chosen.
 - In evaluating the quality of a prior, the authors focused on agreement of the interquartile regions.
 - These situations require a more detailed treatment
- Prior and posterior distribution of model parameters were compared (section 3.5.6.2) and only in a few cases were the distributions were different.

Parameter calibration

- The analysis seemed to indicate that everything fit as expected, although in most cases the Post distributions were narrower than the Prior distributions,
- **To complete documentation:** Rank parameters based on percent change from prior.
- What we see (figures 3-9, 3-10, A-3 and A-4) suggests that the updated model fit quite well.
- Table 3-45 gives a detailed description of how well the model fit for the individual in vivo studies with discussion of why or why didn't the model fit well for those data.
- Problem is overwhelming amount of information, difficult to identify the key issues
- Especially problematic (decreases transparency) is that several key aspects are not presented or not clearly presented. This includes missing
- Use of some rat study data only for model validation and not used in the estimation of model parameters helped to provide confidence in the model as well as point out areas where the model may still be inadequate.

Model predictions of the available in vivo data

- **Sequential analysis** mouse-rat-man is good
- **Good with summary tables, e.g. Table 3-42.** Summary comparison of updated PBPK model predictions and in vivo data in mice
- **Not sure how interest in chronic exposure would warrant ignoring variability** over time. If anything, you would want to model the most accurate human experience over an extended period.
- **Need to account for variability over time supported** by paragraph 2 on 3-108: Chui et al. (2007) found that there was variability in urinary excretion from same individual exposed to the same concentration on different occasions.
- Also supported by Table 3-45: there was occasion in which a female was exposed to both 50 and 100 ppm. Assuming the same subject-specific estimates across the two occasions resulted in over prediction at the higher exposure (? Seems like it should be underpredicted).
- **I understand that the models are complex with a lot of parameters.** However, it would be good if they worked out the results more explicitly for a couple of the more important model parameters

Characterization of PBPK model dose metric predictions, including those for the GSH conjugation pathway

- Mass balance equations; Scaling equations
- Graphical comparisons (Obs and Predic conc-time profiles (there are a few))
- Sensitivity analyses, sens of
 - “simple” pbpk model to parameter values (especially GSH-related)
 - Bayesian model to different assumptions about prior
- Description of how they characterization of uncertainty and variability was a bit confusing mainly due to inconsistent use of the terms “population” and “group.”

Recommendations

Panel of CQ1 recognize the huge effort deployed by NCEA to generate this PBPK modeling part of this report with clarity and transparency. These recommendations should help NCEA to improve their document for the final version.

- Need better description of final model and improve PBPK equation description according to the system biology.
- More transparency in the Bayesian analysis is needed
- Provide sensitivity analyses for each parameter.
- Need to account for variability over time supported.

Meta-analysis of Cancer Epidemiology

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].

Panel Response Summary



- The 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] followed the NRC recommendations for conducting a Meta-analysis. Their approach was clearly and transparently described and technically and scientifically adequate for supporting EPA's hazard characterization and dose-response assessment.
- Consistent approach to the meta analysis, performed appropriate literature review and developed clear and appropriate criteria for selection of studies to be included in the meta-analysis.
- Studies included in the meta-analysis were required to have individual TCE exposure estimates in the study.

Panel Response Summary



- **Strong discussion on potential confounding. Lack of effect of TCE for lung cancer, fairly well convincing that confounding by smoking is unlikely.**
- **Age, gender and race confounders were appropriate for the analysis and the meta analysis included effect estimates that were adjusted.**
- **The report characterized strengths and weaknesses of meta-analysis, clear and appropriate about which studies that remained in the meta-analysis. Studies excluded were justified and listed.**

Panel Response Summary



- Discussion on misclassification of disease and exposure, hard to do well, better on outcome end, weak on exposure, clear of what was done and clear on results of bias.
- Analysis performed on three cancers, why these three were picked not clear, history, would have wanted to see other cancers, e.g. Smoking confounding, no studies to have access of lung cancer – meta analysis of lung cancer would make finding clear, drive home point.
- Pleased with use of random effects models and appropriate testing for heterogeneity, sensitivity and publication bias.

Panel Response Summary



- Helpful to see the detailed process of going through the literature, review of all literature relevant to various cancer sites, documenting the rationale for selection of studies for the meta-analysis. Selection criteria were described and justified. Included both incidence and mortality.
- Conservative in the meta-analysis, what used or not used. Effect sizes for the meta-analysis were appropriately conservative.
- Findings of several community studies were very compelling, but choice was to leave these out, huge misclassification errors, lack of control for confounding, but effects were there, decision to keep those out was a good choice,
- EPA appropriately discussed the changing grouping of hematopoietic and lymphatic system tumors and selected lymphoma as an outcome for meta-analysis.

Panel Response Summary



- EPA specifically wanted to get at studies with the best outcome definitions. Rather than pick at studies where the cancers were grouped.
- The panel agrees that the conclusions were appropriate in the meta-analysis for the three cancers that TCE exposure increases risk. Our assessment of 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 cancer sites selected for the meta-analysis. Could be nicely summarized in a table.
- Consider including meta-analysis for lung cancer or other sites for comparison for which some association with TCE exposure has been reported in epidemiologic studies.
- Provide measures of heterogeneity for each meta-analysis such as Q score.
- Use method of Greenland to convert odds ratios to relative risks for consideration of inclusion in the meta-analysis

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:

McMillan & Others - The EPA draft IRIS document in general has provided an accurate, clear and objective assessment of the hazard TCE poses for non-cancer toxicity in humans.

the central nervous system [Section 4.s3]

Dietert - The possibility that some adverse outcomes (considered in the nervous system and developmental sections) may be linked with autoimmune conditions and/or inflammatory dysfunction should be considered as in the case of both sensory problems (e.g., auditory and ocular impairment) as well as sleep problems.

If useful, references describing the comorbid occurrence of these problems in conjunction with immune dysfunction-based disease are: Dietert and Zelikoff, *Curr. Pediatr. Med.* 5(1):36-51, 2009; *World J. Pediatr* 6(2):111-118. 2010.

the kidney [Section 4.4]

McMillan - In regard to the effects of TCE in the kidney, EPA has (again) provided a thorough but clear description of these effects. In particular, the role of GSH-derived metabolites of TCE in mediating cytotoxic effects in the kidney is well described. One issue of concern here is the quantitative aspects of these effects. For example, the question regarding whether or not sufficient DCVC is formed from TCE to

Keil Response: Add 18% increase in kidney weight of male mice only in Peden-Adams et al 2008 in MRL+/- study.

the kidney [Section 4.4]

Weaver - The focus on animal data 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 account for TCE-induced nephrotoxicity (p.4-191) is not clear and requires further investigation. 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). The authors also did not discuss how they addressed exposure to solvent mixtures as they attempted to focus on specific agents.

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.

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the kidney [Section 4.4]

Rankin -Two additional points are worth nothing:

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 lesion 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.

the liver [Section 4.5]

McMillan - 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.

*the male reproductive system [Sections
4.8.1.1.3, 4.8.1.2, and 4.8.1.3.2];*

- No specific changes.

the immune system [Section 4.6]

Keil Response: Page 4-338 – clarify “subpopulation levels” on line 31 and 33

Keil Response: p4-367 – consider maternal exposure 2 years before conception and add to conclusions? See table 4-366 (4-64) as the Relative Risk seems to be high

Keil Response: Consider that immunosuppression is weighing in more than autoimmune effects. Woolhiser at 1000 ppm inhalation = 64% PFC suppression in rats

Sanders at 24 or 240 mg/kg; then 0.1, 1, 2.5 and 5 mg/mL; Female PFC LOAEL is 2.5 mg/mL 4-6 months or 4 AND 6 months (see Table 4-65 on page 4-374)

Keil Response: Fix top line p4-395 – “Gilkeson” cite is NZB-W mice

Dietert – Important to mention that for TCE effects, immune dysfunction includes both improper immune enhancement (evidence of autoimmune predisposition and parameters, some indication of inflammatory misregulation) as well as targeted immunosuppression.

and the developing fetus, including the role of TCE in inducing fetal cardiac defects

- **Poste** - p. 4-506 Summary of human developmental data
- **Poste** - This short section merely lists the types of outcomes that have been studied (e.g. decreased birth weight, congenital malformation, etc.) but does not give an overall conclusion. In section 4.8.3.3 Discussion/Synthesis of Developmental Data, it is stated that the weight of evidence from human and animal studies is suggestive of the potential for TCE toxicity. The language on this topic in the Chapter 6, the summary chapter, is much more definitive: “While comprising both occupational and environmental exposures, these studies are overall not highly informative due to the small numbers of cases and limited exposure characterization or to the fact that exposures were to a mixture of solvents.” (p. 6-9). This important conclusion should also be included in the discussion of human studies in Chapter 4.
- **Poste** - p. 4-506, line 30. Should be changed to “Tables 4-85 and **4-87**”
- **Poste** - More information should be provided in some of the tables to provide key information in context without the need to searching in other chapter. An example is Table 4-86 on ocular defects seen by Narotsky et al., 1995. The species (rat) and route of administration (gavage) should be stated.
- **Poste** - Consistent units should be used in tables so that studies can be compared more easily. For example, in Table 4-87, the TCE concentrations in drinking water are given as mg/ml and ppm for two studies (Collier et al., 2003 and Dawson et al., 1993).
- **Poste** - p. 4-520, lines 3-5. It is stated that Johnson et al., 1998b saw significant effects only when TCE was given pre-pregnancy plus pregnancy. However, Table 4-90 shows significant effects from exposure to TCE in pregnancy only at 1100 ppm.

*and the developing fetus, including the role of TCE in
inducing fetal cardiac defects [Section 4.8.3]?*

Poste - p. 4-498, line 27. There is a word missing after
“spontaneous”.

Selmin - On the issue of cardiac defects, I think the report explained logically why , recognizing the limitations of the study, 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, so may be they could be cited to make a stronger argument.

Overall, the report needs to do a little better job integrating the results from the studies so to make the reasons why some studies are selected or not really transparent.

Here, summarized are the results from three recent publications (PDF files sent to Marc Rigas):

In Rufer et al., 2010 (is there a typo mentioning Rufer et al., 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) ... continued.....

and the developing fetus, including the role of TCE in inducing fetal cardiac defects [Section 4.8.3]?

Selmin continued - TCE effects on 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 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 , confirming 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

Keil Response: Cardiac malformations – personal note: refer to repeat study by Allen and Fisher while at Wright Patterson that identified a reduced effect or no effect in cardiac malformations when compared to first study.

Cancer Hazard Assessment

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*].

Panel Summary Response

- The cancer hazard characterization of the carcinogenicity 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 support the conclusion that TCE is carcinogenic to humans by all routes of exposure as outlined in the US EPA cancer guidelines.
- The report clearly, logically, clearly and objectively presents the methodological review of the epidemiologic evidence, highlights the criteria for study inclusion in meta-analysis, 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 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.

Panel Summary Response

- The pooled risk estimates, although modest, were robust with no indication of publication bias or heterogeneity.
- The report appropriately highlights the causal criteria in support of the conclusion. The biologic plausibility and coherence are supported by the laboratory animal data, and the toxico-kinetic and other epidemiologic data of cancer and immune effects support the carcinogenicity of TCE.
- 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.

Panel Summary Response

Although the summary evaluation focused on the scientific evidence and meta-analysis for kidney, lymphoma and liver cancers, there is limited suggestive evidence for TCE as a risk factor for cancer at other sites including bladder, esophagus, prostate, cervix, breast and childhood leukemia also supports the conclusion.

Add paragraph describing the definition of lymphoma as used in IRIS.

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].

- **The 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 EPA provides a more balanced description in the hazard assessment between TCE's 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.** 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; however, the Board recommends that EPA conducts a thorough dose-response modeling to provide science-based information on the relative contribution of each metabolite, where data is available, to the liver effects of TCE.
- **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's 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.

Charge Question 6

- Drs. Dietert, Keil, Manautou, Rankin, Rusyn, Selmin, Weaver

Charge Question 6

- Overall: commended EPA for comprehensive, accurate discussion of complex topic
- Will address initial overall question in next slide
- 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];
 - Page 4.210: “Although not encompassing all of the actions of TCE and its metabolites that may be involved in the formation and progression of neoplasia, available evidence supports the conclusion that a mutagenic MOA mediated by the TCE GSH-conjugation metabolites (predominantly DCVC) is operative in TCE-induced kidney cancer. This conclusion is based on substantial evidence that these metabolites are genotoxic and are delivered to or produced in the kidney, including evidence of kidney-specific genotoxicity following *in vivo* exposure to TCE or DCVC. Cytotoxicity caused by DCVC leading to compensatory cellular proliferation is also a potential MOA in renal carcinogenesis, but available evidence is inadequate to conclude that this MOA is operative, either together with or independent of a mutagenic MOA. The additional MOA hypotheses of peroxisome proliferation, accumulation of $\alpha_2\mu$ -globulin, and cytotoxicity mediated by TCE-induced excess formic acid production are not supported by the available data.”

Charge Question 6

- Group generally agreed although perhaps more emphasis on cytotoxic MOA
- Rankin: “The MOA for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation should not be totally excluded and should be considered more closely. Weight of evidence does not exclude this MOA and including this MOA may more accurately reflect kidney tumor formation than a mutagenic mechanism alone.”
- Rusyn: “does not feel as strong as the EPA with regards to this MOA as being THE only MOA.” “While it is difficult in general to establish a causal link between cytotoxicity, compensatory proliferation and carcinogenesis, but in addition to the mutagenic MOA, the combination of the cytotoxicity, proliferation and DNA damage together may be a much stronger MOA than the individual components.”
- Dr. Johanson noted cytotoxicity and threshold under consideration in EU

Charge Question 6

- that there is inadequate support for PPAR α agonism and its sequellae being key events in TCE-induced liver carcinogenesis [*Section 4.5.7.2*];
 - Agreed

Charge Question 6

- that there are inadequate data to specify the key events and MOAs involved in other TCE-induced cancer and non-cancer effects;
 - Group agreed
- 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 –
- Group Agreed for All with recommendations below:
 - *Section 4.3.10 (Neuro);*
 - *Section 4.4.7 (Kidney); extent of GSH pathway in humans may be overestimated and impact of this must be transparent*
 - *Section 4.5.7 (Liver): change “unknown” to “complex” (section 4.5.7.4)*
 - *Section 4.7.4 (Lung), Rusyn: agreed but noted “There is, however, good data for chloral hydrate and a stronger discussion on the MOA for lung non-cancer and cancer effects should be included.”*
 - *Section 4.8.1.3.3.2 (Reproductive),*
 - *Section 4.8.3.3.2.1 (Fetal cardiac malformations).*

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?
 - Agree with above recommendations

Charge Question 6

- Additional recommendations
 - Rusyn: Tabular format for MOAs
 - Rusyn: Graphical or tabular presentation
 - of quantitative differences in the affinity of the various isoforms of PPARs to TCA, DCA and other model peroxisome proliferators
 - quantitative differences in affinity between species
 - Could use material from Guyton
 - Manautou: “Primary hepatocellular carcinoma and cholangiocarcinoma (intrahepatic and extrahepatic bile ducts) are the most common primary hepatic neoplasms (El-Serag, 2007; Blehacz and Gores, 2008). These are clearly distinct from a PPAR-alpha dependent mode or action. Would like to see some discussion on how this form of liver cancer is not seen in rodent models of TCE liver cancer where hepatocellular carcinomas are seen primarily in a PPAR-alpha dependent-manner.”
 - Manautou: “The addition of most recent studies with PPAR null mice and the humanized mice and the propensity of these mice to develop HCC in response to PPAR agonist”

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 existing data?

Charge Question 7

- Good review of potentially susceptible populations (section 4.10)
 - We agree that the identified factors (genetics, lifestage, background, may modulate susceptibility to TCE carcinogenicity and non-cancer effects
- Review includes adequate data to support factors that modulate exposure and pharmacokinetics, but few data to support differing susceptibility to TCE exposure effects
 - Conduct a thorough review of the literature to determine whether more data on subpopulation-specific effects are available
 - Discuss explicitly the lack of such data and the need for such data in risk assessment
- Make specific recommendations for studies that would fill this data gap for susceptible groups
 - Epidemiologic studies in which internal comparisons can be made to determine whether there is effect modification
 - Larger studies such as consortia studies (kidney cancer, lymphoma) – particularly studies with stored DNA
 - Animal studies

Charge Question 7

Recommended additions to the report (section 4.10):

- Add exposure to solvent mixtures as potential susceptibility factor
 - Exposure to >1 chemical to the same target organ likely increases risk
- Comments on early-life stages are understated
 - Add literature on importance of obesity epidemic in children here in terms of retaining TCE in vivo
 - We agree with use of standard age-dependent adjustment factors in the protection of children
- For genetic susceptibility section, add study on hypersensitivity dermatitis in Asian workers
 - Li et al. EHP 2007 (HLA-B*1301 as a Biomarker for Genetic Susceptibility to Hypersensitivity Dermatitis Induced by Trichloroethylene among Workers in China)
 - Notable as this type of skin response was not well described in US occupational literature

Charge Question 7

Clarifications to the report (Section 4.10):

- The wording is often not clear about whether you are describing results for a study that looked at effect modification of the TCE effect or not, as opposed to 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.

Charge Question 8:
*Dose-Response Assessment:
Methods and Results for Non-Cancer*

Drs. Emond, Fuentes, Johanson,
Portier, Post, and Weaver

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:

- It is appropriate that all studies showing dose-response for neurological, kidney, liver, immunologic, respiratory system, reproductive and developmental effects, and body weight change were evaluated.
- A list of all non-cancer health effects and studies discussed in Chapter 4, noting those which were considered candidate critical effects and studies, should be included.
- More details of studies selected (gender, strain, duration) when needed, cross references to Ch. 4, consistent dose units, etc. should be provided in Tables 5.1-5.5.
- Definition of dose-response (control and a single dose level?) should be provided.
- Other specific comments related to improving readability will be provided later.
- *Comments on concerns with NCI (1976) mouse nephrosis and NTP (1988) rat nephropathy to be discussed under G. below.*

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

- Well done and well documented.
- Approach, selection criterion and decision points explained in Appendix F provide details of POD selection. Suggest that information from Table F-13 be included in body of Ch. 5.
- Graphs give good presentation of BMD analyses.
- BMD is good approach but does not solve the problem of poor data.
 - Example: Toxic nephropathy in female rats (NTP 1988). Extrapolation from LOAEL at very high doses and a high % of animals affected leads to very uncertain extrapolation. Lower fractions affected among males and other rat strains suggest that the loglogistic BMD analysis might be severely overestimate risk at low doses

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 $3/4$ power scaling for some dose metrics.

- In general, use of PBPK modeling is commended.
- RfDs and RfCs for kidney endpoints highly sensitive to rate of renal bioactivation of DCVC (ABioactDCVCBW34) in human vs. rodents.
 - p-RfDs/RfCs based on this dose-metric are several 100-fold lower than RfDs/RfCs based on applied dose with standard UFs.
 - p-RfDs/RfCs for other endpoints based on other dose metrics are much closer to RfDs/RfCs based on applied dose and standard UFs.
- Basis for renal bioactivation dose metric should be clearly presented and discussed in Chapter 3 and other appropriate sections. If it was derived indirectly, from data on other metabolic pathways leading to and/or competing with bioactivation, this should be clearly discussed.
- Uncertainties about the *in vitro* and *in vivo* data (e.g discrepancy between Lash et al. and Green et al.) used to estimate this dose metric are greater than for other dose metrics. This uncertainty should be highlighted and addressed by sensitivity analysis.

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 $3/4$ power scaling for some dose metrics (Continued).

- Rationale for scaling dose metric to body wt.^{3/4} along with PBPK interspecies extrapolation should be clarified (dose rate to target tissue vs. internal concentration).
- Discussion of “empirical dosimetry” vs. “concentration equivalence dosimetry” should be clarified.

D. The selected uncertainty factors (UFs)

- UFs are consistently applied in Tables 5-8 to 5-13.
- UFs are appropriately applied only if the BMD-PBPK derived 99th percentile (HEC99 and HED99) dose metrics are correct.
- Definitions for subchronic and chronic durations should be provided. Consideration should be given to a partial UF for study duration for studies marginally longer than 90 days (e.g. 18 wks.) Are studies of 4 wks (defined as subchronic) long enough to extrapolate to lifetime?

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.

- It should be noted that 99th percentile is probably very sensitive to choice of prior distribution.
- The selections of idPOD and the extrapolation for rodent to human and then considering the 99th percentile is acceptable to track the sensitive population.
- The approaches to simulate a large range of exposure doses to get the distribution (page 5-68) are adequate.
- To characterize variability/uncertainty for the toxicokinetically-sensitive individual, more than just the distribution of 99th percentile, such as the 95th percentile, could be considered. A quantile regression looking simultaneously at several quantiles could be presented.
- *Concerns on use of PBPK modeling for kidney endpoints already discussed in C. above.*

Additional Issue Related to Points C, D, and E:

Two individuals questioned use of most sensitive animals + BMD + PBPK + UFs as overly conservative. This was also discussed by Dr Rhomberg:

1. BMD analysis on based on most sensitive species-strain-sex
2. idPOD is based on 1% or 5% response in the animals. This is used as a central dose estimate in humans
3. idPOD is even based on the lower bound estimate of the 1% or 5% response
4. Then the 99th percentile of the internal dose is calculated, i.e. for the 1% most sensitive humans, adding on several UFs for interspecies and intra-human pharmacodynamic variability.
5. Thus correction for uncertainty/variability is partly triplicated (lower bound of 1%/5% response and 1%/5% response used as central tendency but 99th percentile and UFs).
6. Is this extra UF necessary with the BMD approach?

Question: Did explanations by Dr. Chiu and other EPA scientists sufficiently address these concerns?

F. The qualitative and quantitative characterization of uncertainty and variability

- Uncertainties related to RfC and RfD adequately discussed.
- Quantitative uncertainty analysis of PBPK model-based dose metrics for LOAEL or NOAEL based PODs (Section 5.1.4.2) needs rewriting to 1) clarify objective of this 2-D type analysis and 2) methodology used.

F. The qualitative and quantitative characterization of uncertainty and variability

COMMENTS FROM DR. FUENTES:

- In the PBPK model the uncertainty and variability are quantified with the posterior distributions (as done in any Bayesian framework). In the more general dose-response framework, the uncertainty is characterized with UFs. The UF selected explain the main sources of variability and uncertainty:
- More sensitivity analysis and model diagnostics are needed to be convincing. More coherence and consistency is recommended. The PBPK was Bayesian, and if the dose-response is not Bayesian (UFs are not) it should be made more clear.
- The goodness of fit presented are limited, and not Bayesian (in part done by eye, and then getting a p-value of the fit). A p-value is not a proper metric in a Bayesian setting.
- Modeling assumptions and choices should be justified, and the statistical framework needs to be more convincing to ensure that we can make proper inference (by presenting sensitivity analysis, convergence analysis, model diagnostics, model validation and goodness of fit). The diagrams presented are very helpful.

G. The selection of the critical studies and effects for non-cancer dose-response assessment

- **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] are supported as critical studies and effects.
 - Questions related to cardiac malformation study (Johnson et al., 2003) were adequately addressed in Charge Question 3.
 - Note: BMD highly sensitive to model choice in Johnson et al.
- Although a tremendous amount of information is presented on liver toxicity, it was not a critical endpoint because it was less sensitive than other endpoints

G. The selection of the critical studies and effects for non-cancer dose-response assessment (Continued):

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 studies**, uncertainties in PBPK modeling based on renal bioactivation of DCVC are discussed above.
- Additional issues re: **NTP (1988)** female Marshall rats – toxic nephropathy:
 - Excessive mortality due to dosing errors and possibly other causes.
 - Very high doses and a high fraction of animals (>60%) with toxic nephropathy results in very uncertain extrapolation to BMD. Lower fractions affected among males and other strains.
 - Renal cytomegaly (not critical effect) in almost 100% treated animals.
 - (Note: Neither renal cytomegaly nor toxic nephropathy was seen in any of 396 control animals in study (8 groups: M and F, 4 strains)).
- Additional issues re: **NCI (1976)** Toxic nephrosis in mice:
 - No BMD analysis.
 - Much uncertainty associated with extrapolation from LOAEL with nearly 100% animals affected.
 - (Note: No M or F control animals had toxic nephrosis.)

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, rather than on the basis of the single most sensitive critical effect.

- This approach is supported because it is a very robust approach that increases confidence the final RfC and Rfd.
- Keil et al. (2009), Peden-Adams et al. (2006), and Johnson et al. (2003) should be used as principal studies supporting the RfD/RfC.
- There is less confidence in the RfDs/RfCs based on the three studies with renal endpoints [(Woolhiser et al., NCI (1976), and NTP (1988)], but they should also be used to provide additional support for the RfC/RfD.
- Use of multiple critical effects reduces uncertainty and better characterizes variability.
- This approach may create more work for the risk assessors and the users of the risk assessment. However, note that a single RfD and RfC is provided to users of the risk assessment.

TCE Charge Question 9

Discussants: Claude Emond
Gunnar Johannson
Michael Pennell

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 & transparently described & technically/scientifically adequate to support EPA's draft inhalation and oral unit risks:

a. Estimation of Unit Risks for RCC from Charbotel et al.

- ▶ We agree that the analysis was well described and scientifically appropriate.
- ▶ Study should be used to estimate unit risks.
 - Some mention should be made of effects of adjustment for cutting oils, though adjusted ORs shouldn't be used for risk estimation since it is not a clear confounder.
 - Include some additional statements about assumptions (linear RR–dose relationship, RR ind of age) and limitations (measurement error of exposure).

b. Adjustments of RCC Unit Risks for Added Risks of Other Cancers.

- ▶ We accept the analysis and presentation as is.

c. Estimation of Human Risks from Rodent Bioassays.

- ▶ We accept the methods and results.
 - Should mention potential biases caused by informative drop out in studies with mortality prior to earliest time to tumor.
 - Provide more details behind Bayesian analysis of combined risk across tumor types.
 - There is a need for data that support a similar MOA across species.

d. Use of Linear Extrapolation

- ▶ We agree that this is probably the best approach given our current knowledge.

e. Applications of PBPK Modeling

- ▶ We found that the PBPK models provided valuable information to the risk assessment and agree that the internal dose should be preferred over applied dose.
 - Assuming that the proper dose metrics were chosen and that human models are correct (particularly the GSH pathway).

f. Uncertainty and Variability Analysis.

- ▶ We agreed that their consideration of uncertainty and variability was for the most part adequate (particularly that pertaining to the PBPK models).
- ▶ Discussion of how exposure variability relates to risk estimates was limited.

g. Conclusion that Unit Risk Estimates Based on Rodent & Human Data are Consistent.

- ▶ We accept this conclusion.

h. Preference for Unit Risk Estimate Based on Human Data.

- ▶ We agreed that the human data should be preferred over rodent data.
 - Within species uncertainty is easier to deal with.
- ▶ Ideally we would like to see estimates based on multiple human studies but understand that no other studies provide quantitative dose measures.

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*].

- EPA did an excellent job of describing and presenting the ADAF computations for both oral and inhalation situations.
 - All the steps are clearly laid out for inhalation exposure but shortened for the oral exposure which makes the presentation less easy to follow.
 - Recommendation is to include all details in the oral exposure description as was done for the inhalation situation.
- Impact of ADAF on total risk in this case is not large.
 - Only one tumor type receives the adjustment, impact would be greater if all tumor types were adjusted.

- An issue relating to the validity of the assumption of equal susceptibility for individuals > 16 years of age was discussed.
 - Age-dependent adjustment factors seems contradictory to the assumption that RR is independent of age that was the foundation of the linear model ($RR = 1 + \text{slope} \times \text{dose}$ [p. 5-131]) used to compute unit risks from the Charbotel et al study.
 - The reasons why EPA uses age-dependent adjustment factors for ≤ 16 years of age, not for the elderly, and does not directly produce age dependent risks per mg/kg/d were discussed.
 - “Because the TCE intake is not constant across 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 for TCE as in Table 5-42, but this is not something that is commonly reported...”
 - Recommendation to modify wording to clarify.

➤ Plea from one Panel member who is a risk practitioner to compute and include risk values for Office of Water standard water consumption levels (using ADAF approach) into the IRIS report for TCE.