NOTICE: **Do Not Cite or Quote.** Preliminary Comments submitted by individual members are for review and to assist meeting deliberations by the CAAC-ETBE/tBA committee. These comments do not reflect consensus advice or recommendations, and have not been reviewed or approved by the chartered SAB and do not represent EPA policy.

Compilation of the SAB Chemical Assessment Advisory Committee augmented for the review of ETBE and tBA (SAB CAAC-ETBE/tBA Committee)
Member Preliminary Comments
submitted in Response to Agency Charge Questions

The following comments from committee members were submitted in response to the Agency Charge available at https://yosemite.epa.gov/sab/sabproduct.nsf//MeetingCalBOARD/AD6DBAAB343F7682852581540063B2A6?OpenDocument. Responses are grouped according to chemical. The compilation contains all comments received as of August 7, 2017.

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Ethyl Tertiary Butyl Ether (ETBE)

Dr. Alan Stern

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer reviewed studies that should be considered for modeling?

I claim no specific expertise in pharmacokinetic modeling and particularly not in PBPK modeling. In my reading of Appendix B as well as the PK/PBPK Model Evaluation for the IRIS Assessments of Ethyl Tertiary Butyl Ether (CASRN 637-92-3) and tert-BUTYL ALCOHOL (CAS No. 75-65-0), I generally find that the use of the model is appropriate and clearly described. However, on page B-23, the following statement, "Finally, because induction is expected to have an equal impact on oral and inhalation exposures—and only in the case that tert-butanol levels or metabolism were used as a dose-metric—induction's

potential impact on risk evaluation for ETBE is considered minimal," regarding induction over time of TBA following ETBE administration, seems to me to implicitly depend on the absence of a first-pass effect of the liver with ingestion (versus inhalation) exposure. This should be addressed. I am not aware of any additional studies that should be considered.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Does the classification give appropriate weight to the results from initiation–promotion studies? Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported.

I agree with the categorization of carcinogenic potential as "suggestive evidence." Although the direct evidence (hepatocellular tumors in Saito et al.2013) of tumors at the high dose only is weak, the data meet the criteria for this designation. This designation is supported (but not advanced to a designation of "likely to be carcinogenic to humans") by the initiation-promotion data. These data provide a plausible mode of action (i.e., promotion) for the carcinogenicity of ETBE. The evidence for a tumor promotion capability of ETBE as opposed to an initiation or complete carcinogenicity capability does not lessen the relevance of the observed carcinogenic potential.

4c. Cancer toxicity values. Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for quantitative analysis, and whether the Saito et al. (2013) study is suitable for this purpose.

While I support the "suggestive evidence" characterization of carcinogenic potential, I do not believe that the Saito et al. (2013) (or any other available data) are sufficiently robust to provide a meaningful quantitative estimate of cancer risk. With a statistically significant increase in tumors at the high dose only, any number of models can provide an adequate fit to the dose-response data. This results in the cancer slope factor having an extreme model dependence such that "best-fit" of the possible models becomes meaningless from a statistical or practical standpoint.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x 10–3 per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for 4 oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

As noted in my response the charge question 4c, I do not believe that the available data are sufficiently robust or have a sufficient dose-response to warrant the derivation of a cancer slope factor. I don't believe that the slope factor that was derived is scientifically supported or can provide useful information.

Dr. Deborah A. Cory-Slechta

1. Literature Search Strategy/ Study Selection and Evaluation-Systematic Review Methods. Please comment on the strategies for literature searches, criteria for study inclusion or exclusion and evaluations of study methods and quality discussed in the Literature Search Strategy/Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

The literature search and study selection were clearly described and objectively applied. In addition, the criteria for inclusion are well described and appropriate inclusion and exclusion criteria, as well as criteria of study quality for inclusion applied.

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

The conclusion that kidney effects as a potential human hazard of ETBE could be a human hazard is both scientifically supported and clearly described as based on dose-related increases in noncancer endpoints in male and female rats. The review has very carefully reviewed and systematically evaluated the evidence for this conclusion, particularly the evidence in relation to the potential mechanisms of α2u-globulin and CPN.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of $5x10^{-1}$ mg/kg-day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012). Please comment on whether this value is scientifically supported and its derivation clearly

described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

There were multiple reasons for the choice of urothelial hyperplasia that are supportive of its choice. This includes the fact that it was the only outcome that derived from a full 2-year study and had the lowest uncertainty factor values associated with it, providing more confidence in the value. Correspondingly, is consistent with epithelial hyperplasia in the renal pelvis in response to chronic tert-butanol exposure, an effect seen in both sexes. It also is a more specific indicator of toxicity than the alternative which was kidney weight changes. These reasons provide sound scientific support for this choice and the derivation of the values is clearly described.

4a. Cancer modes-of-action in the liver. As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPARα, PXR and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

The potential for PPARα, PXR and CAR involvement in ETBE tumorigenesis was considered, based on findings in both cases of effects of ETBE exposure on these pathways. Data supporting a PPARα mechanism for ETBE was limited to begin with, but in addition, data gaps preclude an assessment of a full evaluation of the PPAR mechanism of action. Inconsistencies in a PPAR mechanism were also found, specifically the fact that apoptosis rates were actually increased rather than decreased in response to ETBE. Similarly, some evidence was found to support a potential role of CAR/PXR in ETBE-associated liver tumorigenesis. Here too, however, inconsistencies were noted, such as the discordance in dose response between ETBE metabolism rate relative to liver. Moreover, several data gaps here too preclude a full assessment, as no evaluation of lack of clonal expansion or gap junction communication have been assessed. Thus, the conclusion that liver tumors are relevant to human hazard identification, i.e., not shown to be due to PPAR-mediated or CAR/PXR mediation, is scientifically justified.

Dr. Hugh Barton

2. Chemical Properties and Toxicokinetics.
2a. Chemical properties.

Is the information on chemical properties accurate? No comment.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties?

The description in Section B.1.5 of Appendix B in the Supplemental Information is limited to specific details about the implementation in the dose-response assessment, e.g., how induction of metabolism and drinking water exposures were handled. As such, the description is clear but so

limited that it does not describe the model overall nor its assumptions or uncertainties. Useful information is provided in US EPA 2017, but this document indicates it is a draft. Model code was made available through the HERO database, which is appropriate though also providing limited information especially to those without the modeling software used.

- EPA appears to have carefully considered alternative models that were available as described in US EPA 2017. For the limited application in this assessment of route extrapolation and given the dose metric selected, the model appears appropriate. Descriptions of assumptions and uncertainties are available in US EPA 2017 and the cited peer-reviewed literature publication for each model (e.g., Salazar et al 2015, Borghoff et al 2017).

- Tier 1 Recommended Revisions: It is misleading to say "A more detailed summary of the toxicokinetic models is provided in Appendix B.1.5 (US EPA 2017)" on page 1.3 lines 11-12. The text should indicate that some additional information in available in the appendix, as well as in the cited EPA draft report (US EPA 2017) and the original literature references.

- Tier 3 Future Considerations: For purposes of using PBPK models in IRIS assessments, EPA needs to establish a consistent practice for documentation of both the model itself and the review of the model (and any modifications made by EPA to implement it). It is not desirable for EPA to write long descriptions of the model it is using that would repeat much of what is in published literature, but on the other hand providing more summary information seems desirable. In light of the limited role of the PBPK model in this analysis, the assessment should not be delayed while establishing such best practices.

Are there additional peer-reviewed studies that should be considered for modeling?

None that I'm aware of.

2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

The rate of ETBE metabolism is not an appropriate choice for the dose metric as demonstrated by Fig B-3 (page B-26) and text on B-27, indicating this dose metric dose not result in a "consistent dose-response relationship" when combining oral and inhalation studies to assess liver tumors. No alternative measure of internal dose has been identified that does provide such a consistent dose-response relationship. The choice of dose metric often varies depending upon the model of action of the toxicity and the question being

addressed, so rate of ETBE metabolism could be an appropriate choice for some other currently unspecified purposes.

- Tier 1 Recommended Revisions: It is recommended below that route extrapolation not be implemented for the oral cancer dose-response analysis, so selection of a dose metric will not be necessary.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported.

- It is appropriate and justified to consider initiation-promotion studies as well as other mechanistic studies that may inform cancer hazard assessment. Tumors observed in initiation-promotion studies generally reflect the sites sensitive to the initiating mutagenic carcinogen, so initiation-promotion studies should not generally be used to understand the cancer site for a compound that can cause cancer when administered in the absence of other initiators. As such, these studies generally should not inform criteria for EPA's cancer descriptors such as whether tumors arise at more than one site.
- Tier 3 Future Considerations: Since environmental exposures are inevitably to mixtures, EPA needs to develop a consistent process for considering the potential for tumor promotion. Such a process, would include compounds that are not only complete carcinogens (i.e., exposure to the single compound induces cancer), but also those that are solely promoters. However, the scientific justifications for applying low dose linear analyses are largely inappropriate for assessing tumor promoters, which tend to involve biological processes that behave as biological thresholds even across populations with sensitive individuals. Therefore, a process for addressing tumor promotion as part of a cancer evaluation should address both hazard identification and dose-response analysis.

Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

- It is unclear whether ETBE could cause cancer by the oral route. The oral initiation-promotion studies may suggest this is a possibility. A variety of evidence is discussed in the toxicological review but appears to raise a range of possibilities rather than demonstrating a consistent direction. The lack of consistent dose-response when evaluating both inhalation and oral studies using the ETBE metabolic rate or other

measures of internal dose raises major doubts about applying perspectives from the inhalation study to the oral route.

- Tier 1 Recommended Revision: For the oral route of exposure the scientific evidence is best characterized as "inadequate information to assess carcinogenic potential".

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x 10^{-3} per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

- Extrapolation of the inhalation cancer findings to the oral route using ETBE metabolic rate as the dose metric is not supported by the available scientific information, though it is clearly described. For purposes of further commenting on this question, it is assumed that the liver tumors are relevant for human dose-response analysis, but if it were determined that that was not scientifically supported, then route extrapolation would not be scientifically supported either. Figure B-3 (page B-26) shows, as described in text on B-27, that use of the internal dose metric does not result in a "consistent dose-response relationship". The text indicates that "internal dose is inadequate to explain different in tumor response across these studies", so why would one use it for route extrapolation? At least three possibilities exist that could contribute to the apparent inconsistencies: 1) the internal dose metric used is not the correct representation of the dose (and what would be correct may be difficult to determine absent a clear understanding of the mechanism), 2) the differences may arise from the toxicodynamic processes, and/or 3) interstudy variability, which can be substantial in 2-year studies.

- Tier 1 Recommended Revisions: Eliminate the route extrapolation. Report that the oral study was negative and assessments of internal dose did not obtain a consistent dose-response relationship across the oral and inhalation studies, so route extrapolation was not considered appropriate.

Dr. Lorenz Rhomberg

ETBE Charge Question 1 – Literature Search/Selection/Evaluation

Overall, the structure of the search, selection, and evaluation is clearly presented. The Summary Chart (Fig.LS-1) shows the overall patterns of disposition. What is difficult to get at, however, is the reasoning behind disposition of individual identified literature items. That is, one is not readily able to check on the scoring of individual studies on the named criteria or to see the reasons for which individual studies were put in one or another category. This makes critical comment on selection and disposition difficult. The issues about evaluation of quality and impacts on interpretation listed 'under "Database Evaluation" are said to be discussed in the text, as the studies come up. But again, this scattering and partial reporting does not allow an

observer to follow (and comment upon, if appropriate) the treatment of individual issues on individual studies. There is no ready way to find such discussion of any one study (other than by searching the whole assessment document) and the reader is limited to the aspects that the Agency chooses to discuss. It does not seem that the Supplemental Information includes this information either. It would be good to have a way to capture the evaluations on specific criteria and to see the reasons for disposition of each study. All of the evaluation criteria in Tables LS-3 and LS-4 should have evaluations available for each study to which they were applied. [Tier 2]

ETBE Charge Question 3c – Oral Reference dose for noncancer kidney outcomes

Given the endpoints analyzed, the calculations of BMDs, BMDLs, and oral reference doses are done by straightforward usual methods. The questions, then, are (1) are appropriate endpoints chosen for analysis? (2) among those chosen, which should be used to define the RfD? and (3) are there opportunities and reasons to depart from standard methods?

Although the first question is perhaps more in the province of other Charge Questions, the issue of the appropriateness of the oral RfD calculations cannot be addressed without confronting it. In public comments, some strong views, supported by analysis of a specifically convened PWG, are expressed regarding whether the kidney endpoints are separable, whether they are better considered as various aspects of Chronic Progressive Nephropathy (CPN), and whether they are relevant to processes that could occur in humans. Importantly, the endpoint chosen as critical, urothelial hyperplasia, is characterized by the PWG as a stage in CPN. In sum, the question of the validity and applicability of the endpoints analyzed for the oral RfD needs to be carefully examined. [Tier 1] Even if the decision is to use them, that use must be couched in prominent caveats that acknowledges a significant dissenting body of expert opinion.

Even if one decides to employ these endpoints, it has been said by knowledgable public commenters that, because the endpoints are seen as a suite of CPN manifestations, not all appearances will necessarily be noted in pathological examination, and the counts (and denominators) may be inappropriate. This question needs a clear resolution if the data are to be taken as valid for analysis. [Tier 1]

The reasons given for not considering liver hypertrophy as an endpoint for dose-response analyses seem valid. If the kidney effects are in the end deemed irrelevant, however, the liver effects may be all that is available, and some discussion of the alternative they might present, even if not a full analysis, seems warranted. [Tier 2]

It is difficult to address the second question – which among the analyzed endpoints should form the basis for the RfD – because of the issues of severability among endpoints and their human relevance, noted above. It is noteworthy that the values obtained from different choices are not markedly different.

The third part of the question – departing from defaults – raises the issue of dosimetry. It is noteworthy that the kidney effects of ETBE and tBA are similar, and that tBA is a principle

metabolite of ETBE, strongly suggesting that metabolism of ETBE to tBA is key to ETBA's effects. Yet the dose measures and extrapolation method for animal-to-human are simply the defaults, with no allowance for metabolism. Granted, there is no validated human PBPK model, but there are quite a few studies characterizing human metabolic activity. It would be of great value to explore how the rates of metabolic activation, given what can be discerned about them, could compare with the default dosimetry considerations. The pharmacokinetics of ETBE and tBA do not seem especially complex, and it seems likely that rates of metabolic activation (and how they may differ among dose levels, between sexes, and between rats and humans) may have a lot to say about expectations of relative toxicity. For instance, there does not seem to be much issue of saturable metabolism that might affect the shape of the dose-response when compared to metabolized dose vis-à-vis administered dose, and this is worth explicitly noting (and noting the limits of the inference). Rat-human differences in metabolic activity toward ETBE would be especially useful to have characterized more thoroughly. [Tier 2]

[Although the current Charge Question is about noncancer effects, it is worth noting that ETBE produces liver tumors but tBA appears not to do so. This suggests that, unlike the kidney effects, tBA as a metabolite of ETBE is not likely to be responsible for ETBE liver effects, and it suggests that perhaps those effects might be attributable to the acetaldehyde moiety rather than the tBA moiety formed by spontaneous decomposition of the initial hemiacetal in the first step of metabolism of ETBE.]

Although there is a lot of tabulation of experimental results, it is difficult to trace the information on any one study from its discussion in the text to the tabulations in the Supplementary Material to the dose-response analysis elsewhere in the Supplementary Material. This is complicated by the fact that there are different analyses of the same data (absolute and relative organ weights, for example), different durations of exposure for otherwise similar experiments, and lack of uniqueness of "author (date)" designations, making it challenging to be sure that one is examining corresponding data in the different places they are discussed or presented.

Critically, there does not seem to be any reporting of statistical analysis of individual studies – trend tests or pairwise significance tests, *etc*. – and this hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role of such statistical analysis in identifying results to report and decisions to include or exclude them from analysis is not very clearly stated.

There is no reporting of units for the responses (as opposed to the exposures) in the Supplementary Material tables, and this also leads to difficulty in interpretation. For instance, in Table C-1, the listing for relative kidney weight by Fujii *et al*. (2010) shows "means" but does not say to what the relative increased kidney weight is relative. Presumably, it is body weight, but the values given suggest otherwise, since (for instance) the control value is 0.546, which would imply that over half of body weight consists of one kidney. An attempt to decipher the reported measure by seeking values in other tables was fruitless.

ETBE Charge Question 3d – Inhalation Reference dose for noncancer outcomes

Given the endpoints analyzed, the calculations of BMDs, BMDLs, and oral reference doses are done by straightforward usual methods. The questions about the inhalation RfC determination are essentially the same as those for the oral RfD a set out for Charge Question 3c, above, and they are consequently repeated here by reference to that response. [Tier 1]

Given that the kidney responses are similar for inhalation and oral exposure to ETBE, and are presumably attributable to metabolic generation of tBA, and also given that the exposure to tBA is systemic from metabolism occurring elsewhere (the liver), it would be valuable to assess how similar the apparent potencies are for inhalation and oral exposure if rendered in terms of projected tissue concentrations for tBA. The sex difference in response seems a bit more marked for inhalation exposure than for oral, and an interpretation of possible reasons for this (including mere statistical fluctuation, which if responsible would suggest averaging across sexes) would be informative. [Tier 2]

On p.2-18, line 8, the document notes that the range among candidate values for an inhalation RfC vary over a 100-fold range. Examination of Table 2-5 suggests that much of this range is actually a consequence of differing choices of UFs (depending on what is done for UFs) as well as of the male-female differences. The PODs themselves are rather similar (for shorter and longer durations) within a sex, though somewhat different for males and females. [Tier 2]

As already noted for Question 3c, although there is a lot of tabulation of experimental results, it is difficult to trace the information on any one study from its discussion in the text to the tabulations in the Supplementary Material to the dose-response analysis elsewhere in the Supplementary Material. This is complicated by the fact that there are different analyses of the same data (absolute and relative organ weights, for example), different durations of exposure for otherwise similar experiments, and lack of uniqueness of "author (date)" designations, making it challenging to be sure that one is examining corresponding data in the different places they are discussed or presented. [Tier 2]

Critically, there does not seem to be any reporting of statistical analysis of individual studies – trend tests or pairwise significance tests, *etc*. – and this hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role of such statistical analysis in identifying results to report and decisions to include or exclude them from analysis is not very clearly stated. [Tier 1]

There is no reporting of units for the responses (as opposed to the exposures) in the Supplementary Material tables, and this also leads to difficulty in interpretation. [Tier 1]

ETBE Charge Question 6 – Executive Summary

The specifics of the Executive Summary clearly depend on the content of the draft assessment, and many of those aspects are subject to debate and comment through other Charge Questions. Accordingly, the Executive Summary will need to be changed to reflect any changes elsewhere

in the document. Comments here are therefore on overarching aspects of what the Executive Summary addresses and in what depth of detail, rather than the specifics it sets out.

The Executive Summary offers statements about the questions considered and summarizes the findings that were in the end chosen. Little is said about alternatives that, even though they may have some merit and provide important context for interpretation and application of the main findings. The section on "Key Issues" is helpful, but it could do more to highlight the consequences of alternative choices for the final assessment. This is especially so because (based on the history of public comment) the interpretation and relevance of key toxicity endpoints driving the analysis has been sharply contested. However these issues are resolved, it is important for the assessment – and for the Executive Summary – to forthrightly deal with the contingency of final conclusions on decisions to accept or not accept debated arguments, and simply not meeting the level of certainty to dismiss an endpoint as irrelevant to human risk does not result in certainty of relevance. [Tier 1]

The connection between the assessment of ETBE and tBA needs to be explicitly discussed (in the full document as well as in the Executive Summary). Since metabolic activation of ETBE is invoked in its assessment, and since tBA is a main metabolite, the interpretation of toxicity, dosimetry, and dose-response for the two compounds ought to inform one another, and the consistency of interpretations needs to be more thoroughly addressed. [Tier 1]

The Executive Summary should provide more specifics about the chosen dose metrics, explaining (in summary form) the basis for calculation, the role of metabolism in activation and clearance, major assumptions or use of alternatives to defaults, and the basis for cross-species dose equivalency. The dosimetry considerations applied to each endpoint should be clear (*e.g.*, dose-metric definition, role of tissue specificity, *etc.*). The simple reference to "PBPK" being used, or to the Category 3 gas approach, are not sufficiently specific. [Tier 1]

Dr. Stephen Roberts

3a. Noncancer kidney toxicity. (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

The assessment of potential kidney toxicity from ETBE is clearly described, both in terms of observations from the literature and evaluation of the potential human relevance of key endpoints. There is scientific support for kidney effects as a potential human hazard, but the support is not strong. Several renal endpoints are proposed as related to ETBE exposure, including a change in absolute kidney weight, urothelial hyperplasia, and increased blood markers. However, it is difficult to determine the extent to which these are the result of CPN, and perhaps 2u-globin-related events, rather than effects of the chemical through a human-relevant MOA. Exacerbation of CPN is presented in the assessment as a rat-specific mechanism of nephropathy <u>not</u> relevant to humans in one location (page 1-34), and elsewhere as a spectrum

of effects, some of which may well occur in the human kidney (pg 1-6) and therefore be relevant. As the draft assessment points out, endpoints such as increased kidney weight and blood markers cannot be definitively explained based upon CPN, but they are not easily separated from the CPN that is occurring in rat study populations either. This makes it difficult to articulate clear scientific support for the conclusion.

3c. Oral reference dose for noncancer outcomes. Section 2.1 presents an oral reference dose of 5 $x10^{-1}$ mg/kg-day, based on urethelial hyperplasia in male rats (Suzuki et al., 2012). Please comment on whether this value is scientifically supported and its derivation is clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

The derivation of the oral reference dose of 5 $x10^{-1}$ is clearly described. The PODs were derived using benchmark dose modeling according to U.S. EPA guidance, and the UFs selected are appropriate. I note that the RfD is based upon a change in incidence of hyperplasia graded "slight or minimal" (page 1-6), and the draft assessment does not indicate that significant increases in more pronounced hyperplasia were observed in any of the ETBE-treated animals. Increased kidney weight was also considered, and although the pathophysiologic implications of this effect aren't always clear, it might be considered a stronger endpoint.

4a. Cancer modes-of-action in the liver. As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPAR□, PXR, and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

A conclusion that the liver tumors in male rats are relevant to human hazard identification is scientifically supported given ambiguity regarding the potential role of PPAR□, PXR, or CAR nuclear-receptor mediated events and inability to eliminate other plausible MOA's based upon observations in the literature. The draft assessment evaluated several potential MOA's for liver cancer in ETBE-treated animals, including modulation of receptor-mediated events (specifically, pathways associated with PPAR□, PXR, and CAR). This potential MOA was important to address for the purposes of hazard identification as it has been postulated that liver tumorigenesis by this MOA is not relevant to humans. [See, for example, *Proposed OPPTS Science Policy: PPAR□-mediated Hepatocarcinogenesis in Rodents and Relevance to Human Health Risk Assessments*, OPPTS, U.S. EPA, November 5, 2003, pg 15: "When liver tumors are observed in long term studies in rats and mice, and 1) the data are sufficient to establish that the liver tumors are a result of a PPAR□ agonist MOA and 2) other potential MOAs have been evaluated and found not operative, the evidence of liver tumor formation in rodents should not be used to characterize potential human hazard."] Although the study by Kakehashi et al. (2013) identified a number of PPAR□, PXR, and/or CAR key events in ETBE-treated rats, evidence for others was missing or inconsistent with expectations, and liver hypertrophy was transient and not well

correlated with liver tumorigenesis. Further, other potential MOAs that could be relevant to humans, such as metabolism to acetaldehyde, could not be ruled out.

4b. Cancer characterization**.** As described in sections 1.2.1, 1.2.2., 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation-promotion data in the cancer hazard characterization is scientifically supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

As the draft assessment notes, the selection of "suggestive evidence …" is based primarily on the positive carcinogenic response in the liver at one dose in a single animal study, along with significant increases in focal pre-neoplastic liver lesions and mechanistic data, including the metabolism of ETBE to acetaldehyde in the liver, and the mutagenic and genotoxic effects of acetaldehyde." Initiation-promotion bioassay data are considered supporting, i.e., "consistent with the descriptor of 'suggestive evidence of carcinogenic potential.'" The very limited emphasis given to the initiation-promotion studies is appropriate and scientifically supported. In fact, had consideration of this information been excluded altogether, the choice of descriptor would be the same.

The "suggestive evidence" descriptor is clearly appropriate for the inhalation route. Because the site of the tumorigenic responses is beyond the point of initial contact with the chemical, this usually argues strongly for application of the descriptor to all routes of exposure. However, in this case, negative findings from oral exposures call that assumption into question. EPA notes that positive findings in other tissues in initiation-promotion studies and evidence implicating acetaldehyde in the human carcinogenicity of ethanol (and genotoxicity of ETBE in ALDH2-deficient mice) were deciding factors in extending the descriptor to the oral route. This rationale is not particularly convincing, in my opinion.

Dr. W.M. Foster

1. Literature Search Strategy/Study Selection and Evaluation.

I found the keywords selected for search using the online venues of scientific databases (PubMed, Toxline, Web of Science, and TSCATS) to be appropriate for ETBE. The resulting Table information defining the searched literature, Tables LS-1, LS-2 (review reports), and LS-3, were lucid and identified the temporal end point (Nov, 2015) of the applied searches, and the inclusion/exclusion criteria utilized. The overall success of the search approach as presented in Fig. LS-1 seemed adequate. The search selections appeared appropriate and on target for determining health effects of exposure to ETBE in animal models for extension to humans.

3b. Non-cancer toxicity at other sites. Identified in Sections: 1.2.2 for liver, 1.2.3 for reproductive, and 1.2.4 for developmental, toxicities.

For the liver, liver weight outcomes increased in male and female rats for sub-chronic oral, and inhalation exposures as presented in summary figures: Figs. 1-9, and 1-10. Additional liver histopathology was not evident for reviewed reports and although a serum marker of liver toxicity (GGT) was elevated, additional vascular markers (AST, ALT, and ALP) were not. This led to non-cancer liver effects being considered as modest and, except for organ weight data, exposure effects did not appear to be dose-related. As there was uncertainty if liver weight increases induced a hazard, liver effects were not considered for dose-related analysis and the derivation of reference values. These conclusions appear warranted.

Reproductive studies were accomplished in rat and mice models via oral and inhalation routes of exposure, and reported effects included indices of delivery and fertility, post-implantation loss, litter size, oocyte viability, sex hormone concentrations, seminiferous tubule histopathology, and sperm effects. Based upon sub-chronic studies definitive conclusions were not developed in regards to reproductive toxicity. For the reproductive endpoints examined, little consistently in rat and mice models was apparent across studies or doses. These conclusions are likely open to further consideration if/when future studies were to be entertained.

Developmental studies reviewed animal models in the rat and rabbit using oral exposure route and developmental responses included fetal and pup survival and growth. In summary the reported evidence was found to be slight and uncertain, and thus at this point developmental effects were not considered in hazard evaluations. Additional studies, perhaps in rodent species, would seem to be necessary for further/final assessments of non-cancer, developmental toxicity.

3d. Inhalation reference concentration (RfC) for non-cancer outcomes. Identified in Section 2.2, an RfC is proposed and based on urothelial hyperplasia in male rats.

For non-cancer outcomes, evidence found to be consistent and support the RfC, relied upon kidney effects of organ weight, and histopathology (urothelial hyperplasia). Organ weight changes for chronic and sub-chronic exposures by the inhalation route were clearly reproducible across many reports in the rat. For urothelial hyperplasia, chronic 2-year studies using inhalation exposure established that this response increased with treatment in male rats. The urothelial hyperplasia data were the only endpoint from chronic exposure (2-year) studies, and organ weights were the only endpoint from sub-chronic (13-week) studies that were considered for dose-response analysis (Saito et al., 2013; JPEC, 2010b). This approach seemed appropriate.

Based on the confidence placed in the principal research report by Saito et al. (2013), the overall confidence was high for the calculated RfC (9 × 100 mg/m3). The Saito et al. research study was well conducted, strictly adhering to GLP guidelines, and including evaluations of sufficient numbers of animals per group (both sexes), that lead to the broad collection, and assessment of appropriate kidney tissue samples, and relevant outcome measures (organ weight, histopathology). The kidney-specific RfC value of 9 × 100 mg/m3, as the overall RfC, representing an estimated exposure level below which deleterious effects from ETBE exposure are not expected to occur, appears to be warranted.

4e. Inhalation unit risk for cancer. Identified in Section 2.4, an inhalation unit risk is proposed of 8 x 10–5 per mg/m3 and based on the response of liver tumors in male rats.

Chronic inhalation exposure enhanced relative organ liver weights (both sexes) in a rat model, and increased hepatocellular adenomas and carcinomas only in the males (Saito et al., 2013). In addition, centrilobular hypertrophy occurred transiently (both sexes, at sub-chronic end points, however not at chronic end points i.e., at 2 years). Centrilobular hypertrophy suggested induction of liver enzymes (possible link to tumorigenesis); however, the centrilobular hypertrophy was observed in oral and inhalation models only at the sub-chronic time points. ETBE is metabolized by cytochrome P450 enzymes to an unstable form that decomposes spontaneously into tert-butanol and acetaldehyde (Bernauer et al., 1998). Acetaldehyde is further metabolized in the liver by ALDH2, while tert-butanol undergoes systemic circulation and ultimate excretion in urine. With ETBE exposure the liver is also exposed to both acetaldehyde and tert-butanol (tBA), so the liver effects caused by tBA and acetaldehyde are relevant to evaluating the liver effects observed from ETBE.

Based on the liver tumor data in male rats (Saito et al., 2013) a single inhalation unit risk was derived and a recommended inhalation unit risk value of 8 × 10-5 per mg/m3, was proposed as an index of the potential carcinogenic risk associated with lifetime inhalation exposure to ETBE). Table 2-10 lists numerous valid uncertainties that are influential to the proposed inhalation unit risk. At this time, there are no data for humans that confirms a general cancer response or the specific tumors observed in the rat chronic inhalation model (Saito et al., 2013). Thus a degree of uncertainty is warranted with respect to the proposed unit value.

Dr. Alan Hoberman

3b. Noncancer toxicity at other sites (Sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1). The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.

- Liver effects: Suggestive evidence
- Developmental toxicity: Inadequate evidence
- Tier 1: Recommended Revisions –

The overall evidence appears to be inadequate to provide a precise evaluation of the hazard but the data is adequate to indicate that any hazard would be at a dose higher than those for other endpoints. No terata and no effects below 1000 mg/kg from various routes.

Reproductive effects were evaluated based on four studies. (Faulkner et al., 1989; Nelson et al., 1989; Daniel and Evans, 1982)

- Tier 1: Recommended Revisions –

The overall evidence appears to be inadequate to provide a precise evaluation of the hazard but the data is adequate to indicate that any hazard would be at a dose higher than those for other endpoints.

Developmental effects were evaluated based on three studies (Huntingdon Life Sciences, 2004; Faulkner et al., 1989; Daniel and Evans, 1982). Daniel 33 and Evans (1982)

Neruodevelopmental effects were evaluated based on three studies (Nelson et al., 1991; Daniel and Evans, 18 1982)

- Male and female reproductive toxicity: Inadequate evidence

Tier 1: Recommended Revisions

Reproductive Effects – one generational (Huntingdon Life Sciences, 2004) and subchronic effects in rats and mice following oral and inhalation exposure (NTP, 1997, 1995)

The overall evidence appears to be inadequate to provide a precise evaluation of the hazard but the data is adequate to indicate that any hazard would be at a dose higher than those for other endpoints.

Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x 10–3 per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The value seems to be scientifically supported and its derivation is clearly described.

6. Question on the Executive Summary
The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

The summary states:
“Inadequate information exists to draw conclusions regarding male reproductive effects, female reproductive effects developmental effects, changes in body weight, adrenal function, immune status or mortality. “

Tier 1: Recommended Revisions
It would be more accurate to state that “no selective developmental or reproductive toxicity was found”. Evidence of any developmental and reproductive toxicity was complicated by maternal toxicity and/or issues with the maternal animals being exposed to the test material. In general any sign of developmental or reproductive toxicity occurred only at doses at or above 1000 mg/kg.

Tier 3: Future Considerations
Developmental neurotoxicity should also be included in this statement.

Dr. Maria Morandi

Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods. Question 1: Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

As is the case with tert-Butyl Alcohol (tBA), the strategy for literature searches does not follow all the recommendations in NRC (2011), except for the selection of studies to be included in the evidence tables. However, it is highly unlikely that following strictly all the NRC (2011) guidelines would have resulted in findings of additional relevant studies.

The general strategy used by EPA consists of starting with a very broad, ETBE-specific search of scientific literature databases, and other sources, and then perform screenings for identifying and selecting the studies relevant to the risk assessment, and classifying them into well-defined categories. The search in scientific literature indexes was updated continually through November 2015 or December 01, 2016 (for TSCATS2). Overall, this approach is well explained and appropriate for the goal of maximizing the likelihood of identifying all relevant studies for the assessment. Studies were systematically included in HERO in the corresponding categories. A clarification is needed as to why the search was not updated to 12/2016 across the board for all sources of potentially relevant citations.

The four scientific databases for the chemical-specific search likely encompass the large majority of the ETBE-relevant, peer reviewed articles, but there should be a statement for why these were the only indexed databases used. Consistent with the broad search strategy, searches were not topic-limited in the initial state of the search. The search keywords do not include all synonyms for ETBE, so a clarification should be provided for why these names were selected among all of those others available for ETBE.

Additional research strategies included manual searches of citations in two review articles, and a personal communication from the Japan Petroleum Energy Center. This is appropriate. However, there should be clarifications for: 1) why citations were only searched manually in two review articles and not other peer-reviewed sources publication, and 2) searches in other sources of citations such as reviews by other national and international agencies or publications (e.g. master/doctoral theses, grey literature, etc.). A short explanation should be provided if or how searches for these additional sources were not performed, beyond public requests for information.

Screening for pertinence and categorization are well described in the narrative, and are clearly depicted in Figure LS-1. Screening criteria are presented in detail in Table LS-3 and are appropriate. The results are accessible in HERO. Clarification should be provided for the exclusion of all three studies (not just for Dorman et al., 1997) from the 33 studies categorized as Sources of Health Effects Data (page xxvii and Figure LS-1) leading to final list of 30 to be included in the database for risk assessment.

Criteria for quality evaluation of studies are well described and appropriate, and they were been applied to the selected animal studies. As is the case with risk assessment of TBA, it is not possible to affirm that the criteria were applied objectively without performing an in-depth review of the quality evaluation process as applied to each study, but there is no evidence that of differential application of study quality criteria.

Hazard Identification - Chemical Properties and Toxicokinetics

Question 2a: Is the information on chemical properties accurate?

Chemical properties presented for ETBE are accurate except for the following:

1. The description of the odor and taste of TBET in lines 5-6, page 1-1, is not completely accurate. Odor is not described in the literature as “highly objectionable”, but as being reminiscent of gasoline or ether vapor. The qualifier of “highly objectionable” applies only to the taste of ETBE.

2. The molecular weight of ETBE should be rounded to 102.18 because the citation provided, NLM (2016), and other sources list it as 102.176.

3. A density of 0.74g/cc at 20C is correct but note that the reference and link for the density of ETBE [ECHA (2016) -https://echa.europa.eu/registration-dossier/-/registered-dossier/15520] does not show this density value for 20C but a density of 0.75g/cc at 15C. An alternative citation to the density of ETBE at 20C is NLM (2016), which shows a density of 0.74 g/cc.

4. The solubility of ETBE in water appears not to be correct. The Dragos and Diaz (2001) citation does not show a value of 2.37 g/l (several values are shown in Table 1 of the publication), so this may not be the appropriate citation. NLM (2016) shows a value of 1.2 g/l at 20C. ECHA (2016); https://echa.europa.eu/registration-dossier/-/registered-dossier/15520/4/9) shows a range of experimental water solubility values measured at 8 different temperatures ranging from 278.15K (5C) to 313.15 K (40C) at a pH of approximately 7 (primary reference is Gonzalez-Olmos & Iglesias (2008), Chemosphere 7, 2098-2105). ETBE solubility in water at 20 and 25C was measured at 16.4 g/l and 14.3 g/l, respectively.

5. The listings for log octanol/water partition coefficients (Kow) are not clear. The Montgomery, (1994) report derived a Kow of 1.48 as the average of two measurements made at two different ETBE concentrations and 25C. This value and the original report are also cited by ECHA (2016). However, Table 1 also shows a value for log oil/water partition of 1.74 at 25C

cited to (2001) which designates it as a Kow. Log oil/water is an old term that does not correspond to the measurement or specific application of this property. The 1.74 is citied was cited from a government report (reference # 30 in Dragos and Diaz) not available to this reviewer, and that there is no notation about the temperature at which it was determined, or if it represents an estimated rather than an experimental value, the latter being preferable. Unless the origin of the 1.74 value is ascertained, it would be advisable to list only the 1.48 value.

6. The value for viscosity at 40C is correct but showing viscosity at 20C (0.540 mm2/s) would be more consistent with the temperatures at which other properties are listed.

7. Rows/cells listing odor-related properties need to be revised to list clearly the "Odor detection threshold" (0.013ppm, which is correct), the "Odor recognition threshold" (0.024ppm, which is also correct) in two separate rows. The odor detection threshold in water is listed twice with two different values. The first value of 0.049ppm is correct but the second of 0.005ppm is not. Instead, this row/cell should list the "Odor recognition threshold (in water)" which is 0.106ppm (106µg/l).

Inhalation reference concentration for noncancer outcomes.

Question 3d. Section 2.2 presents an inhalation reference concentration of 9 x 100 mg/m3, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

As is the case with tBA, there is essentially no discussion of the comparative pathology between the rat and the human kidney for the specific lesions selected for deriving candidate reference values for non-cancer effects. EPA argues that individual lesions, rather than the cluster of lesions that defines CPN in the rat, may occur in the human kidney, and that exacerbation of any of these lesions is likely to reflect cell injury also relevant to humans. This is a reasonable argument, but it should be better supported by some explicit discussion of the comparative nephropathology in both species in order to further support their use in reference value derivation.

If EPA's argument for selecting some of these outcomes for RfC derivation is accepted based on the human relevance of the individual lesions, the value of 9 x 100 mg/m3 is supported by the selection of key studies, and the clear description of its derivation in the main document with details provided in the Supplemental Information. Lacking relevant human studies, EPA appropriately selected the set of ETBE inhalation studies suitable for derivation of the RfC, and identified key outcomes for modeling across these studies. These included increases in absolute kidney weights in males and females in two subchronic studies, and urothelial hyperplasia in male rats and increased CPN severity in males and female rats in one chronic study. Adjustments for intermittent concentrations, human equivalent concentration derivation, and benchmark modeling or extrapolation for deriving PODs was done following EPA guidelines. Uncertainty

factors were applied to the PODs also following EPA guidelines. The justification for selection of urothelial hyperplasia as the basis for the RfC derivation instead of change in kidney weights because of specificity is reasonable. In addition, the RfC was derived from the only chronic study available, which is a high quality study. The assessment describes clearly all the limitations and the estimates. All the candidate values except for one (based on increased CPN severity in male and female rats in the chronic study) are in the order of a factor of two from the selected RfC, so it is relatively consistent with most of the others. This reviewer does not have an alternative approach for this derivation.

Inhalation unit risk for cancer.

Question 4e: Section 2.4 presents an inhalation unit risk of 8 x 10–5 per mg/m3, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

Given the limitations of the data available for deriving an inhalation unit risk for ETBE to just one study; lack of chemical-specific MOA, and in light of the multiple lines of evidence for the carcinogenic potential of ETBE, the derivation of and the value for the unit risk are scientifically supported, with all the caveats clearly described in the assessment. The critical uncertainty is that there is only one study available for this derivation, and that tumor incidence was increased at only at one dose level, the highest. Nonetheless, EPA provides guidance for deriving unit risks even in these cases, and this guidance was followed for arriving at the 8 x 10–5 per mg/m3 estimate. The derivation is described clearly in section 2.4 of the assessment and section C.1.2. of the Supplemental Information. This reviewer does not have an alternative approach for estimating the inhalation unit risk factor.

Dr. Karen Chou

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods. Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

Yes, the strategies are clearly described.
Recommended correction, p. 1-2, Line 7: Citation mistake. It should be Nihlen et al., “1998a”.

2a. Chemical properties. Is the information on chemical properties accurate?

Please provide temperature for water solubility.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

The information provided on P. 2-21 and in Appendix B 1.5 is sufficient for understanding the rational of the application of the PBPK model in the current assessment.

2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

Yes, the use of the rate of metabolism of ETBE for route-to-route extrapolation is well justified.

3a. Noncancer kidney toxicity (Sections 1.2.1 (p. 1-4 to p. 1-36; 1.3.1 (p. 1-109 to p. p. 110)). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

The conclusion and supporting rationales are sufficiently described. Following are a few suggested modifications:

Suggested consideration: P. 1-21, Line 5-6: please provide reference(s) for this statement, "Acetaldehyde is metabolized further in the liver and is not thought to play a role in extrahepatic toxicity". Is there a reference to support this statement? Acetaldehyde is in the circulation after exposure to ETBE.

Suggested clarification, P. 1-31, Line 3: "respectively"? Please clarify.

Suggested addition, P. 1-32, Line 37 – P. 1-33, Line 2: An explicit statement here may be helpful: The effect of proximal tubule proliferation is not male-rat alpha2u-globulin specific.

Suggested deletion, p. 1-33, Line 14: "...a dose-response for ~~at~~ all of the endpoints..."?

Suggested addition, p. 1-33, Line 29: ..., suggesting ETBE and/or other metabolites, in addition to tBA, cause hyaline droplet accumulation.

Suggested addition, p. 1-35, Line 19-20: ... be entirely explained by the **male rat-specific** alpha2u-globulin or **rat-specific** CPN processes.

Recommendation: P. 1-110, Line 25, "Appendix B 1.5.4": Should this be Appendix B.1.5?

3b. Noncancer toxicity at other sites (Sections 1.2.2 (p. 1-36 to p. 1-56), 1.2.3 (p. 1-56 to 1-88), 1.2.4, (p. 1-88 to 1-100) 1.2.6, 1.3.1). The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.

- Liver effects: Suggestive evidence
- Developmental toxicity: Inadequate evidence
- Male and female reproductive toxicity: Inadequate evidence

Recommended clarification, P. 1-37, Line 12: ... from "parental and" adult "oral" exposure... . Please clarify that the F-1 were exposed to ETBE and metabolites through F-0 parental exposure during premating, mating, gestation and lactation.

Recommended: P. 1-51, Line 29-30: The information provided in Line 26-29 on this page does not lead to the conclusion on Line 29-30. Centrilobular hypertrophy is one of a sequence of biological responses to ETBE or its metabolites. Some of the biological responses may lead to autophagy-mediated restoration and regeneration. Nonetheless, autophagy could also lead to tumorigenesis. (For the relationships among oxidative stress, autophagy, cellular restoration, tissue regeneration, and tumorigenesis, also see comment below for P.1-55, P. 89, Lines 2-11.

Recommended addition, p. 1-54, Line 28: (1) Please provide description of temporal relationship observed in existing studies. (2) Please describe and explain how and what kind of temporal relationship is needed for establishing evidence for oxidative stress as a MOA for liver effects.

Recommended considerations, P. 1-55, Line 1-2: It would be very helpful if the draft can provide explanation for this claim, "... not relevant to humans". If the explanation was provided earlier in the draft, the reviewer has missed it.

Recommended modification: p. 1-76, Line 11-14: Please provide the following clarification in the discussion. Animals age is a major difference between the studies by JPEC (2010b) and Medinsky et al. (1999). The reproductive organs in JPEC's study were examined in animals older than 2 yr, while the animals in the study by Medinsky et al. (1999) were much younger. These two studies are not comparable in terms of the stages of reproduction organs.
Please include testicular effects, such as mineralization and arteritis in 500 ppm and 1500 ppm treatment groups in the discussion. There are additional observed effects in Table L 1-6 of the report by JPEC (2010b), but omitted in the publication by Saito et al. (2013).

Recommended correction: p. 1-90 Line 23: Please delete "~~or biological~~". Missing right atrioventricular valve is a biologically significant matter.

Suggestion, relevant endpoint for quantitative assessment: Number of fetuses is a relevant endpoint for quantitative analysis, which decrease is observed in two difference species in two different studies.

Suggested addition: Parental male pituitary weight, both absolute and relative weights, increased (14%) in 1000 mg/kg treatment group. See section 3.6.2.1.1 on P. 71 of the report by Gaoua, (2004b).

Suggested future considerations: F-1 pup body weight was significantly increased (10.8% in males and 11.5% in females) in the 500 and 1000 mg/kg treatment group. See original report Table 26 and 27 on p. 150 and 151 (Gaoua, 2004b). These pups, both males and females continuously gain more weight than the controls from day 1 to day 21, although only the gain in Day 4-7 in males reached statistically significance, see Table 19 of report by Gaoua 92004b). Please note that the increase in body weight in the 1000 mg/kg treatment group is not caused by smaller litter size, because, the number of pups surviving 21 days was significantly greater than that of the control, see p.133 of the original report by Gaoua (2004b).

3c. Oral reference dose for noncancer outcomes. Section 2.1 (p. 2-1 to p. 2-10) presents an oral reference dose of $5x10^{-1}$ mg/kg–day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

Suggested addition: P. 2-6, Line 9-10, "... the ETBE oral database adequately covers ...reproductive and developmental effects": The statement is made without supporting evidence. Please conduct dose response analyses (reference doses) for reproductive and developmental effects to support this statement.

Recommended modification, P. 2-6, Line 9-10, "... the ETBE oral database...does not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD": Please modify or delete this statement. This statement deviates from the logic of scientific investigation: additional studies will always carry the possibility of identifying a more sensitive target tissue or a more sensitive species.

Recommended deletion, P. 2-10, Line 3, "only kidney effects were identified as hazard": This is statement is not true; many other effects are observed.

Need correction, P. R-10: The link to US EPA (2002) does not lead to the correct document.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 (p. 2-11 - p. 2-20) presents an inhalation reference concentration of 9 x 10^0 mg/m^3, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

Suggestion: When reproductive and developmental endpoints are relevant to human health and when the data are sufficient for reference dose derivation, reference doses can be developed to support the statement on P.15, Line 21- 23, "The ETBE inhalation database... adequately covers all major systemic effects, including reproductive, developmental, ...effects."

Recommended modification, P. 2-15, Line 23-24, "... the ETBE inhalation database...dose not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD": Please modify or delete this statement. This deviates from the logic of scientific investigation: additional studies will always have the possibility of identifying a more sensitive target tissue or a more sensitive species.

4a. Cancer modes-of-action in the liver. As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPARα, PXR, and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

The conclusion is scientifically supported. Please also see following comments.

P.1-55, P. 89, Lines 2-11, "This observation suggests that these transient effects are not associated with the observed rat liver tumorigenesis": The "transient" effect on liver weight, hepatic cell proliferation, and markers of apoptosis (i.e. lack of consistent temporal trend) may reflect the timing of the protective effect of autophagy on liver pathological effects. CYP2E1 is significantly induced by ETBE in week 1, and less so in week 2 (Kakehashi et al., 2013). Oxidative stress can cause CYP2E1-mediated autophagy. Consequently, autophagy ameliorates inflammatory gene expression and hepatic tissue remodeling. On the other hand, autophagy defects have been related to tumorigenesis (Levine and Kroemer 2008); it seems to play a dual role in cancer, tumor initiation and tumor suppression (Guo et al. 2013). Therefore, these transient effects may or may not be associated with tumorigenesis. Transient effects observed in ETBE studies could be the cause or consequence of the protective autophagy, which itself is a transient biological response. Here is more direct explanation on the transient elevation of PPARalpha observed by Kakehashi et al. (2013): CYP2E1 can down-regulates PPARalpha expression. The increase in CYP2E1 expression could explain the transient elevation of PPARalpha in week 1and subsequent lowered expression of PPARalpha inWeek 2. This

transient expression of PPARalpha is evident in Table 2 of the original publication by Kakehashi et al. (2013).

For future consideration, P. 1-53, Line 33-34: ALDH1b1 polymorphism among Caucasians has been associated with susceptibility to acetaldehyde toxicity.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2 (p. 110 to p. 1-114), and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. ~~Does the classification give appropriate weight to the results from initiation–promotion studies? Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported~~ Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

Inclusion of the 2-stage initiation-promotion studies in human cancer hazard characterization is sufficiently justified and scientifically supported.

Suggested addition, P. 1-112, Line 26: An increase in total oncologically relevant pathologies of the forestomach was observed in male Sprague-Dawley rats of the 250 mg/kg treatment group (Maltoni et al., 1999).

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

~~Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for quantitative analysis, and whether the Saito et al. (2013) study is suitable for this purpose.~~

Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the Saito et al. (2013) study is a suitable basis for this quantitative analysis.

US EPA's Guidelines for Carcinogen Risk Assessment does not prevent or prohibit the derivation of quantitative analysis for a suggestive cancer causing agent. In the case of ETBE, it is not assigned the descriptor of "Likely to be carcinogenic to Humans" because of the lack of evidence in a second species other than rats. The existing two 13-week mouse studies have rather small number of observations in each treatment groups. The reviewer agrees that the quantitative analysis should be conductive, when data from one species clearly support the likelihood of carcinogenicity.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1×10^{-3} per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The value is scientifically supported and its derivation clearly described.

4e. Inhalation unit risk for cancer. Section 2.4 presents an inhalation unit risk of 8×10^{-5} per mg/m^3, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The value is scientifically supported and its derivation clearly described.

5. Question on Susceptible Populations and Lifestages. Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

Suggested additions:

An isoform of ALDH, ALDH1B1, is present in the gastric and intestinal mucosa (lamboeuf et al. 1981). The isoform ALDH1B1 is highly expressed in the gastrointestinal epithelium. It plays an important protective role against carcinogenicity of acetaldehyde. The role of ALDH1B1 in acetaldehyde metabolism has been proven in both mice and human. Human ALDH1b1 has at least four variants among Caucasians. Carriers of some of the less active ALDH1b1 variants have been shown to be susceptible to acetaldehyde toxicity.

Placental transfer of acetaldehyde has been demonstrated in both humans and animals (Blakley and Scott 1984; Karl et al. 1988). In addition, fetuses have less capacity to detoxify acetaldehyde. In humans, regardless of the genotype, the amount of ALDH2 expressed at fetal stage is about half of the adult level (Yoshida et al. 1990). When both the pregnant woman and the fetus carry the homozygous ALDH2 of the variant genotype, the fetus would belong to the highest risk group among all genetically identified susceptible populations.

Additional susceptible population include chronic alcohol users, and individuals who are also exposed to MTBE and tBA.

6. Question on the Executive Summary. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

Suggestion: For reference dose derivations, please include all endpoints considered.

Recommended addition, P. xxi, Line 14-15: There are also noncancer liver effects.

Recommended clarification, P. xxi, Line 33-34, and P. xxiii, Line 12-13 "... are <u>appropriate for identifying a hazard to the kidney</u>.": Does this intend to say that these parameters are relevant endpoints for assessing dose-response relationship?

Recommended modification, P. xxi, Line 37: "No additional histopathological findings were observed" is too strong a statement here, because there are other histopathological findings.

Suggested addition, P. xxii, Line 11-13: Please add descriptions of the findings from existing reproductive and developmental studies to the Executive Summary. These may include degenerative spermatocytes, sperm cell DNA breaks, and decrease in sperm numbers from inhalation exposure, and increases in plasma estradiol concentration in male rats, decreases in number of fetuses, missing missing right atrioventricular valve in the offspring in two studies with two species (rabbits and rats) from oral exposure.

Suggestion, P. XXV, Line 18-19: Susceptible population include individuals carrying ALDH2 and ALDH1b1 variants with low catalytic activity toward acetaldehyde, fetuses, alcohol users, and individuals who are also exposed to MTBE and tBA. Please see comments for Question 5

<u>Supporting References</u>

Blakley PM, Scott WJ. 1984. Determination of the proximate teratogen of the mouse fetal alcohol syndrome. Toxicol Appl Pharmacol 72(2): 364-371.

Guo Jessie Y, Xia B, White E. 2013. Autophagy-Mediated Tumor Promotion. Cell 155(6): 1216-1219.
Karl P, Gordon B, Lieber C, Fisher S. 1988. Acetaldehyde production and transfer by the perfused human placental cotyledon. Science 242(4876): 273-275.
Levine B, Kroemer G. 2008. Autophagy in the pathogenesis of disease. Cell 132(1): 27-42.
Yoshida A, Shibuya A, Davé V, Nakayama M, Hayashi A. 1990. Developmental changes of aldehyde dehydrogenase isozymes in human livers: Mitochondrial ALDH2 isozyme is expressed in fetal livers. Experientia 46(7): 747-750.

Dr. Janet Benson

I have read Appendix B – ETBE Supplemental Information with focus on B.1.5, Borghoff et al, 2016 and Salazar et al, 2015.

It is not clear to me at this point what was done by EPA to develop a route to route extrapolation based on the models of Borghoff et all and Salazar et al. It is not clear how an equivalent internal dose was derived – based on what exposure scenario and what doses? There is discussion of the role of induction and de-induction (page B-23) that did not appear to be a big component the models developed by these two investigators.

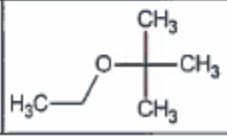
What I didn't see that was certainly present in the results provided by Borghoff et al, is a discussion of non-linear kinetics at higher doses possibly altering mechanisms of toxicity. This would be relevant to how internal doses might be derived.

Is the model being described designed to address liver and kidney tumors only, or toxicity in general. Was binding of TBA or ETBE to α2u-globulin included in the model? Is Figure B-3 the end result of the modeling?

Table 1-1. Physicochemical properties and chemical identity of ETBE

Characteristic or property	Value	Reference
Chemical name	2-ethoxy-2-methylpropane 2-methyl-2-ethoxypropane	NLM (2016)
Synonyms	ethyl *tert*-butyl ether ethyl *tert*-butyl oxide methyl-2-ethoxypropane tert-butyl ethyl ether ETBE	NLM (2016)
Chemical formula	$C_6H_{14}O$	NLM (2016)
CASRN (Chemical Abstracts Service Registry Number)	637-92-3	NLM (2016)
Molecular weight	102.17	NLM (2016)
Melting point	−94°C	Drogos and Diaz (2001)
Boiling point	73.1°C	ECHA (2016)
Density at 20°C	0.74 g/cm³ @ 20°C	ECHA (2016)
Water solubility	2.37 g/L 1.2×10^4 mg/L 20°C	Drogos and Diaz (2001) NLM
Partition coefficients: Log oil/water at 25°C Log K_{ow}	 1.48 1.74 1.92	Montgomery (1994) incomplete reference Drogos and Diaz (2001) NLM
Viscosity at 40°C	0.47 mm²/s	ECHA (2016)
Vapor pressure	17kPa@ 25°C 124 mmHg @ 25°C	NLM (2016)
Henry's Law Constant	1.39×10^{-3} atm-m³/mol @ 25°C	NLM (2016)
Odor detection in air	0.013 ppm (0.054 mg/m³)	Vetrano (1993)

This document is a draft for review purposes only and does not constitute Agency policy.

Characteristic or property	Value	Reference
~~Detection threshold~~ Odor. Recognition threshold in air	0.024 ppm (0.1 mg/m³)	Vetrano 1993
Taste detection threshold (in water)	0.047 ppm (47 µg/L)	Vetrano (1993)
Odor detection threshold (in water)	0.049 ppm (49 µg/L)	Vetrano (1993)
Odor detection threshold (in water)	0.005 ppm (5 µg/L)	Vetrano (1993)
Conversion factors	1 ppm = 4.18 mg/m³ 1 mg/m³ = 0.24 ppm 1 mg/m³ = 102,180 mmol/L	TBA doc has references
Chemical structure		HSDB (2012)

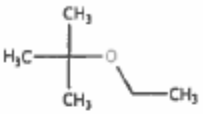
Substance Name: Ethyl tert-butyl ether
RN: 637-92-3
UNII: 3R9B16WR19
InChIKey: NUMQCACRALPSHD-UHFFFAOYSA-N

Note
Gasoline additive

Classification Code
TWA (5 ppm)

Molecular Formula
C6-H14-O

Molecular Weight
102.176



All | Links to Resources | Names & Synonyms | Registry Numbers | Structure Descriptors | Toxicity | Physical Properties

Links to Resources

NLM Resources (File Locators)

CCRIS	HSDB	PubMed	PubMed Toxicology
DrugPortal	ITER	PubMed AIDS	RTECS
EMIC	MeSH	PubMed Cancer	TOXLINE
Haz-Map	PubChem	PubMed Central	

Regulatory Agencies (Superlist Locators)

CA65	MTL	TSCAINV
HPV	TLV	

Other Resources (Internet Locators)

CAMEO	EINECS	IUCLID	SRC BIODEG
CTD	EPA ACToR	NIST WebBook	SRC DATALOG
ECHA	EPA CompTox	NTP DBS	USA.gov
eChemPortal	EPA SRS	OSHA ChemDB	

Search for this InChIKey on the Web

Names and Synonyms

Name of Substance
Ethyl tert-butyl ether

Synonyms

1,1-Dimethylethyl ethyl ether	EC 211-309-7	Ethyl tert-butyl ether	NSC 1069
2-Ethoxy-2-methylpropane	EINECS 211-309-7	Ethyl tert-butyl oxide	tert-Butyl ethyl ether
2-Methyl-2-ethoxypropane	ETBE	HSDB 7867	UNII-3R9B16WR19
CCRIS 6061	Ethyl 1,1-dimethylethyl ether	Methyl 2-ethoxypropane	

Systematic Names

2-Ethoxy-2-methylpropane	2-Methyl-2-ethoxypropane	Ether, tert-butyl ethyl	Propane, 2-ethoxy-2-methyl-

Superlist Names

Ethyl t-butyl ether	Propane, 2-ethoxy-2-methyl-

Registry Numbers

CAS Registry Number
637-92-3

FDA UNII
3R9B16WR19

System Generated Number
0000637923

Structure Descriptors

ChemIDplus - 637-92-3 - NUMQCACRALPSHD-UHFFFAOYSA-N - Ethyl tert-butyl et... Page 1 of 3

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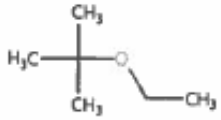
Substance Name: Ethyl tert-butyl ether
RN: 637-92-3
UNII: 3R9B16WR19
InChIKey: NUMQCACRALPSHD-UHFFFAOYSA-N



Note
Gasoline additive.

Molecular Formula
C6-H14-O

Classification Code
TWA (5 ppm)

Molecular Weight
102.176

All | Links to Resources | Names & Synonyms | Registry Numbers | Structure Descriptors | Toxicity | Physical Properties

Names and Synonyms

Name of Substance
Ethyl tert-butyl ether

Synonyms

1,1-Dimethylethyl ethyl ether	EC 211-309-7	Ethyl tert-butyl ether	NSC 1069
2-Ethoxy-2-methylpropane	EINECS 211-309-7	Ethyl tert-butyl oxide	tert-Butyl ethyl ether
2-Methyl-2-ethoxypropane	ETBE	HSDB 7867	UNII-3R9B16WR19
CCRIS 6061	Ethyl 1,1-dimethylethyl ether	Methyl-2-ethoxypropane	

Systematic Names

2-Ethoxy-2-methylpropane	2-Methyl-2-ethoxypropane	Ether, tert-butyl ethyl	Propane, 2-ethoxy-2-methyl-

Superlist Names

Ethyl t-butyl ether	Propane, 2-ethoxy-2-methyl-

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1S/C6H14O/c1-5-7-6(2,3)4/h5H2,1-4H3

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InChIKey

NUMQCACRALPSHD-UHFFFAOYSA-N

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O(C(C)(C)C)CC

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Toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LC50	inhalation	123gm/m3/15M (123000mg/m3)	BEHAVIORAL: GENERAL ANESTHETIC	Anesthesiology. Vol. 11, Pg. 455, 1950.
rabbit	LD50	skin	> 2gm/kg (2000mg/kg)	GASTROINTESTINAL: OTHER CHANGES SENSE ORGANS AND SPECIAL SENSES: OTHER CHANGES: OLFACTION GASTROINTESTINAL: "HYPERMOTILITY, DIARRHEA"	National Technical Information Service. Vol. OTS0557630,
rat	LC50	inhalation	> 5880mg/m3/4H (5880mg/m3)		National Technical Information Service. Vol. OTS0557661,
rat	LD	oral	> 5gm/kg (5000mg/kg)		National Technical Information Service. Vol. OTS0557630,

Physical Properties

Physical Property	Value	Units	Temp (deg C)	Source
Melting Point	-9.40E+01	deg C		EXP
Boiling Point	73.1	deg C		EXP
log P (octanol-water)	1.920	(none)		EST
Water Solubility	1.20E+04	mg/L	20	EXP
Vapor Pressure	124	mm Hg	25	EXP
Henry's Law Constant	1.39E-03	atm-m3/mole	25	EST
Atmospheric OH Rate Constant	8.84E-12	cm3/molecule-sec	25	EXP

Physical property data is provided to ChemIDplus by SRC, Inc.

This is what I get when I click on Drogos + Diaz 2001.

EPA United States Environmental Protection Agency

HERO: Health and Environmental Research Online

You are here: EPA Home » Research » NCEA » HERO » Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation

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Reference Type
Technical Report

Subtype
EPA Report

Title
Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation

Author(s)
U.S. Environmental Protection Agency :: U.S. EPA

Year
2014

Publisher
Risk Assessment Forum, Office of the Science Advisor

Location
Washington, DC

Report Number
EPA/100/R-14/002F

Number of Pages
109

Language

Guidance for applying quantitative data to develop data-derived extrapolation factors for i... Page 2 of 2

English

URL
https://www.epa.gov/risk/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and

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  Not Chemical Specific
(https://hero.epa.gov/heronet/index.cfm/project/page/project_id/36/filtercondition/or/usac
  Guidance, notices
(https://hero.epa.gov/heronet/index.cfm/project/page/project_id/36/filtercondition/or/usac
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(https://hero.epa.gov/heronet/index.cfm/project/page/project_id/2297)

OPPT REs
- OPPT_Asbestos_F. Human Health
(https://hero.epa.gov/heronet/index.cfm/project/page/project_id/2533)
  Libby_IRIS_Final
(https://hero.epa.gov/heronet/index.cfm/project/page/project_id/2533/filtercondition/or/u

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HERO Iteration 45

Dr. Marvin Meistrich

3a. Noncancer kidney toxicity:

I agree. No specific comments at this point, awaiting input from panel members with more expertise in kidney effects.

3b. Noncancer toxicity at other sites:

I agree that these other sites should not be used as the basis for deriving noncancer or inhalation reference concentrations (delete "purposes" from text).

Liver effects: No specific comments at this point, awaiting input from panel members with more expertise in liver effects.

Developmental toxicity: Almost all assays show no significant developmental toxicities of ETBE oral administration at 1000 mg/kg-d

Three skeletal/visceral developmental endpoints showed significant increases at the high dose but the biological/toxicological significance of these was minimized. I think these aspects need further discussion.

Male reproductive toxicity: Most assays show no toxicities but sensitive analyses of sperm DNA damage and motility show effects of ETBE inhalation at 7000 mg/m^3 (~700 mg/kg-d) in mice

No male reproductive toxicity (testis weight sperm number, morphology, motility, histopathology, androgen-dependent accessory organ weights, and fertility) observed in rats with oral administration of up to 1000 mg/kg-d for times up to 6 months. Little weight is given to reproductive toxicity from the reported hormone changes after doses of 1200 or 1800 mg/kg-d because the baseline levels of estradiol were unusually low, changes in testosterone were not significant, and accessory reproductive organ weights did not change.

Inconsistent results were reported after inhalation exposure of rats to ETBE at 7000 or 21000 mg/m^3; one report showed degeneration in about 10% of tubules but another report did not. In either case, the changes were small since testis weights were not affected.

Inconsistent results on testicular histopathology were reported after inhalation exposure of mice to ETBE at 7000 (500-900 mg/kg-d) or 21000 mg/m^3; this could have been due to a strain difference. In either case, the changes were small since testis weights were not affected. Further analyses of the apparently more sensitive inbred strain reveal increase in DNA strand breaks and oxidative DNA damage in sperm, which show a reduced number of sperm with rapid movement. In addition, Aldh2 genetic deficiency these defects and sperm number reductions were observed at 2100 mg/m^3.

Female reproductive toxicity: no toxicities observed at maximal doses used.
Female reproductive toxicity in rats: ovarian weights, counts of primordial and growing follicles, estrous cyclicity, pregnancy rates, embryo survival, and overall pup survival to weaning unaffected by following conditions:

Oral administration of up to 1000 mg/kg-d for up 10 weeks plus during development and pregnancy
Inhalation of up to 5000 ppm (20900 mg/m^3) for up to 2 years
Female reproductive toxicity in rabbits: Fetal implantation, viability, development, and body weight were unaffected by ETBE by gavage to rabbits up to 1000 mg/kg-d during pregnancy

3c. Oral reference dose of 0.5 mg/kg/day based on urothelial hyperplasia in male rats.

Use of kidney endpoint seems appropriate. Reproductive and developmental toxicity (NOEL=1000 mg/kg-d), higher doses than kidney endpoint (LOEL=171 mg/kg-d)

3d. Inhalation reference concentration of 9 mg/m^3 based on urothelial hyperplasia in male rats.

Use of kidney endpoint (LOEL=1500 ppm, 6270 mg/m^3) seems appropriate. However, sperm DNA damage (DNA breaks, 8-oxo-deoxyguanine) in B6 mice were observed at similar exposure levels and could also be considered.

Comments about presentation in Draft Review

Throughout male reproductive text; Aldh2+/- mice are referred to as "heterogeneous"; the correct term is "heterozygous"

Table 1-14; Page 1-60: The dose for inhalation studies is mistakenly given here as "mg/kg-d"; it should be "mg/m^3".

Figure 1-12 (page 1-75): Absolute testis weight is a much better and more precise measure of toxicity. Testis weight does not vary with body weight changes.

Dr. Jeffrey Fisher

2. Chemical Properties and Toxicokinetics
2a. Chemical properties. Is the information on chemical properties accurate?

Add units for molecular weight. If log oil/water is octanol/water, please state as such. Vapor pressure in mm Hg would be useful.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

Instead of using a default method (page 2-3 tox review for ETBE) to calculate HED I would have created an ETBE and TBA model for human inhalation using the published human PBPK model of Nihlen and Johanson, 1999. In this paper serum time course and exhaled breath samples were taken for 5, 25, and 50 ppm 2 hr inhalation exposures.

Was the model of Borghoff modified by EPA? Is so are these changes provided?

Looking at the acslX model code obtained from the HERO data base it appears the CL (concentration in liver) and not CVL (concentration in venous blood) was used to estimate metabolic rate. Since ETBE has moderate lipid solubility, this may skew the metabolic rate estimate some.

2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

My first question is that since the rate (near 2 mg/hr) is for a rather high ETBE inhalation concentration (Table 2.7, near 7000 mg/m3 or 1667 ppm) I would like to see a plot of rate of metabolism on the Y axis and on the X axis inhalation concentration, low ppm to 2000 ppm. After looking at the Salazar et al. 2015 paper, blood concentrations of ETBE exceed the Km value for hepatic metabolism of ETBE for high concentrations. Use of a MM equation to describe metabolism may lead to a non-linear function for rate of metabolism. I believe you assume that this function is linear.

With no clear smoking gun pertaining to MOA or correlations between dose metrics and metabolites (published modeling papers), this dose metric may be adequate. Without accounting for formation of acetaldehyde in the stoichiometry of ETBE metabolism, a more general estimate of total metabolic formation (and clearance of ETBE) makes sense.

4e. Inhalation unit risk for cancer. Section 2.4 presents an inhalation unit risk of 8 x 10–5 per mg/m3, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

Rat to human extrapolation: I would have created an ETBE and TBA model for human inhalation using the published human PBPK model of Nihlen and Johanson. In this paper serum time course and exhaled breath samples were taken for 5, 25, and 50 ppm 2 hr inhalation exposures.

Dr. Lawrence Lash

2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

The issue of the choice of dose metric for the ETBE toxicological review is most concisely discussed on page 2-21:

“A critical decision in the route-to-route extrapolation is the selection of the internal dose metric for establishing “equivalent” oral and inhalation exposures. For ETBE-induced liver tumors, the four options are the (1) concentration of tert-butanol in blood, (2) rate of tert-butanol metabolism in the liver, (3) concentration of ETBE in blood, and (4) rate of ETBE metabolism in the liver (Salazar et al., 2015). The major systemically available metabolite of ETBE is tert-butanol, which has not been shown to cause liver toxicity, so tert-butanol blood concentration and tert-butanol metabolism are not plausible dose metrics. ETBE in the blood also is not supported as a dose metric because liver concentrations of ETBE are more proximal to the site of interest. Liver concentration for ETBE will lead to a similar route-to-route extrapolation relationship as using liver metabolism of ETBE because metabolism is a function of the liver concentration. Since metabolism is saturable and the degree of metabolic saturation can vary with dose and dose-rate,

there is likely to be some difference between using these two metrics for extrapolation. Further, if the BMCL is in the linear metabolic range, then the route-to-route extrapolation will be independent of the choice between ETBE concentration in liver and ETBE metabolism. While this computational equivalence exists, use of the rate of metabolism of ETBE in the liver accounts for the possible role of acetaldehyde, the other metabolite of ETBE produced in the liver, which is a genotoxic carcinogen. Consequently, the rate of metabolism of ETBE was selected as the best available basis for route-to-route extrapolation."

The section noted above clearly explains that although ETBE is primarily metabolized to tBA, blood tBA concentration is not an appropriate dose metric because not all effects of tBA and ETBE are consistent with each other (specifically liver toxicity from ETBE and not from tBA). Blood ETBE concentration is also explained as not being an appropriate or the best choice because liver concentrations would be "more proximal to the site of interest." The document further explains that use of liver ETBE metabolism would be a better choice because of enzyme saturation and the potential to account for a role of acetaldehyde in ETBE toxicity. Overall, I agree that the rate of ETBE metabolism in the liver would seem to be the most appropriate dose metric. However, it does seem somewhat contradictory to exclude blood tBA concentration as a potential dose metric because of the absence of liver toxicity and then exclude blood ETBE concentration because liver ETBE concentration is more proximate to the "site of interest." In other words, the contradiction lies in using the absence of liver toxicity as part of the rationale on the one hand and then calling the liver the "site of interest" on the other hand.

Another contradiction is found in section 1.2.1 when discussing noncancer kidney effects. On page 1-36, lines 7-11, the document states the following:

"Noncancer kidney effects yielded consistent dose-response relationships using tert-butanol blood concentration as the dose metric, consistent with the hypothesis that tert-butanol mediates the noncancer kidney effects following ETBE administration. Based on dose-related increases in these noncancer endpoints in rats, kidney effects are a potential human hazard of tert-butanol exposure."

Unlike the later discussion that was specifically focusing on the dose metric for ETBE hazard, here the document seems to be concluding that blood tBA concentrations are the most appropriate dose metric. Clarification, with either a correction or more explanation of what is actually intended, is needed here.

The issue of the choice of dose metric for the ETBE toxicological review is most concisely discussed on page 2-21:

"A critical decision in the route-to-route extrapolation is the selection of the internal dose metric for establishing "equivalent" oral and inhalation exposures. For ETBE-induced liver tumors, the four options are the (1) concentration of tert-butanol in blood, (2) rate of tert-butanol metabolism in the liver, (3) concentration of ETBE in blood, and (4) rate of ETBE metabolism in the liver (Salazar et al., 2015). The major systemically available metabolite of ETBE is tert-butanol, which has not been shown to cause liver toxicity, so tert-butanol blood concentration and tert-butanol metabolism are not plausible dose metrics. ETBE in the blood also is not supported as a

dose metric because liver concentrations of ETBE are more proximal to the site of interest. Liver concentration for ETBE will lead to a similar route-to-route extrapolation relationship as using liver metabolism of ETBE because metabolism is a function of the liver concentration. Since metabolism is saturable and the degree of metabolic saturation can vary with dose and dose-rate, there is likely to be some difference between using these two metrics for extrapolation. Further, if the BMCL is in the linear metabolic range, then the route-to-route extrapolation will be independent of the choice between ETBE concentration in liver and ETBE metabolism. While this computational equivalence exists, use of the rate of metabolism of ETBE in the liver accounts for the possible role of acetaldehyde, the other metabolite of ETBE produced in the liver, which is a genotoxic carcinogen. Consequently, the rate of metabolism of ETBE was selected as the best available basis for route-to-route extrapolation."

The document is organized according to the standard manner for IRIS assessments. Section 1 presents the hazard assessment, and is appropriately divided into three main sections: 1) overview of chemical properties and toxicokinetics; 2) synthesis of evidence by organ or system; and 3) integration and evaluation. The document clearly describes the nature of the available literature, the criteria used to include or exclude studies, and the rationale for the choice of the principal studies for each effect. Section 2 is the section on dose-response analysis; it is divided into five main sections: 1) oral reference dose for effects other than cancer; 2) inhalation reference concentration for effects other than cancer; 3) oral slope factor for cancer; 4) inhalation unit risk for cancer; and 5) application of age-dependent adjustment factors. There are no additional studies that should or need to be added to the analysis.

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

Although both absolute and relative kidney weights are increased after ETBE exposure, the document clearly explains why absolute organ weight is a more reliable reflection of specific effects on the kidneys in that body weight changes will impact relative organ weights and potentially obscure effects of the chemical exposure.

Consideration of alpha-2u effects and their role in explaining renal tumors in male rats is presented in thorough and systematic manner. The document reviews the specific criteria that the EPA established many years ago regarding alpha-2u as a male rat-specific MOA. The database clearly supports the conclusion that the database is insufficient to conclude that ETBE induces alpha-2u globulin nephropathy.

I am bothered, however, by the use of urothelial hyperplasia as a surrogate for noncancer kidney effects. There is no known mechanistic link between bladder effects such as urothelial hyperplasia and the various types of kidney injury typically observed with chemicals similar to

ETBE. With that said, however, the conclusion in section 1.3.1 on page 1-109, lines 29-32 is appropriate:
The conclusion is:
"Urothelial hyperplasia in male rats, increased severity of CPN, increased blood biomarkers in male and female rats, and increased kidney weights in male and female rats are considered the result of ETBE exposure, independent (of) α_{2u}-globulin, and relevant for assessing human health hazard."

Thus, while I agree that the statement is correct and appropriate, the urothelial hyperplasia effect should really be considered separately from kidney effects. Furthermore, use of exacerbation of CPN and/or increased blood (serum) biomarker levels would seem more appropriate to quantify kidneys as a target organ for noncancer effects.

Specific Comments:
1) On page 1-5, lines 35-36: It is stated that single mouse inhalation study showed "weak increases in kidney weight." I think the text should be more specific as the descriptor "weak" is vague and not really informative.
2) Page 1-35, lines 20-21: Delete "renal" from the phrase "renal nephrotoxicity" as it is redundant.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

There are two primary issues to consider here. The first is the inclusion of 2-stage initiation-promotion studies. It is completely appropriate to include these here as they have relevance to consideration of ETBE hazard. One can consider that such studies can model situations where individuals may be exposed to ETBE subsequent to exposure to other carcinogens. Moreover, these studies exist and provide some of the only positive data.

The second issue concerns the EPA's evaluation for ETBE as having "suggestive evidence of carcinogenic potential in humans." I agree that this conclusion is scientifically supported by the data. This is despite any limitations, uncertainties, and modest inconsistencies in the database.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence." Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the Saito et al. (2013) study is a suitable basis for this quantitative analysis.

Section 2.3 discusses the dose metric (see comments above), application of a PBPK model for ETBE, and uncertainties in the analysis, and finally their use in calculation of an oral slope factor for cancer hazard assessment. Clear and specific references are made to how the present analysis adheres to the EPA's own guidelines, as expressed in their 2005 document. In section 2.4, the inhalation unit risk is discussed. Despite being considered only "suggestive" of being a human carcinogen, the explanation for conducting the quantitative analysis is clear, consistent with past practice, and is scientifically justified. Finally, the appropriateness of using the 2013 Saito et al. study as the basis for the analysis is clear and appropriately explained.

Dr. John Budroe

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

The current ETBE model (Borghoff et al., 2016) seems to be under-predicting the amount of ETBE that metabolizes to *tert*-butanol (tBA). A few adjustments to the model may be sufficient to more accurately predict less loss through exhaled breath and protein binding kinetics.

The following points summarize plausible adjustments to the ETBE model that have biological bases examined for compounds that metabolize to tBA in addition to ETBE, namely, methyl tert-butyl ether (MTBE) and tert-butyl acetate (tBAc).

1) Capacity-limited blood protein binding other than or in addition to α2u-globulin:

Previous studies of MTBE metabolism indicate blood protein binding and/or renal tubular reabsorption of tBA. Johanson et al. (1995) and Nihlen et al. (1998, 1998b) reported toxicokinetics and acute effects of inhalation exposure of 10 male human subjects to MTBE vapor at five, 25, and 50 ppm for two hours during light physical exercise. Authors noted some

exposure dependence for the urinary half-life with shorter values seen at the highest exposure level (50 ppm for 2 hours). A low renal clearance for tBA (0.6 to 0.7 mL/hour/kg) suggests extensive blood protein binding or renal tubular reabsorption of tBA.

The current Borghoff et al. (2016) model incorporates protein binding of tBA to α2u globulin (Williams and Borghoff, 2001) and renal tubular reabsorption of this protein as observed by Neuhaus (1986) in male rats. However, disproportionately less radiolabeled tBAc was found in feces after inhaling 100 ppm versus 1000 ppm for 6 hours in male rats (2.7 and 1% respectively; Cruzan and Kirkpatrick, 2006) which indicates that protein binding of acetates and esters that metabolize to tBA may also be saturating at higher levels of exposure. Over-predictions of ETBE and tBA levels in blood following ETBE inhalation observed in Figure 6 of Borghoff et al. (2016), along with evidence of low renal clearance of tBA in humans, suggests that capacity-limited blood protein binding may be occurring in male rats in addition to the α2u globulin-binding mechanism in male rats.

2) A greater rate of tBA metabolism is observed in male versus female rats.

tBA cannot be oxidized by alcohol dehydrogenase (ADH). tBA elimination occurs by other metabolic and excretion pathways (Plapp, 2015). Gender-stratified data from repeated dose studies of rats (Miller et al., 1997; Leavens and Borghoff, 2010) show that metabolism of tBA is induced by repeated doses in male but not female rats. The induction of tBA metabolism is also shown in mice (McComb and Goldstein, 1979) but the sex of these study subjects was not reported. It has been established that other forms of alcohol inhibit the production and increase the clearance of testosterone (Gordon *et al.*, 1976). It is also established that repeated doses of alcohol reduce the production of testosterone, reduce the inhibition of ADH by testosterone (Rachamin *et al*., 1984) and leads to greater alcohol metabolism. Studies of sex-specific P450 enzymes suggest that estrogen and testosterone play a role in regulating CYP2C7 and 2C11 in the rat (Bandiera and Dworschak, 1992). In the case of tBA, higher levels of these or similar acting CYP450 enzymes may inhibit the metabolism of tBA analogous to the inhibition of ADH in the metabolism of other alcohols.

In the current PBPK model, the omission of this gender-specific effect from repeated doses is to under-represent the rate of urinary clearance and over-represent the rate of clearance of tBA by exhaled breath.

Adjustment of the ETBE model to predict less loss through exhaled breath and capacity limited protein binding might result in the model predicting lower amounts of parent compound (ETBE) being metabolized to tBA, especially at higher doses.

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

Renal toxicity was correctly identified as a potential human hazard of ETBE exposure: effects in rats included increased kidney weight (males and females), urothelial hyperplasia (males), increased blood concentrations of total cholesterol, blood urea nitrogen, and creatinine. Renal toxicity was observed after both oral and inhalation exposures to ETBE. Additionally, a small increase in mouse absolute kidney weight after ETBE inhalation exposure was noted in the Document; however, a description of the study that generated this data was not provided. The consistency of effect across the several studies reported and across routes of administration provides confidence in the conclusion that ETBE is a renal toxicant. This conclusion is both scientifically supported and clearly described.

However, the Document overemphasizes the putative role of chronic progressive nephropathy (CPN) in the induction of renal toxicity by ETBE. It takes the somewhat contradictory position that although the individual renal toxicity endpoints that are claimed to comprise CPN are relevant to human health risk assessment if treatment-related, the "spectrum of effects" as a whole are not. For example, the Document states "Although mortality in the 2-year studies was significantly increased in ETBE-treated male and female rats compared with controls following oral and inhalation exposure (see Appendix B.1.5), causes of death were the result of age-associated diseases, such as CPN." If ETBE treatment causes increased mortality compared to controls as a result of exacerbating renal toxicity, then that increased mortality should be considered to be relevant to human risk assessment. The Document appears to indicate otherwise. Renal failure tends to increase in humans with age. To insist that exacerbation of rat renal dysfunction by a toxicant only be considered to be relevant to human risk assessment if there is an exact histopathological match between rats and humans is unwise.

3d. Inhalation reference concentration for noncancer outcomes**.** Section 2.2 presents an inhalation reference concentration of 9×10^0 mg/m^3, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

The inhalation reference concentration of 9×10^0 mg/m^3, based on urothelial hyperplasia in male rats (Saito et al., 2013) is scientifically supported. Urothelial hyperplasia is a relatively sensitive endpoint, and is relevant to human risk assessment. The derivation of the inhalation RfC using this endpoint is clearly and correctly derived.

However, this lesion endpoint may be a more specific indicator of kidney toxicity, compared with the relatively nonspecific endpoint of kidney weight change, but is not clearly a more sensitive indicator of kidney toxicity. Table 2-5 from the Document lists POD_{HEC} values for increased urothelial hyperplasia from the 2-year male rat study by Saito *et al.* (2013) and increased male rat absolute kidney weight from the 13-week study by JPEC (2008) of 265 and 111 mg/m^3, respectively. The Document also discusses the magnitude of change in absolute kidney weights, which increased in male and female rats exposed for 26 weeks compared with 13–18 weeks, suggesting that toxicity would be expected to increase with exposure durations greater than 13 weeks. This indicates that increased absolute kidney weights are a more sensitive indicator of kidney toxicity than increased urothelial hyperplasia.

Thus, since 13-week increased male rat absolute kidney weight is a more sensitive endpoint than 2-year increased urothelial hyperplasia and produces a more health-protective RfC value (4×10^{-1} mg/m^3 versus 9×10^{-0} mg/m^3 for urothelial hyperplasia), the 13-week increased male rat absolute kidney weight should be used as the candidate value for the RfC.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the Saito et al. (2013) study is a suitable basis for this quantitative analysis.

The "suggestive evidence" descriptor for ETBE is appropriate, as is a quantitative cancer dose-response analysis for ETBE. As noted in Charge Question 4c, "when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks". The study used for quantitative cancer risk assessment analysis, Saito *et al.* (2013) was well conducted and reported, had no confounders and received external peer review. Section 2.3 of the Document adequately describes the scientific quality of the Saito *et al.* 2013 study, but should elaborate on the utility of "providing a sense of the magnitude and uncertainty of potential risks". The Document notes that the United States is a major exporter of ETBE, producing 25% of the world's ETBE in 2012. Worldwide consumption of ETBE is concentrated in Western Europe, Eastern Europe and Japan. This indicates the potential for substantial worker, consumer and residential exposure to ETBE in both the United States and other nations, with potential concomitant health risks. The

quantitative cancer dose-response analysis contained in the Document is needed in order to provide a sense of the magnitude of potential cancer risk associated with ETBE exposure.

Finally, the Saito *et al.* (2013) study is definitely a suitable basis for a quantitative ETBE human cancer risk analysis. That study used a sufficient number of animals, was well designed and conducted, had no apparent confounders, received external peer review, and reported significant and dose-dependent increases in male rat liver tumors.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x 10–3 per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The Saito *et al.* (2013) study reported significant and dose-dependent increases in male rat liver adenomas and carcinomas. Rat liver adenomas are considered to be capable of progressing to carcinomas. These tumors are relevant to human cancer risk assessment. Additionally, the main metabolites of ETBE, acetaldehyde and TBA, are carcinogens, and acetaldehyde is also a known genotoxicant.

The following elements of the dose-response analysis and derivation of the oral slope factor were performed correctly and adequately described:

- The use of the BMDS multistage cancer model for the calculation of an inhalation POD.
- route-to-route extrapolation of the inhalation BMCL using the PBPK model for ETBE in rats described in Appendix B of the Supplemental Information document to derive an oral POD. The use of the liver metabolism rate of ETBE is a logical dose metric for route-to-route extrapolation.
- ¾ power body weight dose scaling between animals and humans
- use of the BMDS multistage cancer model to calculate a cancer slope factor via linear low-dose extrapolation.

There is no obvious alternative approach for developing an oral cancer slope factor for ETBE that would be more appropriate.

4e. Inhalation unit risk for cancer**.** Section 2.4 presents an inhalation unit risk of 8 x 10^{-5} per mg/m^3, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The same comments made in response to charge question 4d. (Oral slope factor for cancer) also apply to this charge question. The only difference in this case was, since the ETBE exposure in the key study was by inhalation, the route-to-route extrapolation of the inhalation BMCL using the PBPK model for ETBE in rats used to derive the oral cancer slope factor was not necessary.

There is no obvious alternative approach for developing an inhalation cancer unit risk factor for ETBE that would be more appropriate than the approached outlined in the Document.

References (not included in the Document or Supplemental Information)

Bandiera S, Dworschak C. (1992). Effects of testosterone and estrogen on hepatic levels of cytochromes P450 2C7 and P450 2C11 in the rat. Arch Biochem Biophys. 296:286-95.

Cruzan G, Kirkpatrick D. (2006).Tertiary-butyl acetate metabolism, toxicity, and human health considerations. Toxicological & Environmental Chemistry. 88:405-21.

Gordon GG, Altman K, Southren AL, Rubin E, Lieber CS. (1976). Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. N Engl J Med. 295:793-7.

Miller MJ, Ferdinandi ES, Klan M, Andrews LS, Douglas JF, Kneiss JJ. (1997). Pharmacokinetics and disposition of methyl t-butyl ether in Fischer-344 rats. J Appl Toxicol. 17 Suppl 1:S3-12.

Neuhaus OW. (1986). Renal reabsorption of low molecular weight proteins in adult male rats: alpha 2u-globulin. Proc Soc Exp Biol Med. 182:531-9.

Nihlen A, Walinder R, Lof A, Johanson G. (1998). Experimental exposure to methyl tertiary-butyl ether. II. Acute effects in humans. Toxicol Appl Pharmacol. 148:281-7.

Plapp BV, Leidal KG, Murch BP, Green DW. (2015). Contribution of liver alcohol dehydrogenase to metabolism of alcohols in rats. Chem Biol Interact. 234:85-95.

Rachamin G, Britton RS, Macdonald JA, Israel Y. (1984). The inhibitory effect of testosterone on the development of metabolic tolerance to ethanol. Alcohol. 1:283-91.

Dr. Isaac Pessah

3b. Noncancer toxicity at other sites (Sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1). The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.

- Liver effects: Suggestive evidence
  Scientifically supported and clearly described.
  Nine chronic oral or inhalation exposure studies ranging from 13 weeks to 2 years in duration are presented and reflect a strong database on liver effects. I agree that liver weight data should not be used for hazard identification due to several confounding issues (mortality, proliferative lesions, tumors (hyperplasia) with inhalation route). Relative liver weights (normalized to BW) were a consistent finding across studies but only reached significance at the highest dose at ≥16 weeks exposure, and were modest ranging from 8% to 27% across sexes-greater in males. Centrilobular hypertrophy and focal lesions seen at highest doses/concentrations that also increased liver weight, and appears to be a transient effect since 2-year oral or inhalation studies showed no evidence of centrilobular hypertrophy. Result from studies measuring serum enzymes were not consistent across studies and do not permit their levels to be used for deriving noncancer RfD(C). Overall, I agree that liver effects reported to date cannot be used reliably to derive oral and inhalation reference dose or concentration. Lack of clearly defined and consistent receptor mediated targets or signaling responses or crosstalk due to ETBE exposures (eg, PPAR□□□PXR, CAR) detracts from using liver effects for using liver effects for deriving noncancer reference doses/concentrations. The fact that mice lacking Aldh2 expression are more sensitive to liver effects of ETBE implicate acetaldehyde as the toxophore mediating both noncancer liver toxicity and induction of liver tumors (via inhalation), though direct MOAs (or AOPs) for each process have not been adequately addressed to support using acetaldehyde for deriving references values.

- Developmental toxicity: Inadequate evidence

- Male and female reproductive toxicity: Inadequate evidence
  At the highest maternal doses, *in utero* exposures resulted in increased relative liver weights in F1, although mechanisms are lacking. Although there are several studies that have addressed ETBE reproductive effects on adult male and female rodent models (mainly rat), the results in most studies did not find evidence of developmental toxicity in males or females. Two studies found suggestive evidence of male reproductive sperm pathology and function, testicular pathology, etc., but these results were variable, seen at high doses/concentrations, and not replicated by several other studies, including a 2-year multigenerational study. Male Aldh2 KO mice showed greater sensitivity to reproductive outcomes of ETBE-exposures, and this issue deserves more investigation since it may lead to understand one or more AOP involved in noncancer toxicity of ETBE. Influences of ETBE on female reproduction are not sufficient to warrant their use to derive a reference dose or concentration.

  There are at least 3 studies that have addressed developmental neurotoxicity in male and female F1, offspring. The results of these developmental studies, which included assessments of early reflexes, avoidance, ASR, and motor activity, were negative. I agree that they are inadequate evidence to inform reference doses/concentrations. However, I would like to emphasize that there are no studies that examine spatial memory or other types of cognition, or measures of activity-dependent plasticity and behavioral outcomes associated with social/anxiety domains. In my opinion, these are major gap in our knowledge about developmental neurotoxicity of ETBE,

and raises several uncertainties, especially since we have no or little information about mechanisms that produce renal and liver toxicities. This may point to future studies. Recent, albeit weak, evidence has been reviewed suggesting a role for ALDH2 polymorphisms as a genetic risk for a variety of noncancer chronic diseases, including cardiovascular disease and late-onset Alzheimer's Disease (reviewed in Zao and Wang, 2015 *Biomed Res Int* 2015:174050).

3c. Oral reference dose for noncancer outcomes. Section 2.1 presents an oral reference dose of $5x10^{-1}$ mg/kg–day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

The RfD for noncancer outcomes of $5.1x10^{-1}$ mg/kg–day based on urothelial hyperplasia rates in males reported by Suzuki et al (2012) seem well supported by the scientific evidence provided. Urothelial hyperplasia is a very sensitive and specific biomarker of kidney injury and the frequency was dose-dependent in this oral feeding (dosed water) study. Independent studies also found evidence of urothelial hyperplasia, and kidney damage via chronic oral as well as inhalation exposure models in rats. These effects were dose- or concentration-dependent, and males were determined to be selectively sensitive. The noncancer RfD of $5.1x10^{-1}$ mg/kg–day is based on an adequate dose-response data with acceptable use of statistical analyses to derive the RfD. Although no AOP (mechanism for nephrotoxicity) is known, it is clear that one or more genetic polymorphism(s) can shift the dose-response relationship for ETBE-triggered urothelial hyperplasia to the left, indicating that impaired metabolism of acetaldehyde (eg, inactive ALDH2*2 that occurs in ~50% of individuals of Eastern Asian decent) confers significant susceptibility to kidney damage associated with ETBE exposures. That said, the lack of clearly defined etiological mechanisms that can account for kidney damage associated with ETBE exposure raises uncertainties, especially if they were to increase susceptibility to females. Thus one could theorize of a number of genes variants that might increase female susceptibility to ETBE, yet these models are not available, nor are human data available, for extrapolation beyond the ALDHE2*2-susceptible population. For example, ETBE is metabolized to TBA via multiple CYP P450 isozymes known to be polymorphic and variable in their catalytic activity.

Note Concerning calculation of Inhalation Unit Risk

There is an apparent error in the equation used to calculate **Inhalation Unit Risk** on page xxv line 2, and again on page 2-26 line 27, or the equation used to derive the Slope Factor for inhalation Risk of liver tumors needs to be corrected.

$0.1/BMCL_{HEC} = 8 \times 10^{-4}\ (mg/m^3)^{-1}$ not $8 \times 10^{-5} (mg/m^3)^{-1}$ (Table 2-9)

A calculation based on $0.1/BMCL_{10}$ is 0.1/1,498 $mg/m^3 = 6.7 \times 10^{-4}\ (mg/m^3)^{-1}$, not $8 \times 10^{-5} (mg/m^3)^{-1}$.

How a slope factor of $8 \times 10^{-5} (mg/m^3)^{-1}$ was derived needs clarification or correction.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. ~~Does the classification give appropriate weight to the results from initiation- promotion studies? Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported.~~ Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

I agree with inclusion of the 2-stage initiation-promotion studies in the human cancer hazard characterization. These studies are robust and clearly demonstrate that ETBE promotes (increases the frequency of) tumors in several tissues when exposure occurs subsequent to initiation with the genotoxic mixture DMBDD. There is also strong evidence that chronic exposure to ETBE by the inhalation route is sufficient to induce liver tumors. I agree that the emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported by available data. The "suggestive evidence" cancer descriptor is scientifically supported and sufficient for both oral and inhalation routes of exposure, considering the absence of any evidence from human epidemiology, e.g. no studies on cancer frequencies associated occupational exposures, which tend to be much lower levels than the doses, concentrations identified to increase tumors in animal studies.

5. Question on Susceptible Populations and Lifestages. Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

I agree that the available data show strong evidence in mice that lack ALDH catalytic activity, as exemplified by ALDH2 KO, have higher sensitivity (susceptibility) to both cancer and noncancer toxicity associated with ETBE. It is also known that humans that express the major ALDH2*2 polymorphism (50% of those having Eastern Asian decent) have dysfunctional ALDH2 and have higher risk for certain cancers. It is reasonable to extrapolate from mice to humans that the ALDH2*2 polymorphism will serve as a predictor of increased susceptibility in those that express the variant. In general, most outcomes that have been measured following ETBE exposures by either inhalation or oral routes indicate males are more susceptible than females, and this should be considered in susceptible population corrections. It should be noted, however, that other genetic polymoprhisms, especially, but not limited to, polymorphisms in the P450 isozymes known to metabolize ETBE to acetaldehyde and TBA.

With regard with regard to susceptible lifestages; the studies to date have not demonstrated that the perinatal period is more susceptible than the adult. Negative results from F1 and F2 offspring exposed during embryonic development (developmental exposures) through the maternal exposure have failed to provide evidence of developmental impairments, but the behavioral endpoints that have measured are limited in scope (early reflexes, avoidance, ASR, and motor activity). However, I would like to emphasize that there are no studies that examine spatial memory or other types of cognition, or measures of activity-dependent plasticity and behavioral outcomes associated with social/anxiety domains. As I mentioned above, the lack of data in my opinion is a major gap in our knowledge and raises several uncertainties, especially since we have no or little information about mechanisms that produce renal and liver toxicities.

Dr. Harvey Clewell

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods. Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

I found the strategies for literature searching, study inclusion and evaluation to be clearly described and objectively applied.

Hazard Identification and Dose-Response Analysis. Chapter 1 (Hazard Identification) and the supplemental materials summarize the chemical properties, toxicokinetics, and health effects associated exposure to ETBE. Chapter 2 (Dose Response Analysis) uses this information to derive an oral reference dose and inhalation reference concentration for noncancer outcomes, in addition to an oral slope factor and inhalation unit risk for cancer.
2. Chemical Properties and Toxicokinetics
2a. Chemical properties. Is the information on chemical properties accurate?

It appears accurate to me.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties?

Section B.1.5 provides only a very brief description of the PBPK model used in this assessment. However, the approach for model evaluation is very clearly described in the USEPA (2017) document "PK/PBPK Model Evaluation for the IRIS Assessments of Ethyl Tertiary Butyl Ether and tert-Butyl Alcohol" cited in Section B.1.5 of Appendix B. This evaluation appears to have been objective and thorough, with a detailed discussion of uncertainties, assumptions and required modifications. I agree with the conclusion of the evaluation that the Borghoff et al. (2016) model, as modified by EPA, is fit for the purpose of supporting the IRIS assessments for ETBE.

Are there additional peer-reviewed studies that should be considered for modeling?

I disagree with the conclusions of the report is the determination that the human model of Nihlen and Johanson (1999) cannot be used for cross-species extrapolation for ETBE.

It is highly unfortunate that the human model of cannot be used to support animal to human extrapolation, and I strongly believe it would be possible to make use of the published model and data from the Nihlen and Johanson (1999) publication to create an acceptable human model.

In particular, I do not agree with the statement in the report that:

"The Nihlén and Johanson model is based on measurements of blood concentrations of eight individuals exposed to 5, 25, and 50 ppm ETBE for 2 hours while physically active. This model differs from conventional PBPK models in that the tissue volumes and blood flows were calculated from individual data on body weight and height. Additionally, to account for physical activity, blood flows to tissues were expressed as a function of the workload. These differences from typical PBPK models preclude allometric scaling of this model to other species for cross-species extrapolation."

Although, as stated in the document, the Nihlen and Johansen (1999) data and modeling approach are not "conventional", they are probably useful. Similar experimental data and PBPK modeling (Johanson et al. 1986; Corley et al. 1994) were used in the IRIS assessment for 2-butoxyethanol.

I also disagree with the statement in the report that:

"As there are no oral exposure toxicokinetic data in humans, this model does not have a mechanism for simulating oral exposures, which prevents use of the model in animal-to-human extrapolation for that route."

The oral route can be described in the model in the same way that it was described in the PBPK models of vinyl chloride (Clewell et al. 1999) and 2-butoxyethanol (Corley et al. 1994), which were used in the development of the RfDs and RfCs for these two chemicals, as well as in both the oral and inhalation cancer assessments, despite the lack of human toxicokinetic data for the oral route. For vinyl chloride, as with ETBE, the dose metric was rate of metabolism of the parent chemical in the liver. For 2-butoxyethanol, as with tBA, the dose metric was the concentration of the active metabolite in the blood.

Therefore, I would recommend that EPA give further consideration to modifying the model of Nihlen and Johanson (1999) in a similar fashion to the way in which Corley et al. (1994) modified the model of Johanson et al. 1986) to support cross-species extrapolations for both inhalation and oral routes of exposure.

2c. Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

I agree with the use of the average rate of metabolism of ETBE in the liver after periodicity is achieved as the dose metric for route-to-route extrapolation in the rat. I am also comfortable with the pulsatile oral ingestion pattern used by EPA for rats exposed via drinking water.

Hazard Identification and Dose–Response Assessment. Comment on EPA's assessment of the toxicological studies and dose-response assessment, including whether there are additional peer-reviewed studies that should be considered.

3. Noncancer
3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

I agree with the conclusion of the assessment that although α2u-globulin and chronic progressive nephropathy may have contributed to the kidney tubular lesions observed in rats, the dose-response data for urothelial hyperplasia are an appropriate basis for calculating BMDs for kidney effects.

3b. Noncancer toxicity at other sites (Sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1). The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.

- Liver effects: Suggestive evidence
- Developmental toxicity: Inadequate evidence
- Male and female reproductive toxicity: Inadequate evidence

I agree with the decision not to perform dose-response assessments for the other sites for noncancer toxicity.

3c. Oral reference dose for noncancer outcomes. Section 2.1 presents an oral reference dose of 5x10–1 mg/kg–day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

I agree that the dose-response data for urothelial hyperplasia in male rats are an appropriate basis for calculating BMDs for kidney effects and I believe the BMD analysis was performed correctly. However as indicated above, I would prefer that the use of PBPK modeling be reconsidered for cross-species extrapolation to replace the BW^3/4 default.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 9 x 100 mg/m3, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

I agree that the dose-response data for urothelial hyperplasia in male rats are an appropriate basis for calculating BMDs for kidney effects and I believe the BMD analysis was performed correctly. However as indicated above, I would prefer that the use of PBPK modeling be reconsidered for cross-species extrapolation to replace the category 3 default inhalation dosimetry calculation.

4. Cancer
4a. Cancer modes-of-action in the liver. As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPARα, PXR, and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

I agree with the conclusion in the assessment that the evidence for a role of nuclear receptor pathway activation in the mode of action for ETBE liver carcinogenicity is inadequate to rule out any human relevance. The evidence suggests that the mode of action is more likely to be associated with the metabolism of ETBE to actaldehyde.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Does the classification give appropriate weight to the results from initiation–promotion studies?

I am comfortable with the manner in which the assessment considers the results of the initiation-promotion studies in supporting the conclusion of suggestive evidence.

Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported.

I agree with the cancer descriptor of suggestive evidence for ETBE based on (1) the observation of liver tumors from inhalation of ETBE, (2) the limited evidence for a role of nuclear receptor pathway activation in the mode of action for ETBE, and (3) the evidence that the mode of action is more likely to be associated with the metabolism of ETBE to actaldehyde.

Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported.

Personally, I find the 2-stage initiation-promotion studies to be unhelpful for the human cancer hazard characterization of ETBE, and would prefer they not be included.

Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

I agree with a cancer descriptor of suggestive evidence for all routes of exposure based on the likelihood that the cancer mode of action may be associated with local metabolism of ETBE to acetaldehyde.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the rationale for including a quantitative analysis given the "suggestive evidence" descriptor.

I could not find any rationale for performing a quantitative analysis for ETBE liver cancer in Sections 2.3 or 2.4, or indeed anywhere else in the document. Rather than providing a rationale for the decision, the assessment merely cites the EPA (2005) Guidelines for Carcinogen Risk Assessment to demonstrate that they do have the option of performing one:
"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however when the evidence includes a well-conducted study, quantitative analysis may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." What is missing in the document is any rationale for performing a quantitative analysis in the case of ETBE. In particular, no rationale is presented to suggest that performing a default low-dose linear dose-response assessment for high-dose-only liver tumors in rats exposed to ETBE would be useful for any purpose.

In Section 1.3.2 (p. 1-112, lines 9-11), the agency summarizes the limited evidence for ETBE carcinogenicity: "*The results for ETBE raise a concern for cancer, but the effects were limited primarily to one tissue (liver), at one dose (highest), and in one sex/species combination (male rats), which were almost entirely benign.*" I do not see any way in which performing a low-lose linear extrapolation of these data could possibly provide a sense of the magnitude and uncertainty of potential risks, help to rank potential hazards, or set research priorities. In fact, I believe that providing only a default linear dose-response assessment for ETBE would be highly misleading.

Also comment whether the Saito et al. (2013) study is a suitable basis for this quantitative analysis.

Saito et al 2013 is a well-conducted and well-reported study, but the data for neoplastic liver lesions are not a suitable basis for the quantitative analyses performed in Sections 2.3 and 2.4.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1 x 10–3 per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

I do not believe that a quantitative analysis for an oral slope factor can be reliably performed at this time.

4e. Inhalation unit risk for cancer. Section 2.4 presents an inhalation unit risk of 8 x 10–5 per mg/m3, based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

I do not believe that a quantitative analysis for an inhalation unit risk can be reliably performed at this time.

5. Question on Susceptible Populations and Lifestages. Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

I agree with the conclusion that individuals with diminished ALDH2 activity may be a susceptible population due to an increased internal dose of acetaldehyde, a primary metabolite of ETBE.

6. Question on the Executive Summary
The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

I found the Executive Summary to be a clear and accurate synopsis for the key findings and conclusions of the assessment.

Tert-butanol Alcohol (tBA)

Dr. Alan Stern

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

I claim no specific expertise in pharmacokinetic modeling and particularly not in PBPK modeling. My reading of Appendix B as well as the PK/PBPK Model Evaluation for the IRIS Assessments of Ethyl Tertiary Butyl Ether (CASRN 637-92-3) and tert-BUTYL ALCOHOL (CAS No. 75-65-0) suggests that the EPA gave careful consideration to the necessary and appropriate parameters.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported.

I agree with the characterization of carcinogenic potential for tBA as "suggestive evidence." The evidence is relatively weak, however. This reflects the potential involvement of $\alpha_{2\mu}$globulin in the renal tubule tumors in male rats, and the high-dose-only thyroid follicular cell tumors in the male and female mice. The data are particularly weak for the thyroid tumors in the male mice, which consisted of a single carcinoma. However, the threshold for this designation is intentionally low, and the occurrence of statistically significant tumors whose potential relevance to humans cannot definitively be ruled out, particularly based on evidence in two species as there is here, is sufficient to warrant this designation.

4c. Cancer toxicity values. Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for quantitative analysis, and whether the NTP (1995) study is suitable for this purpose.

While I support the "suggestive evidence" characterization of carcinogenic potential, I do not believe that the NTP (1995) (or any other available data) are sufficiently robust to provide a meaningful quantitative estimate of cancer risk. With a statistically significant increase in tumors at the high dose only, any number of models can provide an adequate fit to the dose-response data. This results in the cancer slope factor having an extreme model dependence

such that "best-fit" of the possible models becomes meaningless from a statistical or practical standpoint.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 5 x 10–4 per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

As noted in my response the charge question 4c, I do not believe that the available data are sufficiently robust or have a sufficient dose-response to warrant the derivation of a cancer slope factor. I don't believe that the slope factor that was derived is scientifically supported or can provide useful information.

Dr. Deborah A. Cory-Slechta

1. Literature Search Strategy/ Study Selection and Evaluation-Systematic Review Methods. Please comment on the strategies for literature searches, criteria for study inclusion or exclusion and evaluations of study methods and quality discussed in the Literature Search Strategy/Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

Yes, the strategies were clearly described and objectively applied. An extensive search was undertaken after which inclusion and exclusion criteria were applied and these criteria were appropriate, including elimination of studies of mixtures exposures as the toxicological review is specific to tert-butyl alchohol and thus mixture studies introduce problems of defining sources of the mixture responsible for an effect. In addition, the criteria used for evaluation were also appropriate with respect to suitability for inclusion in derivation of reference doses/concentrations.

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

The conclusion that kidney effects as a potential human hazard of tert-butanol could be a human hazard is both scientifically supported and clearly described. The relevant studies are included in the analysis as based on effects in females that cannot be ascribed to CNP as well as the potential for non α2u-globulin-mediated toxicity in males. The review has very carefully reviewed and systematically evaluated the evidence for this conclusion, particularly the evidence in relation to α2u-globulin.

One aspect of the presentation that could be better described are the analyses presented in Tables 1.7 and 1.8 correlating severity of nephropathy with epithelial hyperplasia. Specifically, some additional detail on the methods of correlation testing would assist.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of $4x10^{-1}$ mg/kg-day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

The derived oral RFD of $4x10^{-1}$ mg/kg-day for non-cancer kidney outcome is scientifically supported by the data, and the derivation of the value and in general is clearly described.

One point that deserves clarification is the basis of the choice for the 15 mos data for the organ weights. While it is indicated in the supplemental data that organ weights were not obtained past that time point, it would be good to include it in this section.

4a. Cancer modes-of-action in the kidney. As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving α2u-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

The conclusion that kidney tumors in male rats should be considered relevant to human hazard identification is scientifically justified based on some of the inconsistencies in timing and missing steps in a solely α2u-globulin and/or chronic progressive nephropathy as previously reported.

Dr. Hugh Barton

2. Chemical Properties and Toxicokinetics
2a. Chemical properties. Is the information on chemical properties accurate? No comment.
2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of *tert*-butanol in rats (Borghoff et al., 2016).
Is use of the model appropriate and clearly described, including assumptions and uncertainties?

- The description in Section B.1.5 of Appendix B in the Supplemental Information is limited to specific details about the implementation in the dose-response assessment, e.g., how drinking water exposures were handled. As such, the description is clear but so limited that it does not describe the model overall nor its assumptions or uncertainties. Useful information is

provided in US EPA 2017, but this document indicates it is a draft. Model code was made available through the HERO database, which is appropriate though also providing limited information especially to those without the modeling software used.

- Text in the Toxicological Profile 1.1.3 (page 1-3) needs to be revised so as not to be misleading and to be consistent with the Supplement. While no models of TBA have been created independently of other chemicals from which it arises as a metabolite (e.g., MTBE, ETBE), the TBA model has "been developed specifically for administration of ter-butanol". Pharmacokinetic studies with TBA exposures are how the TBA model was parameterized so the text needs rewording.
- Use of the model for route extrapolation of kidney endpoints from chronic studies for comparison with the kidney effects observed in the subchronic inhalation study is appropriate. There are generally consistent findings of kidney toxicity following subchronic and chronic exposures by different routes, so it is appropriate to evaluate consistency among these and whether route extrapolation of a chronic study is a desirable option compared to application of a subchronic to chronic uncertainty factor in the derivation of a protective exposure level. The model does reasonably well capturing TBA pharmacokinetic data following intravenous, oral, and inhalation exposures supporting its use for route extrapolation for this endpoint.

Tier 1 Recommended Revisions: Reword text in Section 1.1.3 of the Toxicological Profile.
Are there additional peer-reviewed studies that should be considered for modeling?

- None that I'm aware of.

2c. Choice of dose metric. Is the average concentration of *tert*-butanol in blood an appropriate choice for the dose metric?

The average concentration of TBA in blood is an appropriate choice for the dose metric. While the effects occur in kidney use of blood concentration is reasonable given the it is related to kidney concentration by the partition coefficient in female rats. Since the analysis only used effects in females, any issues of modeling male rat specific protein binding do not impact these analyses.

4d. Oral slope factor for cancer**.** Section 2.3 presents an oral slope factor of 5×10^{-4} per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

EPA has derived an oral slope factor based upon female mice using their standard analysis methods in the absence of specific pharmacokinetic analyses (i.e., allometric scaling of administered dose) and mode of action information (i.e., benchmark dose modeling to obtain a

POD and linear extrapolation below that) as supported by their 2005 guidance. It is clearly described. As a default analysis, it is scientifically supported in the absence of contrary findings by the committee in response to charge questions 4aii and 4c concerning the mode of action and relevance of these thyroid tumors in mice and the development of a quantitative analysis when the "suggestive" descriptor is used.

Dr. Lorenz Rhomberg

The responses to these questions are essentially similar in most aspects to those for the corresponding questions on the ETBE analysis.

tBA Charge Question 1 – Literature Search/Selection/Evaluation

Overall, the structure of the search, selection, and evaluation is clearly presented. The Summary Chart (Fig.LS-1) shows the overall patterns of disposition. What is difficult to get at, however, is the reasoning behind disposition of individual identified literature items. That is, one is not readily able to check on the scoring of individual studies on the named criteria or to see the reasons for which individual studies were put in one or another category. This makes critical comment on selection and disposition difficult. The issues about evaluation of quality and impacts on interpretation listed under "Database Evaluation" are said to be discussed in the text, as the studies come up. But again, this scattering and partial reporting does not allow an observer to follow (and comment upon, if appropriate) the treatment of individual issues on individual studies. There is no ready way to find such discussion of any one study (other than by searching the whole assessment document) and the reader is limited to the aspects that the Agency chooses to discuss. It does not seem that the Supplemental Information includes this information either. It would be good to have a way to capture the evaluations on specific criteria and to see the reasons for disposition of each study. All of the evaluation criteria in Tables LS-3 and LS-4 should have evaluations available for each study to which they were applied. [Tier 2]

tBA Charge Question 3c – Oral Reference dose for noncancer outcomes

Given the endpoints analyzed, the calculations of BMDs, BMDLs, and oral reference doses are done by straightforward usual methods. The questions, then, are (1) are appropriate endpoints chosen for analysis? (2) among those chosen, which should be used to define the RfD? and (3) are there opportunities and reasons to depart from standard methods?

Although the first question is perhaps more in the province of other Charge Questions, the issue of the appropriateness of the oral RfD calculations cannot be addressed without confronting it. In public comments, some strong views, supported by analysis of a specifically convened PWG, are expressed regarding whether the kidney endpoints are separable, whether they are better considered as various aspects of Chronic Progressive Nephropathy (CPN), and whether they are relevant to processes that could occur in humans. In sum, the question of the validity and applicability of the endpoints analyzed for the oral RfD needs to be carefully examined. [Tier 1]

Even if the decision is to use them, that use must be couched in prominent caveats that acknowledges a significant dissenting body of expert opinion.

Even if one decides to employ these endpoints, it has been said by knowledgeable public commenters that, because the endpoints are seen as a suite of CPN manifestations, not all appearances will necessarily be noted in pathological examination, and the counts (and denominators) may be inappropriate. This question needs a clear resolution if the data are to be taken as valid for analysis. [Tier 1]

It is difficult to address the second question – which among the analyzed endpoints should form the basis for the RfD – because of the issues of severability among endpoints and their human relevance, noted above. It is noteworthy that the values obtained from different choices are not markedly different.

The third part of the question – departing from defaults – raises the issue of dosimetry. It is noteworthy that the kidney effects of ETBE and tBA are similar, and that tBA is a principle metabolite of ETBE, strongly suggesting that metabolism of ETBE to tBA is key to ETBA's effects. Yet the dose measures and extrapolation method for animal-to-human are simply the defaults, with no allowance for metabolism. Granted, there is no validated human PBPK model, but there are quite a few studies characterizing human metabolic activity. It would be of great value to explore how the rates of metabolic activation, given what can be discerned about them, could compare with the default dosimetry considerations. The pharmacokinetics of ETBE and tBA do not seem especially complex, and it seems likely that rates of metabolic activation (and how they may differ among dose levels, between sexes, and between rats and humans) may have a lot to say about expectations of relative toxicity. The question of whether similar tissue exposures to tBA underlie responses to tBA dosing and tBA production from metabolism of ETBE dosing would be valuable to work out in more detail. [Tier 2]

As already noted for Question 3c for ETBE, although there is a lot of tabulation of experimental results, it is difficult to trace the information on any one study from its discussion in the text to the tabulations in the Supplementary Material to the dose-response analysis elsewhere in the Supplementary Material. This is complicated by the fact that there are different analyses of the same data (absolute and relative organ weights, for example), different durations of exposure for otherwise similar experiments, and lack of uniqueness of "author (date)" designations, making it challenging to be sure that one is examining corresponding data in the different places they are discussed or presented. [Tier 2]

Critically, there does not seem to be any reporting of statistical analysis of individual studies – trend tests or pairwise significance tests, *etc*. – and this hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role of such statistical analysis in identifying results to report and decisions to include or exclude them from analysis is not very clearly stated. [Tier 1]

There is no reporting of units for the responses (as opposed to the exposures) in the Supplementary Material tables, and this also leads to difficulty in interpretation. [Tier 1]

tBA Charge Question 3d – Inhalation Reference dose for noncancer outcomes

Given the endpoints analyzed, the calculations of BMDs, BMDLs, and oral reference doses are done by straightforward usual methods, with the exception of the use of route-to-route extrapolation from oral exposure study analyses, some questions about the inhalation RfC determination are essentially the same as those for the oral RfD a set out for Charge Question 3c, above, and they are consequently repeated here by reference to that response. [Tier 1]

The use of route-to-route extrapolation for inhalation non-cancer effects is a notable deviation from usual practice. Given that there are some inhalation data (though not for full lifetime) the discussion of use of route-to-route extrapolation in ought to say some more about why the choice was made to use the extrapolated oral results over inhalation results. More specifics need to be given as the method for using the PBPK for route-extrapolation are discussed on p.2-12. In particular, the choice of the dose metric to represent "internal dose" (line 12) needs to be more fully explained and justified.

The use of route-to-route extrapolation for the inhalation RfC seems reasonable, given the circumstances, but it does result in considerable uncertainty (and contingency on the validity of the chosen method), and it is important to fully noted these caveats in summarizing the findings, in this section and in the Executive Summary.

As already noted for Question 3c for ETBE, although there is a lot of tabulation of experimental results, it is difficult to trace the information on any one study from its discussion in the text to the tabulations in the Supplementary Material to the dose-response analysis elsewhere in the Supplementary Material. This is complicated by the fact that there are different analyses of the same data (absolute and relative organ weights, for example), different durations of exposure for otherwise similar experiments, and lack of uniqueness of "author (date)" designations, making it challenging to be sure that one is examining corresponding data in the different places they are discussed or presented. [Tier 2]

Critically, there does not seem to be any reporting of statistical analysis of individual studies – trend tests or pairwise significance tests, *etc*. – and this hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role of such statistical analysis in identifying results to report and decisions to include or exclude them from analysis is not very clearly stated. [Tier 1]

There is no reporting of units for the responses (as opposed to the exposures) in the Supplementary Material tables, and this also leads to difficulty in interpretation. [Tier 1]

tBA Charge Question 6 – Executive Summary

The specifics of the Executive Summary clearly depend on the content of the draft assessment, and many of those aspects are subject to debate and comment through other Charge Questions.

Accordingly, the Executive Summary will need to be changed to reflect any changes elsewhere in the document. Comments here are therefore on overarching aspects of what the Executive Summary addresses and in what depth of detail, rather than the specifics it sets out.

The Executive Summary offers statements about the questions considered and summarizes the findings that were in the end chosen. Little is said about alternatives that, even though they may have some merit and provide important context for interpretation and application of the main findings. The section on "Key Issues" is helpful, but it could do more to highlight the consequences of alternative choices for the final assessment. This is especially so because (based on the history of public comment) the interpretation and relevance of key toxicity endpoints driving the analysis has been sharply contested. However, these issues are resolved, it is important for the assessment – and for the Executive Summary – to forthrightly deal with the contingency of final conclusions on decisions to accept or not accept debated arguments, and simply not meeting the level of certainty to dismiss an endpoint as irrelevant to human risk does not result in certainty of relevance. [Tier 1]

The connection between the assessment of ETBE and tBA needs to be explicitly discussed (in the full document as well as in the Executive Summary). Since metabolic activation of ETBE is invoked in its assessment, and since tBA is a main metabolite, the interpretation of toxicity, dosimetry, and dose-response for the two compounds ought to inform one another, and the consistency of interpretations needs to be more thoroughly addressed. [Tier 1]

The Executive Summary should provide more specifics about the chosen dose metrics, explaining (in summary form) the basis for calculation, the role of metabolism in activation and clearance, major assumptions or use of alternatives to defaults, and the basis for cross-species dose equivalency. The dosimetry considerations applied to each endpoint should be clear (*e.g.*, dose-metric definition, role of tissue specificity, *etc*.). [Tier 1]

The use of route-to-route extrapolation for inhalation non-cancer effects is a notable deviation from usual practice. Given that there are some inhalation data (though not for full lifetime) the discussion of use of route-to-route extrapolation in the executive summary ought to say some more about why the choice was made to use the extrapolated oral results over inhalation results (the existence of which, but not the shortcomings, is briefly noted). Simply saying that the extrapolation approach is "more specific and sensitive" does not sufficiently explain the rationale.

Dr. Stephen Roberts

3a. Noncancer kidney toxicity. (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of □2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

Scientific support for kidney effects as a potential human hazard of tert-butanol is limited. The draft assessment examines in detail evidence that kidney effects from t-butanol are due at least in

part to □2u-globulin, concluding that t-butanol has a weak effect through this MOA. Other kidney effects are related to CPN. As with ETBE, distinguishing effects that are due to CPN versus chemical effects versus chemical effects on CPN is difficult.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of $4x10^{-1}$ mg/kg-day, based increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation is clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

Given evidence that renal effects in male rats might be related to some extent to □2u-globulin, only data from female rats were considered, which is appropriate. Specific endpoints from female rats considered were absolute kidney weight, kidney suppurative inflammation, kidney transitional epithelial hyperplasia, and increases in severity of nephropathy. PODs for these endpoints were determined using benchmark dose modeling according to EPA guidance. Choices regarding UF values appear appropriate, with the possible except of the UF_D. The report acknowledges that there are gaps in the database for effects of tert-butanol by the oral route, but contends that they are filled by information available for ETBE. There are enough differences in the toxicity of tert-butanol and ETBE (evident from the two draft toxicological reviews) that the EPA should consider applying a UF_D of 3. Among the candidate RfDo values for tert-butanol based upon kidney effects, the lowest ($4x10^{-1}$) was selected. Part of the argument in favor of this choice to represent kidney toxicity is that it is more sensitive and specific than the relatively non-specific endpoint of absolute kidney weight. Candidate RfDo values based upon the two other endpoints (suppurative inflammation and transitional epithelial hyperplasia) were presumably not chosen because they are higher than the RfDo values for the other endpoints. It is interesting to note that both the draft assessment for ETBE and this one include acknowledgement that there is not consensus that exacerbation of CPN is relevant to humans, which would appear to undermine support for the endpoint chosen.

4a (i). Cancer modes-of-action in the kidney. As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving □2u-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

Renal tubule tumors were observed following drinking water exposure in male but not female rats. The draft assessment concludes that some of the tumors might be associated with □2u-globulin nephropathy. Because the evidence for □2u-globulin was in some respects incomplete, the EPA concludes that some of the tumors might be attributed to "some other, as yet unspecific processes" (page 1-66). On the basis of the assumed existence of other MOA's, the kidney tumors in male rats are considered to be relevant to human hazard identification. Evidence for one or more other MOA's for renal tubule tumors in male rats presented in the draft assessment

is indirect at best, and as a result, the scientific support for the conclusion of human health hazard posed by these tumors is not particularly convincing.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

The draft assessment states that increased tumor incidences were reported for two species, two sexes, and two sites – which would normally be consistent with a descriptor of "likely to be carcinogenic to humans" – but that none of the tumor responses was strong or coherent with ETBE, leading them to select the "suggestive evidence" descriptor. I concur with weaknesses in the tumor data (see comment on 4a(i), above), and consider the descriptor chosen by EPA to be scientifically supported. Because the tumor sites are distant from the point of contact with the chemical, application of this descriptor to all routes of exposure is reasonable and is consistent with EPA guidance.

Dr. W.M. Foster

1. Literature Search Strategy/Study Selection and Evaluation.

I found the keywords selected for search using the online venues of scientific databases (PubMed, Toxline, Web of Science, and TSCATS) to be appropriate for tBA. The resulting Table information defining the searched literature: Tables LS-1, LS-2 (review reports) were lucid and identified the temporal end point (May, 2015) of the applied searches, and Table LS-3 clearly listed the inclusion/exclusion criteria utilized for valuation of reports downloaded from the literature searches. Success of the search approach as overviewed in Fig. LS-1 seems adequate. One concern at this point, is the reliance on older reports, for example, the animal model data base (n=14 reports in total, pg. LS-8) utilized for validity of injury and assessing risk, all were accomplished prior to 1998, except for a single, industry sponsored, reproductive study accomplished in 2004.

The summarization of animal model studies utilized and listed in Table l-5 (pg. LS-8), provide, and establish an understanding of the reproducibility of the animal model data base for modes of exposure (oral, inhalation), duration of exposure (sub-chronic, chronic), and scope or focus (developmental, neurodevelopmental, reproductive) of the subsequent to exposure, tBA-induced injury. The search selections appear appropriate and on target for determining health effects of oral exposure to tBA in animal models for extension to humans.

A report that appeared later in time (online, 2016) than the May, 2015, cut-off date for search inventory, given its subject matter would be reasonable to review for information pertinent to pharmacokinetic models of tBA using oral exposure; additionally, the report

supports a male-rat-specific mode of action for tBA-induced kidney tumors [SJ Borghoff et al, J. Appl. Toxicol. 37: 621–640 (2017)].

3b. Non-cancer toxicity at other sites. Identified toxicities (non-cancer) in Sections: 1.2.3 for developmental, 1.2.4 for neurodevelopmental, and 1.2.5 for reproductive,

For developmental responses, exposure to tBA during gestation led to fetal losses, impairment of fetal body weight, and increases in skeletal variations in exposed offspring. However, dams had changes in body weight and reduced food consumption at tBA doses similar to doses causing fetal effects. This made it difficult to define a clear indication that tBA was causal to specific developmental toxicity or whether the fetal effects reflected maternal toxicity. Based upon the quality of the reports (4 listed on pg. LS-8), this is a reasonable conclusion.

Neurodevelopmental effects to tBA relied upon 2 older research reports (pg. LS-8, one using a mouse model with exposure by diet, the other rat by inhalation) with end point responses based on brain weight, changes in brain biochemistry, and alteration of behavioral performance, effects were found/observed. At this point it is difficult to link induction of neurodevelopmental injury and exposure to tBA in studies with limited determinations; and at this point additional studies with reproducibility of response based on dose-response, and route of exposure upon toxicity end points, are warranted.

For reproductive effects, to date only a single research report investigated the potential for tBA injury and by design was limited to a one-generation study in rats (pg. LS-8). No two-generation reproductive studies are available that evaluate oral or inhalation exposure. In males by oral exposure, the only observed effect was a slight decrease in sperm motility for F0 males in the highest dose group of rats treated with tBA. However, this response was not observed in an evaluation with orally treated rats and mice or in rats exposed via inhalation. Due to limitations of research design, and the need for reproducible outcomes, conclusions with respect to reproductive toxicity have not been defined, and this is appropriate.

3d. Inhalation reference concentration (RfC) for non-cancer outcomes. As identified in Section 2.2 an RfC is proposed (5×10^0 mg/m^3) that relied upon oral exposure in female rats and the severity of nephropathy injury, and was then converted for inhalation exposure using a recently developed physiological based pharmacokinetic (PBPK) model (SJ Borghoff et al., 2017).

A PBPK model was employed to perform a route-route extrapolation to determine a point of departure (POD) for the derivation of the RfC for tBA that is based upon an NTP (1995) oral exposure that was causal to significant levels of organ injury. The study used sub-chronic, and chronic durations of oral exposure (drinking water) to tBA. Doses in male rats ranged between 0 and 420 mg/kg-day, and female rats between 0 and 650 mg/kg-day. Kidney effects, including changes in organ weight, histopathology, or both, were observed in both sexes of rats after 13 weeks, 15 months, and 2 years of treatment. Confidence in the principal study (NTP, 1995) was considered to be high, based upon an adequate research design, and that was accomplished with FDA GLP regulations, and utilized a sufficient number of animals per group (both sexes).

Although a degree of uncertainty does exist and surrounds the application of the PBPK model for the purposes of a route-to-route extrapolation.

4e. Inhalation unit risk for cancer. In Section 2.4 an inhalation unit risk is not proposed.

In the current research study data base, lifetime oral exposure to tBA is associated with increased renal tubule adenomas and carcinoma in male rats, and increased thyroid follicular cell adenomas in female B6C3F1 mice, and increased thyroid follicular cell adenomas and carcinomas in male B6C3F1 mice. However and as stated in the charge question 4e, the lack of a pharmacokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α_{2u}-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats.

Dr. Alan Hoberman

3b. Noncancer toxicity at other sites. (Sections 1.2.3-6, and 1.3.1). The draft assessment finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with tert-butanol exposure, please identify them and outline the rationale for including them in the assessment.

Tier 1: Recommended Revisions

The overall evidence appears to be inadequate to provide a precise evaluation of the hazard but the data is adequate to indicate that any hazard would be at a dose higher than those for other endpoints. No terata and no effects below 1000 mg/kg from various routes.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 5 x 10–4 per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The value seems to be scientifically supported and its derivation is clearly described.

5. Susceptible Populations and Lifestages. As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

Tier 1: Recommended Revisions

The overall evidence appears to be inadequate to provide a precise evaluation of the hazard but the data is adequate to indicate that any hazard would be at a dose higher than those for other endpoints.

Tier 3: Future Considerations

Developmental neurotoxicity should be evaluated

6. Question on the Executive Summary. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

The summary states:

"At this time, evidence of selective developmental toxicity and reproductive system toxicity following tert-butanol exposure is inadequate. Information also is inadequate to draw conclusions regarding neurodevelopmental toxicity, liver toxicity, and urinary bladder toxicity."

Tier 1: Recommended Revisions

It would be more accurate to state that "no selective developmental or reproductive toxicity was found". Evidence of any developmental and reproductive toxicity was complicated by maternal toxicity and/or issues with the maternal animals being exposed to the test material. In general any sign of developmental or reproductive toxicity occurred only at doses at or above 1000 mg/kg.

Tier 3: Future Considerations

Developmental neurotoxicity should also be included in this statement.

Dr. Maria Morandi

Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods. Question 1: Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

It is not clear from the Preamble or from Literature Search Strategy/Study Selection and Evaluation section whether EPA intended to comply fully with the NRC (2011) recommendations regarding search strategies and study selection protocols. There is variance between the NRC recommendations and some of the approaches utilized in the literature search, but there is no statement as to why NRC (2011) guidelines were used in some aspects of the

search and identification protocols and not others. However, and despite some of the additional clarification described below, it is highly unlikely that the strategy adopted by EPA would have missed one or more critical studies.

The overall strategy of starting from a broad, chemical-specific search of relevant scientific literature databases and other sources of information to maximize identification of all publications potentially relevant to the risk assessment, followed by successive screenings for the pertinent studies. This strategy is appropriate. The search was continually updated overtime with a clear stated ending of December 2016, and consistently maintained overtime in HERO. In addition, the agency issued multiple public requests for relevant documentation.

The four indexed scientific databases for the chemical-specific search are appropriate and likely to encompassed most of the tBA-relevant published and peer-reviewed articles, although the text does not indicate if others were considered. The search keywords were typically used synonyms for tBA and the CAS number, but given the range of names for this compound, there should be a rationale as to why these specific names were selected from the universe of those available. Consistent with the broad search strategy, the indexed literature searches were not topic-limited except for Web of Science, and Toxline. The narrative is not clear why these limits were applied so early in the search strategy to these two databases. This may be a good approach for avoiding excessive duplication or non-relevant publications, but it does not seem consistent with the intended chemical-specific broad search of the strategy. A brief justification would help clarify this issue.

Additional research strategies included manual search of citations from review articles, public comments, and reviews performed by other federal and international agencies (OSHA and IPCS, respectively). This is appropriate. However, there should be clarifications, including: 1) why citations were only searched manually in review articles and not in other publications; 2) if there was a search for all federal and international agencies that have performed assessments or other health reviews of tBA (for example, ATSDR), and why only IPCS (1987) and OSHA (1992) were included, and 3) if manual citation searches were performed in other sources such as master/doctoral theses, or reports available in the grey literature.

Study categories and inclusion/exclusion criteria are described clearly and are appropriate to the objectives to the search. They can be easily accessed in HERO, and the results are depicted clearly in Figure LS-1.

Criteria for quality evaluation of studies are well described, and the important features of the 12 studies included in the final database are presented in the assessment and the Supplemental Information documents as part of the narrative and in illustrative tables. Without performing a detailed review of the specific study evaluation process, it is not possible to affirm that these criteria were applied objectively across the board, but there is no evidence in the document that they were not.

Note that tert-butyl alcohol is repeated twice in the password cell for the Toxline database in Table LS-1

Hazard Identification – Chemical Properties and Toxicokinetics
Question 2a: Is the information on chemical properties accurate?

Information on the properties of tBA is correct except as indicated below:

1. In Table 1-1, page 1-1: 2-methyl-2-propanol and 2-methylpropan-2-ol may be more appropriate IUPAC names than tert-Butanol, which can be considered more of a synonym. NCI-C55367 should be removed from the list of synonyms/trade names. It is a NCI designation number, akin to a CAS number.

2. The values shown for melting and boiling points are as listed by ECHA (2017), not HSBD (2007) which lists a melting point of 25.81 and a boiling point of 82.3.

3. The value for density/specific gravity should add the notation that it is at 20C.

4. Note that the reference and URL for the ECHA (2017) citation are nor shown in the reference list.

Inhalation reference concentration for noncancer outcomes. Question 3d: Section 2.2 presents an inhalation reference concentration of 5x100 mg/m3, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

If severity of tBA-induced nephropathy in the female rat is accepted as relevant to humans, the estimated 5 X 100 mg/m3 RfC is scientifically defensible. Evaluation of the literature for non-cancer effects supports the selection of kidney effects for establishing the overall RfC. Selection of the NTP (1997) subchronic inhalation study rats, adjusted for duration, instead of the results for mice is well justified because of the lack of a PBPK model for mice. Selection of the NTP (1995) chronic oral study results for the same species of rat with route-to-route extrapolation and because of the availability of a PBPK model for tBA in the rat is also well justified. Selection of results for female rather than male rats is also well justified. Steps in the derivation of human-equivalent inhalation, points of departure and derivation of candidate values are explained clearly with rationales based on EPA guidance. Specific modeling results are presented in the Supplemental Information and are clearly depicted. The range of candidate values is not excessively large, i.e., within a factor of 7.5, which adds some measure of reliability to the estimated RfC. In particular, the difference in the candidate RfC derived from the subchronic inhalation study based on increases in kidney weight is approximately only 20% lower than the selected RfC.

A concern with the choice of effects on the kidney related to CPN is that the assessment does not provide a clear summary description of histological changes observed in the development of nephropathies in humans as compared to the changes observed in rats, both age-related only and consequent to chemical exposure. The stated assumption that some of the individual tissue outcomes observed in the rodent studies (associated with CPN) could also be relevant to humans even if CPN is not relevant is reasonable, but the document does not provide a line of scientific evidence of comparative histological changes in rodent and human nephropathies to help buttress this statement.

Inhalation unit risk for cancer.

Question 4e: Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α2u-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

The decision of not deriving an inhalation unit risk for tBA is well justified. This reviewer does not have an alternative approach.

Dr. Karen Chou

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods. Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

Yes, the strategies are clearly stated.

2a. Chemical properties. Is the information on chemical properties accurate?

Yes, the Chemical properties are accurate.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

The information provided in Appendix B 1.5 is sufficient for understanding the rational of the application of the PBPK model in the current assessment.

2c. Choice of dose metric. Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?

Yes, average concentration of tBA butanol in blood is an appropriate choice.

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

The conclusion and supporting rationales are sufficiently described.

3b. Noncancer toxicity at other sites. (Sections 1.2.3-6, and 1.3.1). The draft assessment finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with tert-butanol exposure, please identify them and outline the rationale for including them in the assessment.

The conclusions are scientifically supported and clearly described.

Suggestion: p. 1-55, Lines 4-14: Please state dosages as a part of the deliberation of the "Integration of Developmental Effects."

Suggested clarification: p. 1-50, study by Huntingdon Life Science (2004): Should the extraordinary finding, a 100% maternal body weight gain during PND 1-21, be highlighted in the text?

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of $4x10^{-1}$ mg/kg–day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

Yes, the value is scientifically supported and its derivation clearly described.

Recommendation: p. 2-16, Line 10: "44" is likely a mistake, should it be "4"?

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of $5x10^{0}$ mg/m^3, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

Yes, the value is scientifically supported and its derivation clearly described.

4a. Cancer modes of action.

(i) Cancer modes-of-action in the kidney. As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving α_{2u}-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported

Yes, the conclusion is scientifically supported.

This recommendation related to statements in two places: (1) p. 1-44, Lines 23-25 and (2) p. 1-45 Line 37 to p. 1-46 Line 1:

> In this section, the sensitivity between rats and mice are compared. The draft seems to conclude that the data from NTP (1995) study shows that mice are more sensitive than rats, because follicular cell hyperplasia and follicular cell adenoma are only present in mice not in rats. This assertion is incorrect, because all mice received higher doses than rats. The dataset, therefore, cannot be used to draw any understanding of the sensitivity of mice and rats. This incorrect assertion of mice-an- rats comparison appeared at least twice in this section.

P. 1-46, Line 36 "No evidence" is available: Should be "insufficient evidence"?

4a (ii) Cancer modes-of-action in the thyroid. As described in section 1.2.2, thyroid tumors were observed in male and female mice following tert-butanol exposure, and an anti-thyroid mode-of-action was evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid follicular cell tumors in rodents (U.S. EPA, 1998), found the information inadequate to determine whether an anti-thyroid mode-of-action was operating and considered the thyroid follicular cell tumors in male and female mice to be relevant to humans. Please comment on whether this conclusion is scientifically supported.

The conclusion is scientifically supported.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female $B6C3F_1$ mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. ~~Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported. Is the use of and interpretation of initiation-promotion studies for carcinogen classification consistent with the principles for carcinogen classification provided in the 2005~~

~~Guidelines?~~ Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

Yes, the suggestive evidence is scientifically supported for all route of exposure.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data generally would not support one, however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for quantitative analysis, and whether the NTP (1995) study is suitable for this purpose. Please comment on whether Section~~s~~ 2.3 ~~and 2.4~~ of the draft assessment adequately explains the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the ~~Saito et al. (2013)~~NTP (1995) study is a suitable basis for this quantitative analysis.

Yes, the rationale for including a quantitative analysis is sufficiently justified, and the NTP (1995) study is appropriately applied.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 5×10^{-4} per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The value is scientifically supported and its derivation clearly described.

4e. Inhalation unit risk for cancer. Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α_{2u}-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

The reviewer has no alternative suggestions.

5. Susceptible Populations and Lifestages. As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

Suggestions:

Fetal loss and low birth weights are relevant fetal and infant health concerns in humans. Existing studies of TAB suggests potential developmental effects at high doses, including potential neurodevelopmental effects. There is sufficient information for including fetus, as a susceptible population.

People with existing nephropathy or pre-nephropathy conditions (such as suppurative inflammation, transitional epithelial hyperplasia), and thyroid related conditions are likely susceptible populations.

Furthermore, people who are also exposed to MTBE and ETBE may be considered as susceptible populations.

6. Question on the Executive Summary. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

Recommended clarification, P. xiii, Line 14: This reference (HSDB 2007) is cited for t-butanol in human milk. In HSDB (2007), two articles are cited for this claim. (1) Pellizzari ED et al; Bull Environ Contam Toxicol 28: 322-8 (1982) (2) Erickson MD et al; Acquisition and Chemical Analysis of Mothers Milk for Selected Toxic Substances. USEPA-560/13-80-029 (1980). None of them showed presence of tBA in milk, although 1-butanol was present.

General Assessment Comments

P. 1-2, Line 9-11: Recommended corrections: (1) Please note that a TBA equivalent based on 14C is not TBA, it is the total radioactivity, including 14C containing metabolites. Please correct the statement. (2) An important finding in the study by Williams and Borghoff, (2001), single oral 500 mg/kg in Fischer 344 rats), is higher urinary excretion of radioactive metabolites in males than in females, over 12 h. See Table 1 of the publication by Williams and Borghoff (2001).

Supporting Reference

Beaugé F, Clément M, Nordmann J, Nordmann R. 1981. Liver lipid disposal following t-butanol administration to rats. Chemico-Biological Interactions 38(1): 45-51.
HSDB. 2007. t-Butyl alcohol. Bethesda, MD: National Library of Medicine.

Suggested addition: A single oral exposure (1853 mg/kg) in female Wistar rats demonstrated a slow clearance of blood tBA; Less than 15% of blood tBA was cleared in 20 hrs (Beaugé et al. 1981).

Dr. Janet Benson

Table 1-1. Physicochemical properties and chemical identity of *tert*-butanol

Characteristic	Information	Reference
Chemical name	*tert*-Butanol	HSDB (2007)
Synonyms/Trade names	*t*-Butyl alcohol; *tert*-Butanol; *tert*-Butyl alcohol; *t*-Butyl hydroxide; 1,1-Dimethylethanol; NCI-C55367; 2-Methyl-2-propanol; *tertiary* Butanol; Trimethyl carbinol; Trimethyl methanol; *t*-butyl alcohol; TBA	HSDB (2007) IPCS (1987b)
Chemical formula	$C_4H_{10}O$	HSDB (2007)
CASRN	75-65-0	HSDB (2007)
Molecular weight	74.12	HSDB (2007)
Melting point	25.7°C 25.84°C Toxnet or CRC HSDB	HSDB (2007)
Boiling point	82.41°C 82.3°C HSDB	HSDB (2007)
Vapor pressure	40.7 mmHg @ 25°C	HSDB (2007)
Density/Specific gravity	0.78581 0.7886 @ 20°C	HSDB (2007)
Flashpoint	15–23°C	ECHA (2017)
Water solubility at 25°C	1×10^6 mg/L	HSDB (2007)
Octanol/Water Partition Coefficient (Log Kow)	0.317 0.35 Toxnet HSDB	ECHA (2017)
Henry's Law Constant	5.05×10^{-6} atm·m³/mole	HSDB (2007)
Odor threshold	219 mg/m³	HSDB (2007)
Conversion factors	1 ppm = 3.031 mg/m³ 1 mg/m³ = 0.324 ppm	IPCS (1987b)

2 values given in Toxnet

This document is a draft for review purposes only and does not constitute Agency policy.

toxnet

The following information was generated from the
Hazardous Substances Data Bank (HSDB),
a database of the National Library of Medicine's TOXNET system
(http://toxnet.nlm.nih.gov) on July 25, 2017.

Query: The chemical name t butyl alcohol was identified.
The following terms were added from ChemIDplus:
2 methyl 2 propanol
trimethyl carbinol
tert butanol
t butyl hydroxide
t butanol
2 methylpropan 2 ol
1 1 dimethylethanol
CAS Registry Number: 75-65-0

1 - HSDB

MOLECULAR FORMULA:
C4-H10-O **PEER REVIEWED**

MOLECULAR WEIGHT:
74.12[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 272] **PEER REVIEWED**

COLOR/FORM:
A colorless liquid, which forms rhombic crystals melting at 25 to 25.5 deg C[Snyder, R. (ed.). Ethel Browning's Toxicity and Metabolism of Industrial Solvents. Second Edition. Volume 3 Alcohols and Esters. New York, NY: Elsevier, 1992., p. 65] **PEER REVIEWED**

Colorless liquid or rhombic prisms or plates[Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. V2: 602] **PEER REVIEWED**

Crystals[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 272] **PEER REVIEWED**

Colorless liquid or solid (above 77 deg F) [Note: Often used in aqueous solutions].[NIOSH. NIOSH Pocket Guide to Chemical Hazards. Department of Health & Human Services, Centers for Disease Control & Prevention. National Institute for Occupational Safety & Health. DHHS (NIOSH) Publication No. 2010-168 (2010). Available from: http://www.cdc.gov/niosh/npg] **PEER REVIEWED**

ODOR:
Camphor-like odor[NIOSH. NIOSH Pocket Guide to Chemical Hazards. Department of Health & Human Services, Centers for Disease Control & Prevention. National Institute for Occupational Safety & Health. DHHS (NIOSH) Publication No. 2010-168 (2010). Available from: http://www.cdc.gov/niosh/npg] **PEER REVIEWED**

BOILING POINT:
82.3 deg C[Haynes, W.M. (ed.). CRC Handbook of Chemistry and Physics. 94th Edition. CRC Press LLC, Boca Raton: FL 2013-2014, p. 3-392] **PEER REVIEWED**

MELTING POINT:
25.81 deg C[Haynes, W.M. (ed.). CRC Handbook of Chemistry and Physics. 94th Edition. CRC Press LLC, Boca Raton: FL 2013-2014, p. 3-392] **PEER

Page 1

REVIEWED**

DENSITY/SPECIFIC GRAVITY:
0.7886 g/cu cm at 20 deg C[Haynes, W.M. (ed.). CRC Handbook of Chemistry and Physics. 94th Edition. CRC Press LLC, Boca Raton: FL 2013-2014, p. 3-392] **PEER REVIEWED**

DISSOCIATION CONSTANTS:
pKa = 19.20[Serjeant, E.P., Dempsey B.; Ionisation Constants of Organic Acids in Aqueous Solution. International Union of Pure and Applied Chemistry (IUPAC). IUPAC Chemical Data Series No. 23, 1979. New York, New York: Pergamon Press, Inc., p. 989] **PEER REVIEWED**

HEAT OF VAPORIZATION:
39.07 kJ/mol[Kirk-Othmer Encyclopedia of Chemical Technology. 4th ed. Volumes 1: New York, NY. John Wiley and Sons, 1991-Present., p. V4 (92) 692] **PEER REVIEWED**

OCTANOL/WATER PARTITION COEFFICIENT:
log Kow = 0.35[Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995., p. 10] **PEER REVIEWED**

SOLUBILITIES:
In water, 1X10+6 mg/L at 25 deg C /Miscible/[Riddick, J.A., W.B. Bunger, Sakano T.K. Techniques of Chemistry 4th ed., Volume II. Organic Solvents. New York, NY: John Wiley and Sons., 1985.] **PEER REVIEWED**

Miscible with ethanol, ethyl ether; soluble in chloroform[Haynes, W.M. (ed.). CRC Handbook of Chemistry and Physics. 94th Edition. CRC Press LLC, Boca Raton: FL 2013-2014, p. 3-392] **PEER REVIEWED**

SPECTRAL PROPERTIES:
Index of refraction: 1.3878 at 20 deg C/D[Haynes, W.M. (ed.). CRC Handbook of Chemistry and Physics. 94th Edition. CRC Press LLC, Boca Raton: FL 2013-2014, p. 3-392] **PEER REVIEWED**

Index of refraction: 1.38468 at 20 deg C/D; 1.38231 at 25 deg C/D[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 272] **PEER REVIEWED**

VAPOR DENSITY:
2.55 (Air = 1)[Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. V2: 602] **PEER REVIEWED**

VAPOR PRESSURE:
40.7 mm Hg at 25 deg C[Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989.] **PEER REVIEWED**

OTHER CHEMICAL/PHYSICAL PROPERTIES:
Specific gravity: 0.78581 at 20 deg C/4 deg C; 0.78086 at 25 deg C/4 deg C; (calcd) 0.78080 at 25 deg C/4 deg C[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 272] **PEER REVIEWED**

Maximum vapor concentration: 5.53% at 25 deg C[Bevan C; Monohydric Alcohols: C1 to C6. Patty's Toxicology. 6th ed. (1999-2013). New York, NY: John Wiley & Sons, Inc. On-line posting date: 17 Aug 2012] **PEER REVIEWED**

Dr. Marvin Meistrich

3. Noncancer
3a. Noncancer kidney toxicity:

I agree. No specific comments at this point, awaiting input from panel members with more expertise in kidney effects.

3b. Noncancer toxicity at other sites:

I agree that there is inadequate evidence to assess developmental, neurodevelopmental, and reproductive toxicity. The studies referenced in the report are quite limited.

Developmental toxicity: Only small reductions in rat fetal and pup survival and pup body weight were observed at an oral gavage tBA dose of 1000 mg/kg-d. These effect occurred at doses in which there was some maternal toxicity in the form of reduced weight gain

Male reproductive toxicity: Most endpoints show no toxicities at maximal doses used.
One study indicated a ~15% decline in mouse testis weight at an oral (drinking water) dose of 3600 mg/kg-d for 13 weeks. But the statistical significance of this result was presented differently in different parts of the report, and there was also >50% mortality at this dose.

Female reproductive toxicity: Most endpoints show no toxicities at maximal doses used.
One study indicated an extension of the mouse estrous cycle from 4 to 5 days after oral exposure of female mice to 3600 mg/kg-d of tBA for 13 weeks. A non-significant trend towards lengthening the estrous cycle was observed in rats at the maximal exposure level of 1600 mg/kg-d.

3c. Oral reference dose of 0.4 mg/kg/day based on severity of nephropathy in female rats.

Use of kidney endpoint seems appropriate. Reproductive and developmental toxicity (NOEL=400 mg/kg-d), higher doses than kidney endpoint (LOEL=180 mg/kg-d)

3d. Inhalation reference concentration of 5 mg/m^3 based on the severity of nephropathy in female rats

The concentration for the POD (491 mg/m^3) had to be extrapolated from the drinking water study. In that case, the kidney toxicity occurs at a lower dose than reproductive toxicity.

Dr. Jeffrey Fisher

2. Chemical Properties and Toxicokinetics
2a. Chemical properties. Is the information on chemical properties accurate? Is the information on chemical properties accurate?

Looks ok.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

No response.

2c. Choice of dose metric. Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?

At this time, I think so. I need to review TBA again as I am confusing it with ETBE.

4e. Inhalation unit risk for cancer. Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α2u-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

No response.

Dr. John Budroe

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

The Document correctly and clearly identifies severity and incidence tert-butanol (tBA)-induced renal toxicity exhibited by female rats as being relevant to human health risk assessment. Renal toxicity endpoints included increased kidney weights, suppurative inflammation, transitional epithelial hyperplasia, and nephropathy (severity and incidence). These data were obtained primarily from the NTP 1995 tBA drinking water and NTP 1997 tBA inhalation studies. Other studies provided similar data on kidney weight (Huntingdon Life Sciences, 2004) and histopathology (Acharya et al., 1995; Acharya et al., 1997). The consistency of effect across the several studies provides a level of confidence in the conclusion that tBA is a renal toxicant.

In contrast, the Document takes the position in Section 1.2.1 that α2u-globulin induction in male rats exacerbates CPN, making any increase in renal histopathological endpoints in male rats characterized as being part of the CPN "spectrum of effects" not relevant to the characterization

of human health hazard for noncancer kidney toxicity. This rationale is used to justify not considering the increased incidence of transitional epithelial hyperplasia demonstrated by TBA-treated male rats in the NTP 1995 study for either hazard identification or dose-response estimation purposes.

However, the relationship between α2u-globulin induction and elicitation of putatively CPN-related effects is inconsistent. For example, renal suppurative inflammation, which is believed to be part of the CPN spectrum, was not significantly increased in the NTP 1995 male rats and was only weakly correlated with CPN.

It is also illogical to take the position that tBA-induced renal effects such as suppurative inflammation and transitional epithelial hyperplasia are relevant to hazard identification and dose-response assessment if observed in female rats but are not relevant if observed in male rats. These effects are obviously independent to some degree of α2u-globulin induction. The transitional epithelial hyperplasia incidence and severity increase observed in the NTP 1995 male rats should be considered relevant to human hazard identification, and should be modeled for a dose-response assessment. This would at least allow comparison to the same toxicity endpoint in the female rats, and would be consistent with the use of urothelial hyperplasia in deriving the candidate ETBE RfC, since the ETBE draft Document states that urothelial hyperplasia "is synonymous with the transitional epithelial hyperplasia in the renal pelvis observed after chronic *tert*-butanol exposure in both male and female rats".

The Document should not allow concerns regarding "disentangling mechanisms of kidney toxicity and carcinogenicity" to result in eliminating useful toxicity data (tBA-exposed male rat kidney toxicity/carcinogenicity) from consideration. Such an action would not be health protective.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for *tert*-butanol, based on thyroid follicular cell tumors in male and female $B6C3F_1$ mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

Section 2.5 of the U.S. EPA 2005 Guidelines for Carcinogen Risk Assessment document (U.S. EPA, 2005) includes a discussion of the "Likely to Be Carcinogenic to Humans" descriptor. Included in examples of supporting data for this descriptor is the following: "an agent that has

tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans".

The NTP 1995 *tert*-butanol (tBA) drinking water cancer bioassay reported an significant increase in male rat renal tumors, and male and female mouse thyroid tumors. The NTP study was well designed, conducted and reported, and received external peer review. The Toxicological Review of *tert*-Butyl Alcohol document (the Document) states correctly that α2u-globulin-associated nephropathy and chronic progressive nephropathy (CPN) are not likely to be solely responsible for the induction of male rat renal tumors, indicating that those tumors are relevant to tBA human cancer risk assessment.

Additionally, the Document states that the available data are inadequate for concluding that an anti-thyroid MOA is operating in tBA-induced mouse thyroid follicular cell tumorigenesis, no other thyroid tumor MOAs were identified, and the mouse thyroid tumors are relevant to tBA human cancer risk assessment. That assessment is also correct.

tBA has been demonstrated to be carcinogenic in more than one sex (male and female mice), and more than one species (rats and mice). Thus, the "Likely to Be Carcinogenic to Humans" descriptor would be more appropriate for tBA.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data generally would not support one, however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for quantitative analysis, and whether the NTP (1995) study is suitable for this purpose. Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the NTP (1995) study is a suitable basis for this quantitative analysis.

As noted above in the Response to Charge Question 4b, tBA has been demonstrated to be carcinogenic in more than one sex (male and female mice), and more than one species (rats and mice). Thus, the "Likely to Be Carcinogenic to Humans" descriptor would be more appropriate for tBA. The EPA 2005 cancer guidelines states that dose-response assessments are generally completed for agents considered "Carcinogenic to Humans" and "Likely to Be Carcinogenic to Humans". This indicates that a quantitative cancer dose-response analysis should be included in the Document.

However, even if the "suggestive evidence" descriptor is retained in the Document, a quantitative cancer dose-response analysis is still appropriate. As noted in Charge Question 4c, "when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks". The NTP 1995 tBA study was well designed, conducted and reported, had no confounders, and received external peer review. Section 2.3 of the Document adequately describes the scientific quality of the NTP 1995 study, but should elaborate on the utility of "providing a sense of the magnitude and uncertainty of potential risks". The Document notes that the domestic production volume of tBA was approximately 4 billion pounds in 2012, and it is used in lacquers, paint removers, and nail enamels and polishes. This indicates the potential for substantial worker and consumer exposure to tBA, with potential concomitant health risks. The quantitative cancer dose-response analysis contained in the Document is needed in order to provide a sense of the magnitude of potential cancer risk associated with tBA exposure.

Finally, the NTP 1995 study is definitely a suitable basis for a quantitative tBA human cancer risk analysis. That study reported significant and dose-dependent increases in male and female mouse thyroid tumors, and male rat renal tumors. The studies by Doi *et al.* (2007) and Melnick *et al.* (2012) suggest that neither α-2u globulin nephropathy nor chronic progressive nephropathy are valid MOUs for male rat kidney tumor induction. EPA policy notwithstanding, all of those tumor data sets can and should be used for quantitative cancer dose-response analysis.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 5 x 10–4 per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

The female mouse thyroid tumor data set from the NTP 1995 study demonstrates a significantly increased tumor incidence compared to controls and a positive dose-response, and can be used to derive an oral slope factor. The available data on factors that would be required for an chemically-induced anti-thyroid tumor MOA (decreased T3 and T4 levels, increased TSH levels) do not support that MOA. The use of ¾ power body weight dose scaling between animals and humans and the use of the BMDS multistage cancer model to derive the oral cancer slope factor is appropriate, done correctly, and clearly derived.

It would be useful if a more explicit explanation of why the PODs for estimating low-dose risk are different for male and female mice (5% and 10% extra risk, respectively) was provided in either the Document or the attached Supplemental Information.

Additionally, the male rat kidney tumor data set from the NTP 1995 report can be used for deriving a tBA oral slope factor. It has been proposed that α2u globulin induction causes renal

tubule cytotoxicity, which leads to regenerative cell proliferation and ultimately renal tumor production. However, tBA is a weak α2u globulin inducer, and does not induce α2u globulin or cytotoxicity at tBA doses that induce cell proliferation and renal tumors, making this cancer MOA unlikely for tBA. Also, studies by Doi *et al.* (2007) and Melnick *et al.* (2012) suggest that neither α-2u globulin nephropathy nor chronic progressive nephropathy are valid MOAs for male rat kidney tumor induction.

A health-protective choice in this case would be to assume that the relative contribution of α2u-globulin nephropathy and other processes to the NTP 1995 male rat kidney tumor incidence was zero, and develop an oral slope factor for tBA from the male rat kidney tumor data set. That slope factor could then be considered along with the oral slope factor derived from the female mouse thyroid tumor data set.

4e. Inhalation unit risk for cancer. Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α2u-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

The lack of a tBA toxicokinetic model for mice did not per se preclude the use of the NTP 1995 female mouse thyroid tumor data to develop an inhalation unit risk factor for tBA. EPA does not have an obvious stated policy that a PBPK model is required in order to perform a route-to-route extrapolation. The Document should include a route-to-route extrapolation from the 5×10^{-4} $(\text{mg/kg-day})^{-1}$ oral slope factor developed in Section 2.3.3 of the Document, using a default human body weight of 70 kg, a default human inspiration rate of 20 m^3/day, and appropriate inhalation absorption fractions for mice and humans, to develop an inhalation unit risk factor for tBA.

Additionally, as mentioned in the Response to Charge Question 4d, the studies by Doi *et al.* (2007) and Melnick *et al.* (2012) suggest that neither α-2u globulin nephropathy nor chronic progressive nephropathy are valid MOUs for male rat kidney tumor induction. The Document states: "CPN is a common and well-established constellation of age-related lesions in the kidney of rats, for which no known counterpart in aging humans exists. CPN is not a specific diagnosis per se but, rather, an aggregate term describing a spectrum of effects. Individually, these lesions or processes could occur in a human kidney, and their occurrence as a group in the aged rat kidney does not make each one rat-specific if a treatment effect occurs for one or more of them." This leads to the self-contradictory position that the collection of renal histopathological effects designated as CPN are not relevant to human health risk assessment even if treatment-related, but the individual effects are.

A health-protective choice in this case would be to assume that the relative contribution of α2u-globulin nephropathy and other processes to the NTP 1995 male rat kidney tumor incidence was zero, and develop a inhalation unit risk factor for tBA from the male rat kidney tumor data set. That slope factor could then be considered along with the inhalation unit risk factor that should be derived from the female mouse thyroid tumor data set.

References

Doi, AM; Hill, G; Seely, J; Hailey, JR; Kissling, G; Bucher, JR. (2007). α2u-globulin nephropathy and renal tumors in National Toxicology Program studies. Toxicol Pathol. 35: 533-540.

Dr. Lawrence Lash

2c. Choice of dose metric. Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?

The average concentration of tBA in blood is chosen as a dose metric. The clearest explanation for this is provided in Section 2.2 (page 2-12; lines 22-30) ("Methods of Analysis"):

"A critical decision in the route-to-route extrapolation is selection of the internal dose metric that establishes "equivalent" oral and inhalation exposures. For *tert*-butanol-induced kidney effects, the two options are the concentration of *tert*-butanol in blood and the rate of *tert*-butanol metabolism. Note that using the kidney concentration of *tert*-butanol will lead to the same route-to-route extrapolation relationship as *tert*-butanol in blood because the distribution from blood to kidney is independent of route. Data are not available that suggest that metabolites of *tert*-butanol mediate its renal toxicity. Without evidence that suggests otherwise, *tert*-butanol is assumed the active toxicological agent. Therefore, the concentration of *tert*-butanol in blood was selected as the dose metric."

The rationale makes complete sense and is clearly presented. The key points are that renal toxicity appears to be due to the parent compound rather than any metabolites and that blood:kidney distribution is independent of route of exposure.

The document is organized according to the standard manner for IRIS assessments. Section 1 presents the hazard assessment, and is appropriately divided into three main sections: 1) overview of chemical properties and toxicokinetics; 2) synthesis of evidence by organ or system; and 3) integration and evaluation. The document clearly describes the nature of the available literature, the criteria used to include or exclude studies, and the rationale for the choice of the principal studies for each effect. Section 2 is the section on dose-response analysis; it is divided into five main sections: 1) oral reference dose for effects other than cancer; 2) inhalation reference concentration for effects other than cancer; 3) oral slope factor for cancer; 4) inhalation

unit risk for cancer; and 5) application of age-dependent adjustment factors. There are no additional studies that should or need to be added to the analysis.

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described**.**

Just as with ETBE, the EPA thoroughly evaluated the role of alpha-2u globulin in male rat kidney effects of tBA according to the well-establish criteria. Similarly, the issue of CPN and its potential role and relevance to humans was also discussed methodically. I believe that the conclusions reached in the tBA IRIS toxicological review are indeed scientifically supported and clearly described. The primary conclusions are as follows:

1) Kidney effects were identified as a potential human hazard of *tert*-butanol exposure based on several endpoints in female rats, including suppurative inflammation, transitional epithelial hyperplasia, severity and incidence of nephropathy, and increased kidney weights. These effects are similar to the kidney effects observed with ETBE exposure (e.g., CPN and urothelial hyperplasia) and MTBE (e.g., CPN and mineralization).

2) Any kidney effects associated with α_{2u}-globulin nephropathy are not considered relevant for human hazard identification.

3) CPN played a role in the renal tubule nephropathy observed following *tert*-butanol exposure in female rats. Because female rats were not affected by α_{2u}-globulin nephropathy and the individual lesions associated with the spectrum of toxicities collectively described as CPN can occur in the human kidney, exacerbation of one or more of these lesions might reflect a type of injury relevant to the human kidney. Effects associated with such nephropathy are considered relevant for human hazard identification and suitable for derivation of reference values.

4) Overall, the female rat kidney effects (suppurative inflammation, transitional epithelial hyperplasia, increased severity of CPN, and increased kidney weights) are considered the result of *tert*-butanol exposure and relevant to human hazard characterization. These effects, therefore, are suitable for consideration for dose-response analysis and derivation of reference values.

A couple of minor concerns are that suppurative inflammation and transitional epithelial hyperplasia may both have multiple, poorly defined etiologies and may not be mechanistically linked to nephropathy, certainly nephropathy associated with proximal tubular cell injury. Some additional discussion of this may be warranted. Overall, however, the conclusion that kidney effects are a potential human hazard associated with tBA exposure is appropriate.

Comment:

1) On page 2-16, lines 10-11: The document describes, in this paragraph, the derivation of an RfC value based on increased kidney weights and other kidney effects from a chronic oral exposure study in female rats, and states that the effects occurred

> "spanning a range from 44×10^0 to 3×10^1 mg/m^3, for an overall 7-fold range." This is not a 7-fold range, but is only about 1.5-fold.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F$_1$ mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

With regard to kidney cancer, a major finding that makes conclusions about kidney cancer and tBA exposure is that while renal tubule tumors are observed in male F344 rats, the alpha-2u response is operative (albeit weakly). Additionally, CPN occurs in both male and female F344 rats, is nearly as severe in female rats as in male rats, but renal tubule tumors are not observed in the female rats. Based on these apparent conflicting observations and the limitations and uncertainties in the database, I believe the conclusions reached by the EPA and the rationale used to reach these conclusions are clear, logical, and well-justified.

The data on tBA-induced thyroid follicular tumors in both male and female mice are clearer in terms of the ability to make a conclusion about relevance to humans. The document also points out in a concise and clear manner that the database has significant deficiencies. Thus, while it is clear that thyroid growth is stimulated, the database are either negative or equivocal regarding effects of tBA on thyroid and pituitary hormone levels, sites of anti-thyroid action, and reversibility of effects on the thyroid gland at early times after exposure.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data generally would not support one, however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence." Please comment on whether Section~~s~~ 2.3 of the draft assessment adequately explains the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the NTP (1995) study is a suitable basis for this quantitative analysis.

Although the evidence leads the EPA to conclude that the "suggestive evidence" descriptor is appropriate; the document describes the rationale for why a quantitative analysis is conducted. The discussion summarizes the rationale based on dose-response data, MOA information for renal and thyroid tumors, and the absence of a PBPK model for mice. Based on all these considerations, linear low-dose extrapolation was used to estimate human carcinogenic risk.

All these considerations are standard and according to established EPA protocol. I am not convinced, however, that the issue of doing a quantitative analysis for a chemical for which the

descriptor "suggestive evidence" is used, is clearly discussed in Section 2.3. The NTP (1995) study is also clearly and thoroughly described with respect to all the criteria that make it appropriate as the basis of a quantitative analysis.

Dr. Isaac Pessah

3b. Noncancer toxicity at other sites. (Sections 1.2.3-6, and 1.3.1). The draft assessment finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with *tert*-butanol exposure, please identify them and outline the rationale for including them in the assessment.

Based on the available literature, I would have to agree that there is inadequate information to evaluate and make conclusions about developmental, neurodevelopmental, and reproductive toxicity of tBA. Te studies that are available suggest. None of these studies performed detailed behavioral testing in the cognitive, social, anxiety, hyperexcitability, domains. Given the high doses chronic exposures tested, the data that is provided suggest no to minimal developmental effects that was not related to maternal toxicity (altered behavior, nutrition, etc). None the less, important behavioral data is missing and is necessary to completely discount risk associated with exposures that alter maternal TH homeostasis and influences on offspring developmentally exposed to tBA (even though TH criteria for cancer set out by EPA are not met). The absence or inconsistency of results obtained from motor tasks/activity is unlikely to predict other neurodevelopmental domains mentioned above, certainly this pattern is often seen with other chemicals. Neurochemical measures reported by Nelson et al (1991) are suggestive of some neurological consequences, and although the study has weaknesses I cannot discount their observations completely. Moreover, the cross-foster mouse study by Daniel and Evans (1982) may indeed have a component of maternal toxicity contributing to psychomotor deficits observed, yet key behavioral test performed at lower more relevant doses simply have not been done. The lack of any mechanistic information, especially on the relationship between altered TH functioning and behavioral outcome raises uncertainty about the conclusions inferred about the lack of developmental neurotoxicity (or at least raises uncertainties rather than lack of scientific evidence). Better studies directly relevant to DNT are needed.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of $4x10^{-1}$ mg/kg–day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

I concur that the available data presented in the report scientifically supports a RfD of $4x10^{-1}$ mg/kg-d based on nephropathy in female rats exposed via drinking water. The data on which the RfD is based shows dose-dependence, most likely reflects direct kidney damage progressing with over a chronic timeframe relevant to human exposures, and is the most sensitive POD in the available data.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for *tert*-butanol, based on thyroid follicular cell tumors in male and female $B6C3F_1$ mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. ~~Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported~~. **Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.**

Although carcinogenesis is not my expertise, my conclusions based on the available data presented in the report is that the "suggestive evidence" cancer descriptor is scientifically supported for both oral and pulmonary routes of exposure. The high doses on which this conclusion is based may raise uncertainties about relevance, never the less the data support a designation "suggestive evidence" based on EPA guidelines.

5. Susceptible Populations and Lifestages. As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

Clearly data is lacking to make a scientifically valid conclusion about susceptible population; especially given the concerns I raised under **3b**. This topic will need more discussion by the workgroup.

Dr. Harvey Clewell

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods. Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

I found the strategies for literature searching, study inclusion and evaluation to be clearly described and objectively applied.

Hazard Identification and Dose-Response Analysis. Chapter 1 (Hazard Identification) and the supplemental materials summarize the chemical properties, toxicokinetics, and health effects associated exposure to tert-butanol. Chapter 2 (Dose Response Analysis) uses this information to derive an oral reference dose and inhalation reference concentration for noncancer outcomes, in addition to an oral slope factor for cancer.

2. Chemical Properties and Toxicokinetics
2a. Chemical properties. Is the information on chemical properties accurate?

It appears accurate to me.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties?

Section B.1.5 provides only a very brief description of the PBPK model used in this assessment. However, the approach for model evaluation is very clearly described in the USEPA (2017) document "PK/PBPK Model Evaluation for the IRIS Assessments of Ethyl Tertiary Butyl Ether and tert-Butyl Alcohol" cited in Section B.1.5 of Appendix B. This evaluation appears to have been objective and thorough, with a detailed discussion of uncertainties, assumptions and required modifications. I agree with the conclusion of the evaluation that the Borghoff et al. (2016) model, as modified by EPA, is fit for the purpose of supporting the IRIS assessments for tBA.

Are there additional peer-reviewed studies that should be considered for modeling?

I disagree with the conclusions of the report is the determination that the human model of Nihlen and Johanson (1999) cannot be used for cross-species extrapolation for tBA.
It is highly unfortunate that the human model of cannot be used to support animal to human extrapolation, and I strongly believe it would be possible for the EPA to make use of the published model and data from the Nihlen and Johanson (1999) publication to create an acceptable human model.

In particular, I do not agree with the statement in the report that:

"The Nihlén and Johanson model is based on measurements of blood concentrations of eight individuals exposed to 5, 25, and 50 ppm ETBE for 2 hours while physically active. This model differs from conventional PBPK models in that the tissue volumes and blood flows were calculated from individual data on body weight and height. Additionally, to account for physical activity, blood flows to tissues were expressed as a function of the workload. These differences from typical PBPK models preclude allometric scaling of this model to other species for cross-species extrapolation."
Although, as stated in the document, the Nihlen and Johansen (1999) data and modeling approach are not "conventional", they are probably useful. Similar experimental data and PBPK modeling (Johanson et al. 1986; Corley et al. 1994) were used in the IRIS assessment for 2-butoxyethanol.

I also disagree with the statement in the report that:
"As there are no oral exposure toxicokinetic data in humans, this model does not have a mechanism for simulating oral exposures, which prevents use of the model in animal-to-human extrapolation for that route."
The oral route can be described in the model in the same way that it was described in the PBPK models of vinyl chloride (Clewell et al. 1999) and 2-butoxyethanol (Corley et al. 1994), which were used in the development of the RfDs and RfCs for these two chemicals, as well as in both the oral and inhalation cancer assessments, despite the lack of human toxicokinetic data for the oral route. For vinyl chloride, as with ETBE, the dose metric was rate of metabolism of the parent chemical in the liver. For 2-butoxyethanol, as with tBA, the dose metric was the concentration of the active metabolite in the blood.
Therefore, I would recommend that EPA give further consideration to modifying the model of Nihlen and Johanson (1999) in a similar fashion to the way in which Corley et al. (1994) modified the model of Johanson et al. 1986) to support cross-species extrapolations for both inhalation and oral routes of exposure.

2c. Choice of dose metric. Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?

I agree with the use of the average concentration of tBA in the blood at steady state (for continuous inhalation) or after periodicity is achieved (for oral exposure) as the dose metric for route-to-route extrapolation in the rat. I am also comfortable with the pulsatile oral ingestion pattern used by EPA for rats exposed via drinking water.

Hazard Identification and Dose–Response Assessment. Comment on EPA's assessment of the toxicological studies and dose-response assessment, including whether there are additional peer-reviewed studies that should be considered.

3. Noncancer
3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of α2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

I agree with the conclusion of the assessment that although α2u-globulin and chronic progressive nephropathy may have contributed to the kidney tubular lesions observed in rats, the dose-response data for female rats are an appropriate basis for calculating BMDs for kidney effects.

3b. Noncancer toxicity at other sites. (Sections 1.2.3-6, and 1.3.1). The draft assessment finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with tert-butanol exposure, please identify them and outline the rationale for including them in the assessment.

I agree with the decision not to perform dose-response assessments for the other sites for noncancer toxicity.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of 4x10–1 mg/kg–day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

I agree that the dose-response data female rats are an appropriate basis for calculating BMDs for kidney effects and I believe the BMD analysis was performed correctly. However as indicated above, I would prefer that the use of PBPK modeling be reconsidered for cross-species extrapolation to replace the BW^3/4 default.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 5x100 mg/m3, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

I agree that the dose-response data female rats are an appropriate basis for calculating BMDs for kidney effects and I believe the BMD analysis was performed correctly. However as indicated above, I would prefer that the use of PBPK modeling be reconsidered for cross-species extrapolation to replace the category 3 default inhalation dosimetry calculation.

4. Cancer
4a. Cancer modes-of-action.
(i) Cancer modes-of-action in the kidney. As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving α2u-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.
I accept the rationale in the assessment for concluding that there is inadequate information to support a mode-of-action for kidney tumors from tBA solely involving α2u-globulin nephropathy. Although the human relevance of these tumors is highly questionable, it cannot be completely ruled out using EPA guidance.

(ii) Cancer modes-of-action in the thyroid. As described in section 1.2.2, thyroid tumors were observed in male and female mice following tert-butanol exposure, and an anti-thyroid mode-of-action was evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid follicular cell tumors in rodents (U.S. EPA, 1998), found the information inadequate to determine whether an anti-thyroid mode-of-action was operating and considered the thyroid follicular cell tumors in male and female mice to be relevant to humans. Please comment on whether this conclusion is scientifically supported.

I accept the rationale in the assessment for concluding that it would be premature to identify thyroid-pituitary disruption as the mode of action for thyroid tumors from tBA inhalation. Although the human relevance of these tumors is highly questionable, it cannot be completely ruled out using EPA guidance.

4b. Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

I accept the rationale for a cancer descriptor of suggestive evidence for tBA based on the observation of thyroid tumors from inhalation of tBA. Although the human relevance of these tumors is highly questionable, it cannot be completely ruled out using EPA guidance.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data generally would not support one, however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

Please comment on whether Sections 2.3 of the draft assessment adequately explains the rationale for including a quantitative analysis given the "suggestive evidence" descriptor.

I could not find any rationale for performing a quantitative analysis for tBA thyroid cancer in Sections 2.3, or indeed anywhere else in the document. Rather than providing a rationale for the decision, the assessment merely cites the EPA (2005) Guidelines for Carcinogen Risk Assessment to demonstrate that they do have the option of performing one:
"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however when the evidence includes a well-conducted study, quantitative analysis may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." What is missing in the document is any rationale for performing a quantitative analysis in the case of tBA. In particular, no rationale is presented to suggest that performing a dose-response assessment for tBA using the default low-dose linear assumption would be useful for any purpose.

In Section 1.3.2 (p. 1-166, lines 16-18), the agency summarizes the limited evidence for tBA thyroid carcinogenicity: "In B6C3F1 mice, administration of tert-butanol in drinking water increased the incidence of thyroid follicular cell adenomas in females and adenomas or carcinomas (only one carcinoma observed) in males (NTP, 1995), as discussed in Section 1.2.2". I do not see any way in which performing a low-lose linear extrapolation of these data could possibly provide a sense of the magnitude and uncertainty of potential risks, help to rank potential hazards, or set research priorities. In fact, I believe that providing only a default linear dose-response assessment in this case would be highly misleading.

Also comment whether the NTP (1995) study is a suitable basis for this quantitative analysis.

NTP (1995) is a well-conducted and well-reported study, but the data for neoplastic thyroid lesions are not a suitable basis for the quantitative analysis performed in Section 2.3.

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 5 x 10–4 per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

I do not believe that a quantitative analysis for an oral slope factor can be reliably performed at this time.

4e. Inhalation unit risk for cancer. Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α2u-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

I agree with the decision not estimate an inhalation unit risk for tBA. The potential contribution of α2u-globulin nephropathy and other processes to the observed kidney tumors in male rats precludes conducting an informative quantitative dose-response analysis.

5. Susceptible Populations and Lifestages. As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions

I agree that there is inadequate information to identify a possibly susceptible population or lifestage for tBA toxicity.

6. Question on the Executive Summary. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

I found the Executive Summary to be a clear and accurate synopsis for the key findings and conclusions of the assessment.

Committee Members with no Preliminary Comments

1. Dr. Bevin Engelward
2. Dr. Tamarra James-Todd