NOTICE: **Do Not Cite or Quote.** Preliminary Comments submitted by individual members are for review and to assist meeting deliberations by the CAAC-ETBE/tBA committee.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)
Revised Member Comments

The following revised comments from individual committee members were submitted in response to the In-Person committee meeting held August 15 – 17, 2017. Responses are grouped according to chemical. The compilation contains all comments received as of September 5, 2017.

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Ethyl Tertiary Butyl Ether (ETBE)

Dr. Isaac Pessah

Q1: Literature Search Strategy/Study Selection… 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.

Overall I agree with the panel that the search strategies, criteria for study inclusion/exclusion and evaluation and for study quality are described clearly. The complete exclusion of effects reported for non-mammalian species (e.g., the available peer reviewed literature using embryonic zebrafish exposure models) may be a consequence of not adhering fully to the NRC (2011) recommendations for systematic review in the IRIS program. Recommend that EPA incorporate the available data from non-mammalian model organisms at some point for hazard assessment of ETBE.

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.

I agree that kidney urothelial hyperplasia at an oral dose of 170 mg/kg-d and inhalation at 6,000 mg/m^3 is scientifically supported and clearly described for deriving RfD and RfC, respectively. These are the more defensible data for deriving reference doses.

- Liver effects:

Agree with *suggestive evidence, with the following qualifications*

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 $\geq$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:

Although *inadequate evidence* is a reasonable descriptor of the available data, I strongly support the panel's recommendation of describing the available data as *minimal effects at otherwise toxic doses* and that zebrafish data (and data from other non-mammalian models) be included in the assessment as they become available.

Considering the possible biding of ETBE with nuclear receptor (PXR, CAR, etc) it is advisable the further evaluate more sensitive and relevant endpoints for possible developmental neurotoxicity mediated by ETBE interference with these receptors. Although the developmental studies cited in EPA report were convincingly negative, it is not clear that the most relevant neurological and behavioral endpoints were assessed in these older studies. EPA should consider recent evidence showing that both PXR and CAR knockout mice have profound neurological impairments (see the two recent papers from Nicoli Marchi's lab this year). These types of outcomes have not been addressed in the existing developmental data on ETBE or TBA, and thus convey a significant uncertainty in my mind.

Absence of PXR or CAR was found to be associated with anxiety-like behavior and recognition memory impairment adult mice. The latter was simultaneous to an altered EEG signature (lower theta frequency) during sleep and abnormal delta waves. Neurophysiological changes did not correspond to significant structural changes (i.e. no overt neuropathology) in the adult brain. This is strong evidence of a link between NR expression/function and brain physiology. The relationship to ETBE/TBA exposures during embryonic development, binding of these NR and such outcomes seem to me to be a missing piece of developmental neurotoxicity that may be important to protecting human health since they are likely to occur at much lower levels of exposure than the other endpoints use are evaluating (1, 2).

1. Boussadia, B., *et al* (2016) Lack of CAR impacts neuronal function and cerebrovascular integrity *in vivo. Experimental Neurology* **283,** 39-48.
2. Boussadia, B., *et al* (2017) Lack of CAR impacts neuronal function and cerebrovascular integrity *in vivo. Neuroscience* epub July 23 PMID: 27240521

- 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). Thus more animal studies are warranted with Aldh2 mutant alleles, including neurodevelopmental endpoints.

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.

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.

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. 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 a HED consider creating 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.

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. Please rerun the simulations.

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

The text in the supplemental section of PBPK model (page B-24 and B-27) needs to be reworded to clarify the units of this dosimetric (average daily rate of ETBE metabolized per day at periodicity).

However, with no clear smoking gun pertaining to MOA or correlations between dose metrics and metabolites (published modeling papers), this dose metric may be adequate if the extrapolation is performed. 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.

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.

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: Consider creating 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. The ETBE inhalation exposure concentrations in human that mimic the rat for predetermined dose metrics could be used for deriving a unit risk.

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?

The strategy for the literature search for the Toxicological Review of Ethyl Tertiary Butyl Ether did not follow all the recommendations in NRC (2011), except for the some aspects of the selection of the studies to be included in evidence tables. While it is unlikely that strict adherence to the NRC (2011) guidelines would have identified additional critical studies, there is a potential for having missed information, either supportive or non-supportive of EPA's evaluation of ETBE, as discussed below.

The general strategy used by EPA consisted of starting with a very broad, ETBE-specific search of scientific literature databases and other sources, followed by screenings for identifying and selecting the studies relevant to the risk assessment, and classifying the studies into well-defined categories. Searches in scientific literature indexes were updated continually through November 2015 or December 01, 2016 (for the TSCATS2 database). Studies were systematically included in HERO according to the corresponding categories. Overall, the approach is well explained and

appropriate for the goal of maximizing the likelihood of identifying relevant, ETBE-specific studies for consideration in the toxicological review. EPA should clarify 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 ETBE-specific search likely encompass the large majority of the ETBE-relevant, peer reviewed articles, but there should be a clarification 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 synonyms were specifically selected from among the pool of names 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 why: 1) citations were searched manually only in two review articles and not other peer-reviewed publications, and 2) searches in sources of citations other than peer-reviewed publications, such as reviews by other national and international agencies, master/doctoral theses, grey literature, etc., were apparently not performed or the document fails to describe them. Clarification is needed for why the search strategies were more restrictive than the NRC (2011) recommended.

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 two studies in addition to 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 studies to be included in the database for risk assessment.

Criteria for quality evaluation of studies to be included in evidence tables are well described and seem appropriate. It is not possible to ascertain whether the quality criteria were applied objectively or not because there is no documentation of the specific, detailed description of the quality evaluation for each of the studies. On the other hand, there is no evidence in the document that quality evaluation criteria were applied in a non-objective manner.

As indicated above, it is unlikely that the search strategies used by EPA failed to identify key relevant studies (e.g., a human or rodent study). There is, however, lack of information about the sequence of specific search queries used by EPA in the development of the final database of studies to be included in evidence tables and as supplementary information. Consistent with systematic review and the recommendations of NRC (2011)], new additional questions should have arisen during the process of study review. These questions frequently lead to additional reviews of other studies and likely require performing new, additional literature searches using a different set of search terms than those employed previously. It is not apparent that this process occurred as extensively as needed in the toxicological review of ETBE. It is also apparent that the hazard evaluation component may have been limited. For example, the footnote in Table LS-3 indicates that ecological and non-mammalian species were “not considered a source of health effects or supplementary health effects data/mechanistic and toxic kinetic data, but were considered as sources of contextual information.” However, there is no evidence that studies

with ecological or non-mammalian species were actually considered for any purpose, either in the toxicological review or in the supplemental information. As depicted in the available documentation, it appears that ecological/non-mammalian studies were excluded from any consideration, which is inconsistent with the process of systematic review. EPA should provide further clarification of this issue.

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

There several errors in the reporting of chemical and physical properties for ETBE. Some are bookkeeping-type errors (e.g., the property value cited does not correspond to the one given in the citation referenced for that value). Other errors relate to the lack of critical evaluation of the value provide by a reference, typically a secondary one. Not infrequently, chemical and physical property values found in the literature can differ, sometimes substantially, even for some properties that should be clear-cut, e.g., solubility in water. Inaccuracies are propagated by referencing secondary sources which may not be correct or which may report old data no longer consider optimal. Thus, EPA should adopt quality criteria for reporting physical and chemical properties that are consistent with the rigor used in the quality evaluation of other sources of information used in the toxicological review. The Panel was informed at the meeting that EPA is developing a process for this purpose, which the Panel encourages.

Chemical properties presented for ETBE in Table 1-1 and pages 1-1 to 1-2 are accurate except for the following:

1. The description of the odor and taste of ETBE in lines 5-6, page 1-1, is not 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) as well as other sources, lists it as 102.176.

3. A density of 0.74g/cc at 20C is correct but note that the reference and link provided for the density of ETBE [ECHA (2016) -https://echa.europa.eu/registration-dossier/-/registered-dossier/15520] does not show this value 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. Dragos and Diaz (2001) does not show a value of 2.37 g/l. It is not clear what the source for this value is. Table 1 of this publication shows the following values: 7,650mg/l; 12,000mg/l; 26,000mg/l, and insoluble. 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 a peer-reviewed article by 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. Gonzalez-Olmos & Iglesias (2008) should be considered by EPA as the source for the water solubility of ETBE. Because the solubility of ETBE in water decreases substantially as temperature increases, the value given in the table should indicate the temperature at which solubility was determined.

5. Sources for log octanol/water partition coefficients (Kow) values reported in Table 1-1 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 at 25C. This value and the Montgomery (1994) report are also cited by ECHA (2016). However, Table 1-1 also shows a value for log oil/water partition of 1.74 at 25C cited to Dragos and Diaz (2001) which clearly designates it as a Kow. Log oil/water partition is an old term that does not correspond to the measurement or specific application of this property. The 1.74 coefficient was cited from a government report (reference # 30 in Dragos and Diaz, 2001) 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 clarified, it would be advisable to list only the 1.48 value.

6. The value for viscosity at 40C is correct but listing the viscosity at 20C [0.540 mm^2/s; ECHA, (2016)] 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).

It is not clear why this set of specific properties was selected among the many available for chemical compounds. Presumably, it is because the reported properties are relevant to the toxicological review. However, there is a lack of consistency in the type of properties reported for ETBE and tBA, which suggests that EPA does not have a set of criteria for selection of properties to be included in the toxicological review.

More frequently than not, the value listed derives from a secondary reference (typically values reported by other agencies, listed in databases, manuals, etc.) which is appropriate for IRIS as long as the secondary reference reports the primary source for the value, which can then be verified independently.

Values referenced to peer-reviewed, primary sources should be considered more reliable than those for which the primary sources were not peer reviewed. If there are several peer-reviewed primary sources for the same property but different value, EPA should look at the references in

terms of quality criteria, and select accordingly. This could be important such as in the case of the water solubility for ETBE, because based on the primary reference cited in #4 above [Gonzalez-Olmos & Iglesias (2008)], it could be possible that the Suzuki et al. (2012)/JPIC (2010a) oral intake studies were not performed at the true limit of ETBE solubility in water.

Discrepancies among reported values for any particular property can occur in some cases because the specific value was derived as an estimate and, in others, it resulted from actual measurements. There can be wide differences between estimated values because of variability in the assumptions and/or models used in the derivation. Measurements performed following appropriate protocols are preferable to estimates. If no suitable value measured with an appropriate experimental protocol is available for a specific property, then an estimated value can be used but it should be designated clearly as such and it should be referenced to the primary source for the estimate.

Inhalation reference concentration for noncancer outcomes. Question 3d. Section 2.2 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.

As is the case with tBA, there is essentially no critical discussion of the comparative pathology between the rat and the human kidney for the specific lesions/injuries selected for deriving candidate reference values for non-cancer effects. EPA argues that individual lesions, rather than the cluster of lesions (not necessarily all present simultaneously) that conforms the CPN pathology in the rat, may occur in the human kidney, and that exacerbation of any one of these lesions is likely to reflect cell injury relevant to humans even if CPN is not. This is not an unreasonable argument. However, it should be supported better by providing additional, explicit discussions of the comparative nephropathology in both species that can justify the use of these injuries for derivation of reference values. Otherwise, the argument remains more of an assumption than an evidence-based decision.

If EPA's argument for selecting some of these outcomes in rats for RfC derivation is accepted (i.e., the relevance of the individual lesions observed in the rat kidney to humans), the value of 9 x 10^0 mg/m^3 is supported by the selection of key studies, with a clear description of the derivation for six different outcomes in the main document, and with the modeling 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/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 more specificity of the outcome is reasonable. In addition, the RfC was derived from the only chronic study available, which is a high quality study. The assessment describes clearly the limitations of the estimates. All the candidate values except for the estimated RfC values based on increased CPN severity in male and female rats in the chronic study, and on increased absolute kidney weight in male rats in the 13-week study were within the same order of magnitude. The RfC based on increased CPN severity in both male and female rats was approximately 4 times higher than the selected RfC. Therefore, there is some relatively consistency in the various estimates. 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 8×10^{-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.

Given the limitations of the data available for deriving an inhalation unit risk for ETBE to (1) just one study, (2) lack of chemical-specific MOA, and (3) multiple lines of evidence for the carcinogenic potential of ETBE, the derivation of and the specific value for the unit risk are scientifically supported, with all the uncertainties clearly described in the assessment. The critical uncertainty is that there is only one study available for the derivation, and that tumor incidence was increased at only the highest dose level. Nonetheless, EPA provides guidance for deriving unit risks even in these cases, and this guidance was followed for arriving at the 8×10^{-5} per mg/m^3 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. Marvin Meistrich

3. Noncancer. 3a.

Noncancer kidney toxicity: I agree that a kidney damage, such as urothelial hyperplasia, observed at exposures of 170 mg/kg-d or 6,000 mg/m^3, is a reasonable endpoint. However, there are some questions regarding its applicability to human.

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:
Relative liver weight in rat shows increases after high oral doses (1,000 mg/kg-d) but not after inhalation exposure

Weight increases are non-specific; no consistent effects on liver enzyme markers

Pathological lesions might be precancerous; unlikely to directly indicate liver dysfunction

Agree that there is suggestive evidence for liver effects contributing to noncancer toxicity

Developmental toxicity: Almost all assays show no significant developmental toxicities of ETBE oral administration at 1,000 mg/kg-d

Minor effects (skeletal variations, early postnatal deaths) only observed at high oral dose (1,000 mg/kg-d) in some studies; some of these effects may be associated with maternal systemic toxicity

Male reproductive toxicity: Most assays show no toxicities but sensitive analyses of sperm DNA damage and motility characteristics show effects of ETBE inhalation at 7,000 mg/m^3 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 1,000 mg/kg-d for times up to 6 months. Little weight is given to reproductive toxicity from the reported hormone changes after doses of 1,200 or 1,800 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 21,000 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.

Effects on testicular histopathology were observed in one strain of mice after inhalation exposure to ETBE at 7,000 or 21,000 mg/m^3 but not in another strain. These effects 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,

In mice with Aldh2 genetic deficiency, the DNA damage and sperm number reductions were observed at 2,100 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.

If the use of kidney endpoint is considered appropriate, possible reproductive and developmental toxicity (NOEL=1000 mg/kg-d), occurs at higher doses than kidney endpoint (LOEL=171 mg/kg-d) and are not important for setting an RfD.

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

If the use of kidney endpoint (LOEL=1500 ppm, 6270 mg/m^3) is considered appropriate, the absence of ETBE effects on most reproductive and developmental endpoints at doses of 21,000 mg/m3, indicates they are not important for setting an RfC. However sperm DNA damage (DNA breaks, 8-oxo-deoxyguanine) and minor histopathological changes in B6 mice were observed at similar exposure levels and could also be considered in setting of an RfC.

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. Trish Berger

Q4a

I can understand why EPA is not absolutely convinced that the effects of ETBE on the rat kidney are due to nonhuman relevant mechanisms. However, as a scientist, I don’t see any evidence to support that view.

Q5

In my opinion, all developmental effects might be associated with maternal toxicity.

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?

Response: 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.

Response: 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 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.

Response: The inhalation reference concentration of 9 x 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.

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.

Response: The data for either PPARα or CAR/PXR being operative MOAs for ETBE-induced liver tumors in rats are unconvincing. The PPARα MOA involves a decrease in apoptosis, but 2-week exposures to ETBE resulted in an increase in apoptosis. Additionally, the dose-response concordance between ETBE exposure and induction of either PPARα- or CAR/PXR-mediated gene expression was poor. Although data gaps exist for the acetaldehyde-mediated mutagenic MOA, this MOA is still the most likely to be correct of all the proposed MOAs. U.S. EPA was correct in considering the liver tumors in male rats to be relevant to human hazard identification.

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.

Response: 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.

Response: 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 8×10^{-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.

Response: 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. 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.

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

The information appears reasonably accurate for the purposes of this document.

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. It would be better if the information in this document were included directly in the Appendix. 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 providing pharmacokinetic support for 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. To avoid confusion, the assessment should always use the complete description in the previous sentence wherever the dose metric is defined. 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. However, since it appears that what the agency refers to as urothelial hyperplasia is actually renal pelvic transitional hyperplasia, which is part of CPN, it would be better to describe

the endpoint as “increases in severity of nephropathy” to more appropriately correspond with the characterization in the tert-butanol assessment.

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, since it appears that what the agency refers to as urothelial hyperplasia is actually renal pelvic transitional hyperplasia, which is part of CPN, it would be better to describe the endpoint as “increases in severity of nephropathy” to more appropriately correspond with the characterization in the tert-butanol assessment. Also, 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, since it appears that what the agency refers to as urothelial hyperplasia is actually renal pelvic transitional hyperplasia, which is part of CPN, it would be better to describe the endpoint as “increases in severity of nephropathy” to more appropriately correspond with the characterization in the tert-butanol assessment. Also, 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 limited 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 acetaldehyde.

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.

There is some support for a cancer descriptor of "suggestive evidence" for all routes of exposure based on the possibility that the cancer mode of action may be associated with local metabolism of ETBE to acetaldehyde. However, given the fact that there is a well-conducted oral study that is negative for liver carcinogenicity, I believe that "inadequate evidence" would be a better descriptor for that route.

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.
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." There is also evidence from genotoxicity studies and initiation/promotion studies that the mode of action for ETBE is unlikely to be driven by mutagenicity. I do not see any way in which performing a low-lose linear extrapolation of these data could possibly provide an appropriate sense of the magnitude and uncertainty of potential risks, help to rank ETBE against other potential hazards, or even set research priorities. In fact, I believe that providing a default linear dose-response assessment for ETBE would be highly misleading to the public while serving no useful purpose.

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 due to the lack of correspondence of the PBPK dose-metrics with the liver tumor incidence across routes.

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. The potential contribution of nonlinear processes that are only active at the high concentrations used in rats precludes conducting an informative quantitative dose-response analysis.

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.

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.

Tier 1: Recommended correction, p. 1-2, Line 7: Citation mistake. It should be Nihlen et al., "1998a".

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

Tier 1: 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?

Tier 2: The information provided on P. 2-21 and in Appendix B 1.5 is helpful for understanding the rational of the application of the PBPK model in the current assessment. Nonetheless, additional description can enhance the reviewer's understanding of the model.

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

Among the available choices, average concentration of tBA butanol in blood is the best choice.

3. Noncancer
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 described. Some modifications can be made to increase clarity. Following are a few suggested modifications:

Tier 2, 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 present in the circulation after exposure to ETBE.

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

Tier 2: Suggested addition, p. 1-33, Line 29: ..., suggesting ETBE and/or other metabolites, in addition to tBA, cause hyaline droplet accumulation.

Tier 2: 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. 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

Tier 2, suggested 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.

Tier 1, 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.

Tier 2, suggested 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.

Tier 1, recommended modification: p. 1-76, Line 11-14: Please provide the following clarification in the discussion. Animal's 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.

Tier 1: 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).

Tier 1, p. 1-57, Line28-32 and p.76, Line 17-22: Over 100% and statistically significant increases of plasma estradiol in male rats, $p<0.05$, in both 1200 mg/kg and 1800 mg/kg groups, and concurrent decreases in testosterone is a biologically significant effect (de Peyster et al. 2009). Furthermore, the potential target organs of estradiol/testosterone disruption is not limited to organs of gametogenesis. For example, sex hormones is known factor in the progress of CPN in rats.

Tier 1, recommended correction: p. 1-90 Line 23: Please delete “or biological”. Missing right atrioventricular valve is a biologically significant matter.

Tier 1, 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.

Tier 1, recommended 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).

Tier 1, recommendation: 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.

Tier 1: 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.

Tier 1, 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.

Tier 1: 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) dose not lead to the correct document.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 (p. 2-11 to p. 2-20) 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.

Tier 1: When reproductive and developmental endpoints are relevant to human health and when the data are sufficient for reference dose derivation, reference doses should 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."

Tier 1: 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.

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.

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

Tier 1, 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).

Tier 1, 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.

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

Tier 2, 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 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.

Tier 2, the reviewer supports the decision of conducting a 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 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 is scientifically supported and its derivation clearly described.

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.

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:
Tier 1: 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.

Tier 1: 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.
Tier 2: 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.

Tier 1: Chemical toxicity assessment should begin with a description of all observed effects, including the relationships among many organs and tissues. Very rarely the toxicity of a substance is confined in a single organ or type of tissue. A description of the overall effects across organs and tissues is necessary before the emerging of deliberations on the target organ(s) and MOA analysis. The summary of all effects, at the end of the "Summation of Occurrence and Health Effect in the Draft of Executive Summary, is deficient. In the body of the draft, please also describe all observed effects, before section 1.2.1, before focusing on specific effects and MOA.

Tier 1: For reference dose derivations, please include all endpoints considered.

Tier 1: recommended addition, P. xxi, Line 14-15: There are also noncancer liver effects.

Tier 2: clarification, P. xxi, Line 33-34, and P. xxiii, Line 12-13 "... are appropriate for identifying a hazard to the kidney.": Does this intend to say that these parameters are relevant endpoints for assessing dose-response relationship?

Tier 1, recommended modification, P. xxi, Line 37: "No additional histopathological findings were observed" is too strong a statement, it may not be correct, because there are other histopathological findings.

Tier 2, 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.

Tier 2, 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.

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

de Peyster A, Stanard B, Westover C. 2009. Effect of ETBE on reproductive steroids in male rats and rat Leydig cell cultures. Toxicol Lett 190(1): 74-80.

Glassock RJ, Denic A, Rule AD. 2017. The conundrums of chronic kidney disease and aging. Journal of Nephrology 30(4): 477-483.
Guo Jessie Y, Xia B, White E. 2013. Autophagy-Mediated Tumor Promotion. Cell 155(6): 1216-1219.

Hard GC, Johnson KJ, Cohen SM. 2009. A comparison of rat chronic progressive nephropathy with human renal disease-implications for human risk assessment. Critical Reviews in Toxicology 39(4): 332-346.

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.

Tert-butanol Alcohol (tBA)

Dr. Isaac Pessah

Q1: Literature Search Strategy/Study Selection. 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.

Overall I agree with the panel that the search strategies, criteria for study inclusion/exclusion and evaluation and for study quality are described clearly. The complete exclusion of effects reported for non-mammalian species (e.g., the available peer reviewed literature using embryonic zebrafish exposure models) may be a consequence of not adhering fully to the NRC (2011) recommendations for systematic review in the IRIS program. Recommend that EPA incorporate

the available data from non-mammalian model organisms at some point for hazard assessment of tBA.

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. An important caveat is that nephropathy is an appropriate endpoint. Never the less, the available data on which the RfD is based shows dose-dependence, most likely reflects direct kidney damage progressing 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 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 supports that the descriptor of "suggestive evidence" for cancer risk is scientifically supported for both oral and pulmonary routes of exposure. The high doses on which this conclusion is based may raise uncertainties about human 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 needs additional attention in future Tier 3 assessments as detailed by the workgroup.

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?

Need defined search for obtaining information since the values can vary.

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 use of the ETBE model and submodel for TBA needs to be more clearly explained. Specifically, how the TBA metabolite submodel was modified to become a model used for administration of TBA. The human model published by Nihlen and Johanson (1999) should be considered for the PBPK assessment instead of using the default HED methodology.

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

Yes, given the lack of MOA information and the correlation of this dose metric with kidney noncancer endpoints.

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 am not aware of a robust method to determine an inhalation unit risk estimate.

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 the approaches utilized by EPA in the literature search, but there is no statement as to why the NRC (2011) guidelines were used for some aspects of the study search and identification protocols and not for others. The Panel was informed that EPA is developing guidelines for literature searches in the IRIS program and encourages the Agency to adhere as much as possible to the NRC (2011) recommendations. It is important to indicate that despite some of the additional clarifications described below, it is highly unlikely that the strategy adopted by EPA would have missed human or animal 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 in principle. The search was continually updated overtime with a clear stated ending date of December 2016 The database was consistently maintained and updated 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, peer-reviewed articles, although the text

does not indicate if other databases were considered. The search keywords consisted typically used synonyms for tBA and the CAS number. 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 limits were applied so early in the search strategy for Web of Science. Exclusion of reports in PubMed from Toxline appears reasonable if it is assumed that both databases are error-free. This may be a good approach for avoiding excessive duplication or limiting 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 for: 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 may have performed risk assessments or other health reviews of tBA (for example, ATSDR), and why only IPCS (1987) and OSHA (1992) were included, and 3) if manual searches for citations 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. There is lack of documentations about the detailed review and application of quality criteria for each study, so it is not possible to affirm that the quality criteria were applied objectively across the board. However, here is no evidence in the documents that the criteria were not applied objectively.

As is the case with the ETBE toxicological review, there is limited information about how the search for information evolved through the development of the review. As systematic and critical reviews are performed, there are specific questions that arise that may require further additional searches of the literature using other keywords than those previously applied. It is not clear that this process took place in the tBA Toxicological Review, or to what extent it did. Also as in the ETBE Toxicological Review, there is no evidence that the ecological and non-mammalian species literature was used for any purpose despite the footnote in Table LS-1 (page xxxii), despite the potential relevance of these species for hazard identification.

Editorial note: 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?

Comments in response to Question 2a for ETBE are also relevant to the presentation of chemical and physical properties of tBA. Specifically, EPA needs to have a clear set of guidelines for which chemical-specific properties are included as part of toxicological reviews. Sources of property values should be subject to quality evaluation as are the other sources of information included in the reviews (see answer to Question 2a for ETBE).

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 25.81C and a boiling point of 82.3C.

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

Editorial note: the reference and URL for the ECHA (2017) citation is not included in the reference list.

Inhalation reference concentration for noncancer outcomes. Question 3d: Section 2.2 presents an inhalation reference concentration of $5x10^0$ 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 nephropathies in the rat and the severity of tBA-induced nephropathy in the female rat are accepted as relevant to humans, the estimated $5 \text{ X } 10^0$ mg/m^3 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 rat inhalation study, 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 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 8, 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. Marvin Meistrich

3. Noncancer
3a. Noncancer kidney toxicity

I agree that kidney damage, such as severity of nephropathy observed in female rats at exposures of 180 mg/kg-d for 2 years, is a reasonable endpoint. However, the calculation of equivalent inhalation dose by PBPK modeling, which yielded 472 mg/m3, is open to question as no significant increases in severity of nephropathy were reported at doses of 6,400 mg/m3 for 13-weeks. In addition, there are some questions regarding its applicability to human.

3b. Noncancer toxicity at other sites:

I agree that there is no evidence to clearly showing developmental, neurodevelopmental, and reproductive toxicity. The studies referenced in the report are quite limited. Still (except for neurodevelopmental toxicity) I suggest describing recommendation as "minimal effects at otherwise toxic dose levels", rather than that there is "inadequate" information to assess these toxicities.

Developmental toxicity: Only small reductions in rat fetal and pup survival and pup body weight were observed at an oral gavage tBA doses of 1000 mg/kg-d or 10,600 mg/m3. The toxicity may be related to slight maternal toxicities at theses doses.

Developmental Neurotoxicity: Behavioral deficiencies were observed in mice exposed to 5,000 but not 3,300 mg/kg-d during gestation. Changes in brain neurotransmitters were observed in offspring of rats exposed to 6,000 mg/m3 during gestation. Available studies are limited and further studies are warranted.

Male reproductive toxicity: Most endpoints show no toxicities at maximal doses used
The only reported effect, ~15% decline in mouse testis weight at an oral dose of 3600 mg/kg-d for 13 weeks, is likely indirect as there was also >50% mortality at this dose.

Female reproductive toxicity: Most endpoints show no toxicities at maximal doses used.
The only reported significant effect was an extension of the mouse estrous cycle from 4 to 5 days after oral exposure to 3600 mg/kg-d of tBA for 13 weeks. However, no significant changes were 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.

If the use of kidney endpoint is considered appropriate, possible reproductive and developmental toxicities (NOEL=400 mg/kg-d), occur at higher doses than kidney endpoint (LOEL=180 mg/kg-d) and are not important for setting an RfD.

3d. Inhalation reference concentration of 5 mg/m3 based on the severity of nephropathy in female rats

The concentration for the POD (471 mg/m3) had to be calculated from the LOAEL in a drinking water study by PBPK modeling for route-to-route extrapolation. If that calculation is correct and the kidney endpoint is considered appropriate, then the kidney toxicity occurs at a lower dose than possible reproductive or developmental toxicity (LOAEL=10,600 mg/m3).

Dr. Trish Berger

Q4a

I can understand why EPA is not absolutely convinced that the effects of ETBE on the rat kidney are due to nonhuman relevant mechanisms. However, as a scientist, I don't see any evidence to support that view.

Evidence supporting a role in thyroid tumors in males was unconvincing although an overall toxic effect appears present.

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.

Response: The Document correctly and clearly identifies the severity and incidence of 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 has been postulated 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.

Response: 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 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. U.S. EPA's position is backed up by studies by Doi et al. (2007) and Melnick et al. (2012), which suggest that neither α-2u globulin nephropathy nor chronic progressive nephropathy are valid MOUs for male rat kidney tumor induction. It is noteworthy that induction of some CPN endpoints in male rats exposed to ETBE (which is metabolized to TBA) does not result in the induction of kidney tumors, pointing out the inconsistency of this MOA.

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.

Response: 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.

Response: 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. There is no evidence to indicate that the tBA dose inducing tumors in female mice (20 mg/mL) exceeded the Maximum Tolerated Dose (MTD), and mean survival was not significantly different from controls. 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 should be noted that U.S. EPA policy does not preclude dose-response modeling of tumor data sets where only one dose group demonstrates a significant increase in tumor incidence compared to controls. Also, other regulatory agencies (e.g. the California Environmental Protection Agency) have also done tumor dose-response modeling under similar circumstances.

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 CPN are valid MOAs for male rat kidney tumor induction. It is noteworthy that induction of some CPN endpoints in male rats exposed to ETBE (which is metabolized to TBA) does not result in the induction of kidney tumors, pointing out the inconsistency of this MOA.

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.

Response: 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} (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 an 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 (not cited in Document)

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. 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.

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

The information appears reasonably accurate for the purposes of this document.

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. It would be better if the information in this document were included directly in the Appendix. 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 general purpose of providing pharmacokinetic support for 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 of kidney effects in the rat. To avoid confusion, the assessment should always use the complete description in the previous sentence wherever the dose metric is defined. I am also comfortable with the pulsatile oral ingestion pattern used by EPA for rats exposed via drinking water.

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 bwcause of the potential contribution of nonlinear tumorigenic processes that are not human-relevant.

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. The potential contribution of nonlinear processes that are not human-relevant to the observed thyroid tumors in rats precludes conducting an informative quantitative dose-response analysis.

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 CPN 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.

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, but often additional references may be included in the review.

Tier 1: Dermal exposure is a relevant exposure. Tert-butanol is used in perfume and cosmetic and personal hygiene product. Findings from studies of dermal exposure should be reviewed and stated in the hazard identification. Dermal-exposure should not be categorized as "Not relevant exposure paradigms", as shown in Figure LS-1. Additional dermal studies that should be included are Edwards (1982), for contact dermatitis, and Hoshino et al. (1970), for squamous cell carcinoma.

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

Tier 2: Melting point can be significantly affected by purity. Melting point available for the highest purity should be used in the document. Please state the purity of the melting point.

The reviewer does not know where to provide the following comments? These are not for Chemical properties. They are for Toxicokinetics section.

Tier 1: 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).

Tier 2: 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).

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?

Tier 2: The information provided in Appendix B 1.5 is helpful for the understanding of the rational of the application of PBPK model in the current assessment. Nonetheless, additional description can enhance reviewer's understanding of the model.

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

Among the available choices, average concentration of tBA butanol in blood is the best choice.

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.

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.

Tier 1: p. 1-55, Lines 4-14: Please provide dosages in the "Integration of Developmental Effects."
Comments: Potential health effects in toxicity assessment should be identified independent of possible exposure in the environment. Nonetheless, dosages associated with observed effects should be stated. Observed effects should always be presented with information of exposure scenario, including the dose.

Tier 1: The hazard identification of reproductive and developmental effects is insufficient. Many observations in the available studies suggest potential metabolic and/or neurobehavioral disturbance by tBA at different life stages, these include maternal sedation, (Daniel and Evans 1982) and increased maternal body weight during lactation (Huntingdon_Life_Sciences 2004), accompanied by decreases in delayed eye opening and low body weight gain of pups, as well as dose-related effect on righting reflex, cliff avoidance, open field, and roto-rod behavior test scores, at doses [0, 3324, 4879, and 6677 mg/kg/d (Daniel and Evans 1982)]. These are a few examples of reproductive and developmental effects that should be identified and stated in the

draft. Observed material and fetal effects should be presented regardless of whether or not the maternal effects and fetal/neonatal effects can be delineated.

Tier 1: The 160 mg/kg/day as a NOAEL and 400 mg/kg/day as a LOAEL, identified in the study by Huntingdon_Life_Sciences (2004), and qualitatively supported by higher doses in other studies, should be applied in the dose-response analysis as a reference dose. The endpoint for determining the NOAEL and LOAEL is lethargy and ataxia. Apparent lethargy and ataxia are relevant effects to humans, even when it lasted for only 2 weeks in rats. If increases in relative male kidney weight was considered to be human relevant, 160 mg/kg/day would be an effective dose.

Tier 1: The extraordinary finding of 100% maternal body weight increase in the study by Huntingdon Life Science (2004), suggesting potential maternal metabolic disturbance during lactation, should be stated in the hazard identification.

Tier 1: Dermal exposure is a relevant rout of exposure. Please include contact dermatitis (Edwards 1982) in hazard identification.

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 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.

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

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.

Yes, the conclusion is scientifically supported.

Tier 1: The following recommendation related to statements in two places in the draft: (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-and- rats comparison appeared at least twice in this section.
Tier 1: P. 1-46, Line 36 “No evidence” is available: Should be “insufficient evidence”.

(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 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.

Yes, the reviewer agrees to the descriptor of “suggestive evidence”. It 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 Sections 2.3 aof 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.

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 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 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 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 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.

Tier 1: 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 and infants, as a susceptible population. Neonatal gastric exposure (average daily dose of 1723 mg for 4 days) to tBA causes head bobbing resembling the onset of a grand mal seizure in Long Evans Rats (Grant and Samson 1982), and statistically significant decrease in relative brain weight ($p<0.05$) without any effects on relative liver and heart weights, suggesting CNS the target organ of tBA during neonatal developmental. Grant: tBA-induced microcephaly.
Tier 2: People with existing nephropathy or pre-nephropathy conditions and thyroid related conditions are likely susceptible populations.

Tier 1: 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.

Tier 1: Chemical toxicity assessment should begin with a description of all observed effects, including the relationships among many organs and tissues. Very rarely the toxicity of a substance is confined in a single organ or type of tissue. A description of the overall effects across organs and tissues is necessary before the emerging of deliberations on the target organ(s) and MOA analysis. The summary of all effects, at the end of the "Summation of Occurrence and Health Effect in the Draft of Executive Summary, is deficient. In the body of the draft, please also describe all observed effects, before section 1.2.1, before focusing on specific effects and MOA.

Tier 1, 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 the articles provide evidence for the presence of tBA in milk, although the presence of 1-butanol was demonstrated.

Tier1: For reproductive and developmental effects, results of both the qualitative (hazard identification) and the quantitative (dose-response relationship) assessment should be stated. Here are a few examples: There is a general dose-response relationship between tBA exposure and number of stillborn and live pups (Huntingdon Life sciences, 2004; Daniel and Evans, 1982). There is also a dose-response of pups' postnatal weight gain (Huntingdon Life sciences, 2004; Daniel and Evans, 1982). In addition, 400 mg/kg is an effective dose for CNS effects in F-1, which is a dose below apparent maternal toxicity.

References:

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.

Daniel MA, Evans MA. 1982. Quantitative comparison of maternal ethanol and maternal tertiary butanal diet on postnatal development. Journal of Pharmacology and Experimental Therapeutics 222(2): 294-300.

Edwards EK. 1982. ALLERGIC REACTION TO TERTIARY BUTYL ALCOHOL IN A SUNSCREEN. Cutis 29(5): 476-&.

Grant KA, Samson HH. 1982. ETHANOL AND TERTIARY BUTANOL INDUCED MICROCEPHALY IN THE NEONATAL RAT - COMPARISON OF BRAIN GROWTH-PARAMETERS. Neurobehavioral Toxicology and Teratology 4(3): 315-321.

Compilation of revised comments by individual members of the SAB CAAC-ETBE/tBA Committee. DO NOT CITE OR QUOTE.

Hoshino H, Chihara G, Fukuoka F. 1970. DETECTION OF POTENTIAL WEAK CARCINOGENS AND PROCARCINOGENS .2. CARCINOGENICITY OF TERTIARY BUTYL HYDROPEROXIDE. Gann 61(2): 121-&.
HSDB. 2007. t-Butyl alcohol. Bethesda, MD: National Library of Medicine.

Huntingdon_Life_Sciences. 2004. Reproductive and developmental toxicity screening test in rats by oral gavage. Study No. 03-4254. East Millstone, NJ:Lyondell Chemical Company.