UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

DATE

EPA-SAB-18-0xx

The Honorable Andrew R. Wheeler
Acting Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: Review of EPA's Draft Assessments titled Toxicological Review of Ethyl Tertiary Butyl Ether and Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)

Dear Acting Administrator Wheeler:

The EPA's National Center for Environmental Assessment (NCEA) requested that the Science Advisory Board (SAB) review two draft assessments *Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE) (External Review Draft, EPA/635/R-15/016a, June 2017)* and *Toxicological Review of tert-Butyl Alcohol (tert-Butanol; tBA) (External Review Draft, EPA/635/R-17/015a, June 2017).* The assessments evaluate publicly available scientific literature on the toxicity of each chemical. The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of ETBE- and tBA-induced cancer and noncancer health effects.

In response to EPA's request, the SAB augmented the SAB Chemical Assessment Advisory Committee (CAAC) with subject matter experts to conduct the review. The enclosed report provides the SAB's consensus advice and recommendations. This letter briefly conveys the major findings.

The draft assessments evaluate the available physiologically-based pharmacokinetic (PBPK) models in the literature. The SAB finds that the EPA's application of the PBPK model in dose-response characterization of ETBE and tBA is an appropriate way to incorporate science using state-of-the-art methods. However, the overall presentation of the PBPK modeling should be more cohesive, clear, and transparent.

Regarding dose metrics, the SAB recommends against the EPA's use of the rate of metabolism as the dose metric for extrapolation from inhalation to oral routes of administration of ETBE. The SAB finds that there is no consistent dose-response relationship for ETBE when combining oral and inhalation studies to assess liver tumors. The SAB agrees with the EPA's approach to calculate the dose metric for tBA.

Regarding noncancer kidney effects, the SAB is unable to reach consensus with respect to how the Agency interpreted the ETBE database. There was disagreement as to whether noncancer kidney effects

in rats should be considered a hazard relevant to humans and it is critical that further justification be provided by the EPA f. Although the role of alpha 2μ-globulin in ETBE-induced nephropathy in male rats is thoroughly considered according to the 1991 criteria established by the EPA, the SAB recommends application of the more detailed criteria published by the International Agency for Research on Cancer (IARC) (1999). The SAB also recommends that the EPA consider the use of another parameter, such as increases in blood (serum) biomarkers or exacerbation of nephropathy, besides urothelial hyperplasia, as a surrogate for ETBE noncancer kidney effects. For tBA, the SAB recommends the EPA provide a more detailed explanation for considering chronic progressive nephropathy (CPN) as a kidney effect relevant to human hazard assessment. The EPA could also consider other indicators besides suppurative inflammation and transitional epithelial hyperplasia as indicators of kidney effects for tBA or provide better justification for their choice.

The SAB agrees that noncancer toxicity at sites other than the kidney are less suitable for use as the basis for deriving an oral reference dose (RfD) or an inhalation reference concentration (RfC) for both ETBE and tBA. Nearly all of the possible effects at these sites occurred at much higher exposure levels than did observed effects on the kidney.

Regarding noncancer kidney outcomes from exposure to ETBE, the SAB did not reach consensus on an oral reference dose. The difference in opinion is based on the extent of confidence in a CPN-based mechanism for these ETBE effects. Similarly, the SAB did not reach a consensus regarding the oral reference dose for noncancer kidney outcomes for tBA. The difference in opinion relates to the extent of confidence in CPN and/or alpha 2μ-globulin -based mechanisms for these tBA effects.

The SAB agrees with the EPA's conclusion that liver tumors in male rats from exposure to ETBE are relevant to human hazard identification and are scientifically supported. The SAB also agrees that the ETBE mode of action for the rat liver tumors, at this point, remains undetermined. A consensus was not reached for tBA concerning the scientific support for the conclusion that male rat kidney tumors are relevant to human hazard identification. The SAB acknowledges that there is scientific support for the EPA's conclusion that thyroid follicular cell tumors in mice for tBA are relevant to humans. However, the SAB finds that there is uncertainty as to whether an increase in thyroid follicular cell tumors for tBA was demonstrated in male mice.

The SAB concludes that the cancer descriptor should be retained as "Suggestive Evidence" of the carcinogenic potential for both ETBE and tBA. The SAB finds no rationale provided for the EPA's decision to perform a quantitative analysis of carcinogenic potential for either ETBE or tBA. The SAB noted that it is unlikely that performing a quantitative assessment of the potential carcinogenicity data would be instructive for providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities for both ETBE and tBA.

The SAB agrees that the methodology applied for deriving the oral slope factor chosen by the EPA is scientifically supported for both ETBE and tBA. No consensus, however, was reached regarding the EPA's calculation of inhalation unit risk (IUR) for ETBE. Some members conclude that the data are not suitable for developing an IUR due to a potential lack of biological relevance for ETBE. Other members note that the data are appropriate for dose-response analysis for ETBE. The SAB concludes that the tBA drinking water study is not suitable for developing an IUR. The SAB's concerns include the lack of biological relevance due to the magnitude of the high dose, the lack of a mouse tBA PBPK model, and the possibility of nonlinear metabolism kinetics at that dose.

The SAB appreciates this opportunity to review the assessments for ETBE and tBA and looks forward to the EPA's response to these recommendations.

Sincerely,

Enclosure:

NOTICE

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[to be added]

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ACRONYMS AND ABBREVIATIONS

ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
alpha 2μ-globulin	alpha-2-micro-globulin
ALDH2	Aldehyde dehydrogenase gene
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase/transaminase
AST	Aspartate aminotransferase/transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BW	Body weight
CAR	Constitutive androstane receptor
CAAC	Chemical Assessment Advisory Committee
CASRN	Chemical Abstracts Service Registry Number
CI	Confidence interval
CIIT	Chemical Industry Institute of Toxicology
CL	Confidence limit
Cmax	Maximum plasma concentration
CPN	Chronic progressive nephropathy
CVL	Central venous line
CYP450	Cytochrome P450
DNA	Deoxyribonucleic acid
EPA	United States Environmental Protection Agency
ETBE	Ethyl Tertiary Butyl Ether
GD	Gestation day
GDH	Glutamate dehydrogenase
GLP	Good Laboratory Practices
HED	Human equivalent dose
HERO	Health and Environmental Research Online
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IUR	Inhalation unit risk
JPEC	Japan Petroleum Energy Center
Km	Michaelis constant
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
LOAEL	Lowest observed adverse effect level
MOA	Mode of action
MTD	Maximum tolerated dose
MTBE	Methyl tertiary butyl ether
NCEA	National Center for Environmental Assessment
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No observed adverse effect level

NRC	National Research Council
NTP	National Toxicology Program
ORD	Office of Research and Development
OSHA	Occupational Safety & Health Administration
PBPK	Physiologically based pharmacokinetic
PND	Postnatal day
POD	Point of departure
$POD_{[ADJ]}$	Duration-adjusted POD
PPAR	Peroxisome proliferator-activated receptor
PXR	Pregnane X receptor
RD	Relative deviation
RfC	Reference concentration inhalation
RfD	Reference dose oral
SAB	Science Advisory Board
tBA	Tert-Butyl Alcohol (tert-butanol)
UF	Uncertainty factor
U.S.	United States

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA's National Center for Environmental Assessment (NCEA) to conduct a peer review of two draft assessments, titled *Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE) (External Review Draft, EPA/635/R-15/016a, June 2017)* and *Toxicological Review of tert-Butyl Alcohol (tert-Butanol; tBA) (External Review Draft, EPA/635/R-17/015a, June 2017)* (hereafter referred to as the draft ETBE assessment or draft tBA assessment, respectively). The assessments consist of a review of publicly available scientific literature on the toxicity of each chemical.

The draft ETBE and tBA assessments were developed simultaneously by EPA because they have several overlapping scientific aspects. Specifically, 1) tBA is one of the primary metabolites of ETBE, and some of the toxicological effects of ETBE are attributed to tBA. Therefore, data on ETBE are considered informative for the hazard identification and dose-response assessment of tBA, and vice versa; 2) the scientific literature for the two chemicals includes data on alpha 2μ-globulin-related nephropathy; therefore, a common approach was employed to evaluate these data as they relate to the mode of action for kidney effects, and 3) a combined physiologically-based pharmacokinetic (PBPK) model for ETBE and tBA in rats was applied to support the dose-response assessments for these chemicals. Given the overlapping data and features of ETBE and tBA, the SAB conducted a peer review of both chemicals simultaneously.

The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of ETBE- and tBA-induced cancer and noncancer health effects. The SAB augmented the Chemical Assessment Advisory Committee (CAAC) with additional subject matter experts. An overview of the SAB's recommendations and advice on how to improve the clarity and strengthen the scientific basis of each assessment are presented below and discussed in greater depth in the body of the report.

Literature Search Strategy/Study Selection and Evaluation

In general, the SAB finds that the structure and strategy for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods are clearly presented and appropriate with a few exceptions for both chemicals. Several points of clarification/correction are necessary within this section of draft tBA and ETBE assessments, and specific recommendations are provided for each assessment. The SAB is not able to provide advice on whether the EPA's evaluation of study methods and quality were applied objectively for each assessment because of the lack of documentation within the EPA's draft *ETBE* and *tBA* assessments and supplemental information *(2017a, b, c, d)* documents. There also is no clear documentation on the comparative quality evaluation for each of the studies (e.g., by providing the information for each study/quality criterion in the publicly available Health and Environmental Research Online (HERO) database). On the other hand, the SAB finds no evidence that quality evaluation criteria for each draft assessment were applied in a non-objective manner.

Chemical Properties and Toxicokinetics

Chemical properties

The SAB observed several inconsistencies between the chemical properties provided in both the draft ETBE and tBA assessments and the chemical property values actually reported in the cited source or

found in other sources. Because multiple values for a given parameter could be found in the literature, the EPA should use only primary data sources for citation.

Toxicokinetic modeling

The SAB strongly supports the EPA's application of the PBPK model in dose-response characterization of ETBE and tBA as an appropriate way to incorporate science using state-of-the-art methods. However, the SAB offers a few points of clarification/correction regarding the EPA's application of the PBPK model. The SAB notes that the overall presentation of the PBPK modeling should be more cohesive, clear, and transparent. Furthermore, the EPA could also maximize the potential benefit for dose-response and mechanistic analyses using appropriately selected dose metrics. In future modeling efforts, the EPA could assess whether fits to data would benefit from consideration of capacity-limited blood binding of ETBE or sex differences in metabolism.

Choice of dose metric

The SAB agrees that the rate of metabolism of ETBE is a reasonable dose metric for animal-human extrapolation within a route of exposure; however, it is not recommended for extrapolation between inhalation and oral routes of exposure of ETBE. This consensus is reached because there is no consistent dose-response relationship for ETBE when combining oral and inhalation studies to assess liver tumors using predicted metabolic rate. Route-to-route extrapolation should not be conducted until an alternate metric is identified that provides a more consistent dose-response comparison between experimental data for the two routes. The SAB agrees with the EPA's approach of using blood concentration to calculate a dose metric for tBA.

Hazard Identification and Dose–Response Assessment – Noncancer

Noncancer kidney toxicity

The SAB is unable to reach a consensus with respect to how the EPA interpreted the ETBE database for noncancer kidney effects. There is disagreement within the SAB as to whether noncancer kidney effects for ETBE should be considered a hazard relevant to humans. Although the role of alpha 2μ-globulin in ETBE-induced nephropathy in male rats is thoroughly considered according to the criteria established by the U.S. EPA (1991), the SAB recommends application of the more detailed criteria published by International Agency for Research on Cancer (IARC) (1999). The EPA could consider using another parameter, such as increases in blood (serum) biomarkers or exacerbation of nephropathy, besides urothelial hyperplasia, as a surrogate for noncancer ETBE kidney effects.

The SAB is also unable to reach a consensus with respect to how the Agency interpreted the tBA database for noncancer kidney effects. Similar to ETBE, there is disagreement within the SAB as to whether noncancer kidney effects should be considered a hazard relevant to humans. The SAB suggests that the EPA provide a more thorough explanation for considering the enhancement of CPN as a kidney effect relevant to human hazard assessment. The SAB notes that the EPA could also consider other indicators besides suppurative inflammation and transitional epithelial hyperplasia as indicators of tBA kidney effects or provide better justification for their choice.

Noncancer toxicity at other sites

The SAB agrees with the EPA's approach that use of noncancer toxicity at sites other than the kidney are less suitable for deriving an oral reference dose (RfD) or an inhalation reference concentration (RfC). Nearly all of the possible effects at these sites occurred at much higher exposure levels than did ETBE effects observed on the kidney. Similarly, the SAB agrees that noncancer toxicity at sites other

than the kidney should not be used as the basis for deriving an oral reference dose for tBA. Nearly all of the possible effects at these sites occurred at much higher exposure levels than did tBA effects on the rat kidney.

Oral reference dose for noncancer kidney outcomes

The SAB is unable to reach consensus for ETBE regarding the RfD for noncancer kidney effects. The difference in opinion is based on the extent of confidence in a CPN-based mechanism for these effects. The SAB notes that if the EPA's assertion is accepted about the human relevance of the increased urothelial hyperplasia in the male rat kidneys (Saito et al., 2013), then the methodology applied in the derivation of an oral RfD of $5x10^{-1}$ mg/kg-day would be considered to be scientifically supported and its derivation clearly described as currently presented in the draft EPA assessment of ETBE. Similarly, the SAB has not reached a consensus for tBA. The difference in opinion is related to the extent of confidence in CPN and/or alpha 2μ-globulin-based mechanisms for tBA effects. If the selection of increases in severity of nephropathy in female rats in response to tBA administration via drinking water remains the basis of the oral reference dose, then the SAB considers the methodology applied in the derivation of the oral reference dose of $4x10^{-1}$ mg/kg-day to be scientifically supported and its derivation clearly described.

Inhalation reference concentration for noncancer outcomes

The SAB concludes that the derivation of all RfC candidate values is described clearly. The SAB also states that if the EPA's assertion is accepted about the human relevance of the increased urothelial hyperplasia in the male rat kidneys (Saito et al., 2013), then the derivation of the RfC of 9×10^{0} mg/m^3 is scientifically supported for ETBE. The SAB has examined the issues surrounding the human relevance of the kidney endpoints reported in the rat. The SAB agrees that if the severity of tBA-induced nephropathy in the female rat is accepted as relevant to humans, then the estimated 5×10^{0} mg/m^3 RfC is scientifically defensible, with some caveats that are presented later in this report.

Hazard Identification and Dose–Response Assessment – Cancer

Cancer mechanisms of action

Regarding ETBE, the SAB finds that there is scientific support for the EPA's conclusion that liver tumors in male rats are relevant to human hazard identification. The SAB also agrees that the mechanism of action (MOA) for the rat liver tumors remains, at this point, undetermined. Under these circumstances, tumor responses in animals are assumed to be relevant to human hazard identification. While supporting the EPA's decision regarding the human relevance of the male rat liver tumors, the SAB finds that improvement is needed for aspects of the discussion of MOA for hepatic effects of ETBE which are detailed in this report.

For tBA, the SAB notes divergent views regarding whether the conclusion that male rat kidney tumors are relevant to human hazard identification is scientifically supported. While some tumors might be attributable to alpha 2μ-globulin nephropathy augmented by CPN, others could be due to other unspecified processes that are assumed to be relevant to humans. The SAB is unable to reach a consensus because some members are in agreement with the draft EPA assessment, while others think that renal tumors could be explained by CPN and are, therefore, not relevant to humans. Further discussion on this point is presented in this report. The SAB finds the conclusion that thyroid follicular cell tumors in mice are relevant to humans scientifically supported. However, there is uncertainty as to whether an increase in thyroid follicular cell tumors was in fact demonstrated in male mice.

Cancer characterization

For ETBE, the SAB concludes that the cancer descriptors should be retained as "Suggestive Evidence of the Carcinogenic Potential" since ETBE met the minimal criteria for that designation as described in EPA's 2015 Cancer Guidelines. There is a general consensus that "Suggestive Evidence of Carcinogenic Potential" is the proper descriptor for tBA because tBA was found to cause renal tubule adenomas in male F344 rats and thyroid follicular adenomas in female (and possibly male) B6C3F1 mice. This cancer descriptor is scientifically supported for oral exposure, though there have apparently been no inhalation bioassays for the chemical.

Cancer toxicity values

The SAB notes that no rationale is provided in the EPA's draft assessment for the decision to perform a quantitative analysis in the case of ETBE. The SAB concludes it is unlikely that performing a quantitative assessment of the data on ETBE liver carcinogenicity would be useful for "providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities".

Additionally, there is considerable concern about the ability of dose-response modeling to provide instructive information when there is a flat, unresponsive dose response at all doses except the high dose. Because the EPA's policy is to use only the multistage model for benchmark dose modeling of cancer dose-response, only a single estimate of the benchmark dose lower confidence limit (BMDL) is produced. However, while the rationale stated for this policy is to maintain consistency throughout various IRIS assessments, had other models been investigated, it would have been seen that many different models could adequately fit these data, and would likely yield widely divergent BMDL values.

The SAB agrees that the Saito *et al*. (2013) study is well-conducted and well-reported. However, with a statistically significant increase in tumors at the high dose only, the Saito *et al*. (2013) data are not sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for ETBE.

Similarly, there does not appear to be a rationale for performing a quantitative analysis for tBA. It is unlikely that performing a quantitative assessment of the data on tBA thyroid carcinogenicity would be useful for providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. The SAB agrees that the NTP (1995) study is well-conducted and well-reported. However, with a statistically significant increase in tumors at the high dose only, and evidence from other studies supporting a potentially nonlinear mode of action, the NTP (1995) data are not sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for tBA.

Cancer modes-of-action

The SAB concludes that the methodology that was used to derive the oral slope factor chosen is scientifically supported for both ETBE and tBA. However, notwithstanding that the EPA calculated oral slope factors for both ETBE and tBA according to the EPA's policy, this calculation did not necessarily result in a meaningful finding.

The SAB is not able to reach consensus regarding the EPA's calculation of an inhalation slope factor for ETBE. For some members, the Saito *et al*. (2013) study is not suitable for developing a cancer inhalation unit risk (IUR) due to a potential lack of biological relevance for ETBE. Other members have expressed that the Saito *et al*. (2013) study is appropriate for dose-response analysis, and indicate that the ETBE IUR is scientifically supported, given the quality of the Saito *et al*. (2013) study and the liver

metabolism of ETBE. The SAB has no recommendations for alternative approaches for developing an IUR for ETBE.

The SAB concludes that the NTP (1995) tBA drinking water study is not suitable for developing an IUR. Concerns include the lack of biological relevance due to the magnitude of the high dose, the lack of a mouse tBA PBPK model and the possibility of nonlinear metabolism kinetics at that dose. The SAB also has concerns as to whether modeling a single positive concentration would produce a meaningful oral slope factor. An alternative approach for developing an IUR for tBA would be to perform a route-to-route extrapolation from the oral cancer slope factor using default human body weight and inspiration rate values. However, this approach is not consistent with the best available science.

Susceptible Populations and Life Stages

The SAB agrees that there is "plausible evidence" for a vulnerable subgroup to ETBE exposure. The SAB recommends that the EPA should provide additional details with respect to differences in expected outcomes, vulnerable life stages, and the possibility of other potentially vulnerable population subgroups, such as individuals that have non-coding region variants in ALDH2 (aldehyde dehydrogenase gene).

The SAB agrees that the evidence is minimal with regard to tBA for identifying vulnerable populations and life stages. The draft tBA assessment states that there is no identified susceptible population. However, it is unclear as a metabolite of ETBE why other populations mentioned in the ETBE draft assessment were not considered by the EPA. It is also unclear why vulnerable life stages are highlighted for tBA and not for ETBE.

Finally, the SAB disagrees with certain findings presented by the EPA in Table 1-12 of the draft tBA assessment. The actual body weight for the treated group was not double that of the control group, as implied by Table 1-12. Rather, treated dams gained twice as much weight as the dams in the control group during a specific interval. This difference in body weight gain is reasonable given other characteristics noted within the draft assessment and the final assessment does not need to include additional reasoning on this topic.

Executive Summary

The SAB finds the Executive Summaries in both draft assessments to be clear and include the major conclusions from the draft assessments. As changes are made to the body of the draft assessments for both ETBE and tBA, the Executive Summary of each document will need to be changed accordingly. While the Executive Summaries include statements regarding the questions considered and summarize the findings and approaches selected by the EPA, the sections on "Key Issues" should include additional discussion that highlights the consequences of alternative choices for the final assessments because the interpretation and relevance of key toxicity endpoints driving the analysis have been contested by members of the public. How these issues are resolved is important for the assessments and for the Executive Summaries. The SAB suggests that the final conclusions on decisions to accept or not accept debated arguments need to be clearly explained.

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the National Center for Environmental Assessment (NCEA) to review the EPA's *Draft IRIS Toxicological Review of Ethyl Tertiary Butyl Ether* (ETBE) and *Toxicological Review of tert-Butyl Alcohol* (*tert*-Butanol; tBA) (U.S. EPA 2017a and 2017c). EPA's Intgrated Risk Information System (IRIS) is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants.

The draft ETBE assessment reviewed the publicly available studies to identify its adverse health effects and to characterize dose-response relationships. The assessment examined all effects by oral and inhalation routes of exposure and included an oral noncancer reference dose (RfD), an inhalation noncancer reference concentration (RfC), a cancer weight of evidence descriptor, and a cancer dose-response assessment.

The draft tBA assessment reviewed the publicly available studies to identify its adverse health effects and to characterize exposure-response relationships. The assessment examined all effects by oral and inhalation routes of exposure and included an oral RfD, an inhalation RfC, a cancer weight of evidence descriptor, and a cancer dose-response assessment.

Disposition of the public comments received on earlier assessment drafts are available in the Supplemental Information to the draft ETBE (U.S. EPA 2017b) and tBA (U.S. EPA 2017d) assessments.

The draft assessments for ETBE and tBA were developed simultaneously by the Agency because they have overlapping scientific aspects:

- *tert*-Butanol and acetaldehyde are the primary metabolites of ETBE, and some of the toxicological effects of ETBE are attributed to tBA. Therefore, the Agency decided that certain data on tBA are considered informative for the hazard identification and dose-response assessment of ETBE, and vice versa.
- The scientific literature for the two chemicals includes data on alpha 2μ-globulin-related nephropathy; therefore, the Agency chose to use a common approach to evaluate the data as they relate to the mode of action for kidney effects.
- The Agency applied a combined physiologically based pharmacokinetic (PBPK) model for ETBE and tBA in rats (Borghoff et al., 2016) to support the dose-response assessments for these chemicals.

In response to the EPA's request, the SAB augmented the Chemical Assessment Advisory Committee with subject matter experts to conduct the review. The Augmented CAAC held a teleconference on July 11, 2017, to discuss EPA's charge questions (see Appendix A), and a face-to-face meeting on August 15 - 17, 2017, to discuss responses to charge questions and consider public comments. The Augmented CAAC also held teleconferences to discuss their draft report on March 22, March 27 and June 6, 2018. Oral and written public comments have been considered throughout the advisory process.

This SAB report is organized to follow the order of the charge questions addressing ETBE and tBA. The presented recommendations are prioritized to indicate relative importance during the EPA's revisions. As noted within the the EPA's charge for both ETBE and tBA the following recommendation tiers are defined, as follows:

- Tier 1: Recommended Revisions – Key recommendations that are necessary in order to improve the critical scientific concepts, issues and/or narrative within the assessment.

- Tier 2: Suggestions – Recommendations that are encouraged for the EPA to adopt in order to strengthen the scientific concepts, issues and/or narrative within the assessment, but other factors (e.g., EPA need) should be considered by the EPA before undertaking these revisions.

- Tier 3: Future Considerations – Useful and informative scientific exploration that may inform future evaluations of key science issues and/or the development of future assessments. These recommendations are likely outside the immediate scope and/or needs of the current assessment under review.

3. RESPONSE TO SPECIFIC CHARGE QUESTIONS

3.1. Literature Search/Study Selection and Evaluation - Systematic Review Methods

3.1.1. ETBE

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?

In general, the SAB finds that the structure and strategy for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods are clearly presented and appropriate with a few exceptions. The SAB also finds that the included reference tables are lucid and illustrative. In particular, the SAB is pleased with EPA's decision to perform additional statistical analysis when required and also to have the IRIS Program perform peer-reviews of some of the key studies published as part of reports.

The approach to the study search and identification is illustrated clearly in Figure LS-1 and can be followed in the HERO database for ETBE. The general search strategy consisted of starting with four scientific literature databases (i.e., PubMed, Web of Science, Toxline, and TSCATS2) and other sources, followed by screenings for identifying and selecting the studies relevant to the human health risk assessment. Consistent with the broad search strategy, and as depicted in Table LS-1, initial searches were not topic-limited. Searches in scientific literature indexes were updated continually through November 2015 or December 01, 2016 (for the TSCATS2 database). Clarification is needed to address: 1) why these four databases are the only ones used; 2) why the set of search keywords did not include all synonyms for ETBE, and 3) why the searches were not updated through December 2016, across-the-board for all four databases.

Consistent with systematic review and the recommendations of NRC (2011), a literature search with updates that use the same search keywords is not necessarily comprehensive or sufficient since new questions may arise during the process of study review and toxicology assessment. For example, new inquiries have the potential to consider non-mammalian studies, or more in-depth evaluation of the development of genotoxic-related kidney disease in humans. It is not apparent, however, that this continuing and evolving literature search process occurred as extensively as needed in the draft ETBE assessment and, as a result, the hazard evaluation of ETBE may have been too limited. The SAB Panel was informed by staff of the EPA's IRIS program that EPA is developing IRIS-specific guidelines for literature reviews. The SAB supports this.

The SAB finds it appropriate that an additional literature search included two review articles, and a communication from the Japan Petroleum Energy Center (Table L-S2). However, the Agency should clarify (1) why citations were searched manually only in two review articles and no other peer-reviewed publications, and (2) the searches within sources of citations other than peer-reviewed publications (e.g., overviews and assessments by other national and international agencies) were limited.

Inclusion/exclusion screening criteria are summarized in Table LS-3 and the SAB finds the criteria to be generally appropriate. Identified studies are included or excluded for relevance to population, exposure, and outcome and systematically included in HERO according to these criteria. The SAB finds that the exclusion criteria should be better justified. For example, the exclusion of ecological and non-

mammalian species requires further clarification because this exclusion can result in constraining the hazard evaluation.

The criteria for the quality evaluation of studies included in the evidence tables are well described and appropriate. However, the SAB finds it difficult to ascertain the reasoning behind disposition of individual studies and their classification into a particular category. Additionally, some information pertaining both to the evaluation of quality and to the impacts on interpretation of the sources listed under "Database Evaluations" is presented within the main text. Unfortunately, this scattering and partial reporting of information does not easily permit readers to follow or comment upon, if appropriate, the treatment of individual issues within specific studies. Such discussion of any one study is not easily located (other than by searching the entire draft ETBE assessment) and readers are limited to the aspects that the EPA chooses to discuss. In addition, the Supplemental Information does not appear to include information pertaining to the evaluation of quality or the impacts on interpretation.

The SAB agrees that there are no relevant human chronic studies of the health effects of ETBE and that the sources for health effects data are all animal studies, and that EPA identified the critical set of experimental rat, mouse, or rabbit studies for potential inclusion in evidence tables. One of these studies (Dorman et al., 1997) was excluded because studies of neurotoxic outcomes were found to be inconsistent. The SAB recommends that the Agency clarify a discrepancy in the number of studies identified as potential sources of health effects data between Table LS-1 (33 studies identified with 32 left after removing the Dorman et al., 1997 study), and the 30 studies mentioned in the narrative and listed in Table LS-5.

The SAB is not able to provide advice on whether the EPA's evaluation of study methods and quality are applied objectively because of the lack of documentation within the draft assessment. There is no clear documentation on the comparative quality evaluation for each of the studies (e.g., by providing the information for each study/quality criterion in the HERO database). On the other hand, the SAB found no evidence that quality evaluation criteria were applied in a non-objective manner.

The following recommendations are noted:

Tier 1:

- EPA should address whether the whole range of available literature databases were considered.
- EPA should address why not all synonyms for ETBE were used.
- EPA should address why not all databases were updated through December 2016.
- EPA should provide a rationale for not performing citation searches beyond sources listed in Table LS-2.
- EPA should provide a rationale for what appears to be a limited search for additional citations.
- EPA should address the discrepancy in the number of health effects studies reported in Table LS-1.
- EPA should clarify why ecological/non-mammalian studies were apparently excluded from any consideration (despite the footnote in Table LS-3).

Tier 2:

- EPA should provide more transparent documentation on the process for application of inclusion and exclusion criteria and the quality evaluation of studies, in order to support decision making by the Agency. This could be done through the HERO database.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.1.2. tBA

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?

Overall, the EPA's search strategies, criteria for study inclusion/exclusion and evaluation, and criteria for evaluating study quality are described clearly within the EPA's tBA assessment (2017) document. Both the narrative and the tables presented are informative, clear, and illustrative of the literature search, and study screening criteria and disposition. Several points of clarification/correction are necessary within this section of the assessment. The SAB has not reached consensus on the objectivity with which the strategies are applied.

The SAB finds that the EPA's overall strategy of starting from a broad, chemical-specific search of the scientific literature and other sources followed by successive screenings to identify key studies as appropriate within the draft tBA assessment. The strategy followed by EPA maximized the identification of publications potentially relevant to the assessment. The search was continually updated over time with a stated ending date of December 2016. The database was consistently maintained and updated over time in HERO.

Results from the process of data source identification and study screening and disposition are summarized clearly in Figure LS-1. As illustrated in Table LS-1, data sources included four relevant scientific literature databases (PubMed, Toxline, Web of Science, and TSCATS2). The four indexed scientific databases for the chemical-specific search are appropriate and likely to include most of the tBA-relevant published, peer-reviewed articles. The narrative does not indicate whether other databases were considered. Search keywords are appropriate consisting of typically-used synonyms for tBA and the CAS number. Given the range of names for this compound, the Agency should provide a rationale for why the Agency selected these specific names from the universe of those available for tBA.

The indexed literature searches are not topic-limited except for Web of Science and Toxline. EPA is not clear about why limits were applied in the very early phase of the scientific literature search of these two databases. The EPA's exclusion of reports in PubMed from the Toxline database search appears a reasonable approach for avoiding excessive duplication, but only if it is assumed that both databases (i.e., PubMed and Toxline) are completely error-free. While the EPA's approach to using *a priori* limits for the search in Web of Science to specific research areas helps exclude potential non-relevant publications, this approach appears to be inconsistent with the intended chemical-specific broad search of the strategy and could potentially result in missing information. The Agency should provide a brief justification to clarify both these issues.

Additional research strategies included manual search of citations in review articles, public comments, and reviews performed by other federal and international agencies (OSHA and IPCS, respectively), as

summarized in Table LS-2. This is appropriate. However, some SAB members agree that there should be clarifications, including: 1) why the manual search for additional citations is restricted to review articles and not done for other publications (including peer-review articles); 2) if there was a search for all federal and international agencies that may have performed assessments for tBA (for example, ATSDR), and why only IPCS (1987a, b) and OSHA (1992) are included.

Study inclusion and exclusion criteria are described clearly and summarized succinctly in Table LS-3. The SAB finds that the inclusion and exclusion criteria are generally appropriate, but two of the exclusion criteria require revision and/or further clarification, as described below.

The SAB disagrees with the categorization of dermal exposure as a "Not Relevant Exposure Paradigm" (Figure LS-1). tBA is used in perfumes, cosmetics, and personal hygiene products. EPA should review available dermal exposure studies for tBA and present that information as part of the hazard identification. Additional dermal studies that should be included are Edwards (1982) for contact dermatitis and Hoshino et al. (1970) for squamous cell carcinoma.

As indicated in the footnote of Table LS-3, studies of ecological and non-mammalian species (e.g., zebrafish) are "…not considered a source of health effects data or supplementary data…but were considered as sources of contextual information." However, there is no evidence that such studies are considered for any purpose. The SAB notes that this exclusion yields a hazard identification which is not sufficiently comprehensive. In addition, the lack of discussion on studies with non-mammalian species may be a reflection of insufficient adherence to systematic review principles.

The general approach to database evaluation and the criteria for study quality are described in the narrative and summarized in Table LS-4 for the experimental animal studies. The SAB agrees that the quality evaluation criteria are appropriate, that these criteria follow EPA guidelines, and that their application is illustrated in the draft tBA assessment and the Supplemental Information as individual studies are discussed. However, the SAB notes that it is difficult to ascertain EPA's reasoning behind the disposition of individual identified literature items. That is, it is not possible for the reader to examine the scoring of individual studies for the named criteria or to see clearly the reasons for which individual studies are placed in one or another category. This renders critical comment on study selection and disposition difficult. Issues about evaluation of quality and impacts on interpretation listed under "Database Evaluation" (pp. xxxii-xxxiii of the *Toxicological Review of tert-Butyl Alcohol*) are said to be discussed in the text, as the studies are described. However, scattering of the information and partial reporting does not allow the reader to follow (and comment upon, if appropriate) the treatment of specific issues on individual studies. There is no easy way to find such discussion of any one study (other than by searching the entire draft tBA assessment) and the reader is limited to those aspects selected by EPA to discuss. Supplemental Information does not appear to include this information either. There is no evidence in the EPA's draft tBA assessment that the criteria were not applied objectively. However, with little documentation on the detailed review and application of quality criteria for each study, it is difficult to affirm that the quality criteria were applied objectively across the board. It would be useful to have a way to capture the evaluations on specific criteria and to see the reasons for disposition of each study, perhaps as part of the HERO database, such that the application of the evaluation criteria in Tables LS-3 and LS-4 is more transparent.

There is limited information as to whether the search strategy evolved throughout the development of the draft tBA assessment. For example, in the performance of a systematic critical review, specific questions likely will arise and these frequently require additional searching of the literature with

keywords other than those employed in the initial search. The Agency should clarify if this process occurred (or the extent that it did) in the draft tBA assessment.

The following recommendations are noted:

Tier 1:

- EPA should provide clarification on the rationale of the selection of some synonyms of tBA as key search words and not others.
- EPA should provide clarification of the rationale for imposing limitations on sources in the first stage of the scientific literature search (i.e., PubMed, Web of Science).
- EPA should provide clarification of the rationale for limiting the search for additional citations to only some of the publications available in peer-reviewed literature and secondary sources, but not others.
- EPA should provide a rationale for the exclusion of studies of dermal contact as a relevant route of exposure in light of the occurrence of tBA in many consumer products such as perfumes and cosmetics.
- EPA should provide a justification for the complete exclusion of studies with non-mammalian species, which affects the completeness of the hazard identification.

Tier 2:

- EPA should provide more transparent documentation of the process of application of inclusion and exclusion criteria and the quality evaluation of studies, in order to support decision making by the EPA. This could be done through the HERO database.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.2. Hazard Identification - Chemical Properties and Toxicokinetics

3.2.1. Chemical properties.

3.2.1.1. ETBE

Is the information on chemical properties accurate?

The SAB notes several inconsistencies between the chemical properties data provided in Table 1-1 and values actually reported in the cited source or in other sources. Also, as multiple values for a given parameter (e.g., water solubility) could be found in the literature, data included in the assessment should come from reliable sources that are accurately documented.

The following recommendations are noted:

Tier 1:

- The table entries for odor recognition or detection in different media need corrections/ clarifications.
- EPA should have a template and focus on listing only properties relevant to the compound and the specific assessment.
- References used should be checked for quality control to ensure the accurate citations are presented and that the data in tables match those in the reference provided.

- EPA should use primary data sources, not reviews or other government documents.
- If more than one value is found in the primary peer reviewed sources, EPA should provide a rationale for the choice of the one presented.
- If secondary sources or reviews are used, EPA should cross check values with the original citation provided in the secondary sources.
- If results from published studies are used, the quality of the experiment should be reviewed to determine whether the design/data are sound.
- If values presented are estimates, that fact should be identified.
- Units and conditions under which the data were generated should be identified (e.g., solubility in water at what temperature, etc.).

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.2.1.2. tBA

Is the information on chemical properties accurate?

As with ETBE, the SAB observed several inconsistencies among the chemical properties data provided in Table 1-1.

The following recommendations are noted:

Tier 1:

- EPA should have a template and focus on listing only properties relevant to the compound and the specific assessment.
- References used should be checked for quality control to ensure the accurate citations are presented and that the data in tables match those in the reference provided.
- EPA should use primary data sources, not reviews or other government documents.
- If more than one value is found in the primary peer reviewed sources, EPA should provide a rationale for the choice of the one presented.
- If secondary sources or reviews are used, EPA should cross check values with the original citation provided in the secondary sources.
- If results from published studies are used, the quality of the experiment should be reviewed to determine whether the design/data are sound.
- If values presented are estimates, that fact should be identified.
- Units and conditions under which the data were generated should be identified (e.g., solubility in water at what temperature, etc.).

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.2.2. Toxicokinetic modeling.

3.2.2.1. ETBE

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

The SAB strongly supports EPA's application of the PBPK model for ETBE, including the tBA submodel, in dose-response characterization of ETBE as an appropriate way to incorporate science using state-of-the-art methods. However, a few concerns are noted by the SAB.

The overall presentation of the PBPK modeling should be more cohesive, clear, and transparent. Providing essential information, assumptions, results and conclusions would be most helpful. Section B.1.5 provides only a very brief description of the PBPK model used in this assessment. Model code was made available through the HERO database, which is appropriate though it provides limited information especially to those without access to the modeling software. The approach for model evaluation is very clearly described in the U.S. EPA (2017e) document "PK/PBPK model evaluation for the IRIS assessments of ethyl tertiary butyl ether (CASRN 637-92-3) and tert-butyl alcohol (CAS No. 75-65-0) (Draft)" cited in Section B.1.5 of Appendix B. This evaluation appears to have been objective and thorough, with a detailed discussion of uncertainties, assumptions and required modifications. The SAB identifies several concerns regarding the assessment's text on the PBPK model documentation below. No peer reviewed animal or human studies beyond those cited in the assessment have been identified.

Instead of using a default method to calculate the human equivalent dose (HED), the SAB encourages the Agency to create an ETBE and tBA model parameterization for humans using the published human PBPK model of Nihlen and Johanson (1999) and data in Amberg et al. (2000). In Nihlen and Johanson (1999) serum time course and exhaled breath samples were taken for 5, 25, and 50 ppm, 2-hour inhalation exposures. Although, as stated in U.S. EPA (2017e), the Nihlen and Johansen (1999) data and modeling approach are not "conventional," they are useful for application in the PBPK modeling effort. Similar experimental and PBPK modeling (Johanson et al., 1986; Corley et al., 1994) were used in the *Toxicological Review of 2-butoxyethanol*. The oral route can be described in the model in the same way that it is 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 is rate of metabolism of the parent chemical in the liver. For 2-butoxyethanol the dose metric is the concentration of the active metabolite in the blood; for tBA the dose metric is the concentration of tBA in the blood.

EPA should give further consideration to maximizing the potential benefit for dose-response and mechanistic analyses using appropriately selected dose metrics. Some noncancer kidney effects observed following exposures to ETBE appear to show a consistent relationship to average daily concentration of tBA at periodicity. Evaluation of dose metrics (e.g., average daily rates of metabolism or concentrations) in relation to cancer or noncancer endpoints can be valuable in characterizing the dose response relationship and the involvement of ETBE parent or its metabolites tBA and acetaldehyde in toxicity.

The SAB recommends that the Agency consider dosimetry in both positive and negative studies (e.g., liver cancer in rats following ETBE exposures by inhalation and oral routes and tBA exposures by oral administration), since this can also be informative about potential mechanisms and exposure-response relationships. However, the Agency should also consider that the response may arise from combined contributions of more than one compound (i.e., parent and metabolites).

On page B-23, the statement ("Finally, because induction is expected to have an equal impact on oral and inhalation exposures—and only in the case that tert-butanol levels or metabolism are used as a dosemetric—induction's potential impact on risk evaluation for ETBE is considered minimal") regarding induction over time of tBA following ETBE administration seems to implicitly depend on the absence of a first-pass effect of the liver with ingestion (versus inhalation) exposure. This should be addressed and the description and underlying scientific logic of the handling of induction clarified.

Review of the acslX model code obtained from the HERO database found total concentration in liver (CL), and not concentration in venous blood (CVL) representing free concentration in the liver, was used. Since the metabolic rates are fitted to data, changing the model to describe metabolism as a function of the free liver concentration, CVL, will alter estimated Michaelis constant (Km) values but should not otherwise impact model results. Nevertheless, this technical error needs to be corrected.

The model simulations shown in the draft ETBE assessment and the original peer-reviewed publication generally provide good consistency with the experimental data in blood, exhaled breath, and urine. As is frequently the case when modeling multiple data sets with two compounds (i.e., ETBE, tBA) from multiple laboratories with different measures of compound in tissues and fluids (e.g., concentrations in blood, exhaled breath, urine), some data are not as well simulated as others.

Future modeling efforts could assess whether fits to data, particularly exhaled ETBE, which appear to underestimate ETBE metabolism (i.e., exhaled concentrations of ETBE are higher in simulations than data), would benefit from consideration of capacity-limited blood binding of ETBE or sex differences in metabolism. EPA should consider assessing the following four topics and consider adjusting future modeling efforts in light of the noted findings indicated in the literature:

1) Capacity-limited blood protein binding other than or in addition to alpha 2μ-globulin: Previous studies of methyl tertiary butyl ether (MTBE) metabolism indicated 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 5, 25, and 50 ppm for 2 hours during light physical exercise. The 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 alpha 2μ-globulin (Williams and Borghoff, 2001) and renal tubular reabsorption of this protein as observed by Neuhaus (1986) in male rats. However, disproportionately less radiolabeled tBA (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 alpha 2μ-globulin-binding mechanism in male rats.

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

3) In the current PBPK model, the impact of 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.

4) 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.

The following recommendations are noted:

Tier 1:

- The model code should be revised to describe metabolism as a function of the free liver concentration, CVL, and metabolic parameters (e.g., Km or first order rate constants) should be re-estimated. Metabolism based upon total liver concentration, CL, is not scientifically correct.
- The overall presentation of the PBPK modeling should be cohesive, clear, and transparent, and should provide essential information, assumptions, results and conclusions. It is misleading to say "A more detailed summary of the toxicokinetic models is provided in Appendix B.1.5 (EPA 2017a)" . The text of the draft report (EPA, 2017a) could be included in the Supplement, in which case it would benefit from adding a conclusions section.

Tier 2:

- EPA should 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.

Tier 3:

- For purposes of using PBPK models for the draft ETBE assessments, EPA needs to establish a consistent practice for documentation of both the model itself and the review of the model (and any modifications made by EPA to implement it). It is not desirable for EPA to write long descriptions of the model it is using that would repeat much of what is in published literature, but on the other hand providing clear summary information is desirable. Future model evaluations would benefit from using the organizational structure captured in the IPCS PBPK (2010); variants of this organizational structure were published by EPA in Clark et al. (2004) and Chiu et al. (2007).
- Future modeling efforts could assess whether fits to data, particularly exhaled ETBE, which appear to underestimate ETBE metabolism (i.e., exhaled concentrations of ETBE are higher in simulations than data), would benefit from consideration of capacity-limited blood binding of ETBE or sex differences in metabolism.

3.2.2.2. tBA

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 SAB strongly supports EPA's application of the PBPK model for tBA in dose-response characterization of tBA as an appropriate way to incorporate science using state-of-the-art methods.

The overall presentation of the PBPK modeling should be more cohesive, clear, and transparent. Providing essential information, assumptions, results and conclusions would be most helpful. Section B.1.5 provides only a very brief description of the PBPK model used in this assessment. Model code is made available through the HERO database, which is appropriate though it provides limited information especially to those without access to the modeling software. The approach for model evaluation is very clearly described in the U.S. EPA (2017e) document "PK/PBPK Model Evaluation for the IRIS Assessments of Ethyl Tertiary Butyl Ether and tert-Butyl Alcohol" cited in Section B.1.5 of Appendix B. This evaluation appears to have been objective and thorough, with a detailed discussion of uncertainties, assumptions and required modifications. The SAB identifies several concerns regarding the assessment text on the PBPK model documentation below. No additional peer-reviewed studies were identified.

Text in the draft tBA assessment, section 1.1.3 (page 1-3) needs to be revised so as not to be misleading and to be consistent with the Supplement. While no models of tBA have been created independently of other chemicals from which it arises as a metabolite (e.g., MTBE, ETBE), the tBA model has "been developed specifically for administration of tert-butanol." Pharmacokinetic studies with tBA exposures are how the tBA model was parameterized, so the text needs rewording.

Instead of using a default method to calculate HED, EPA should give further consideration to creating a tBA model parameterization for humans using the published human PBPK model of Nihlen and Johanson (1999).

The following recommendations are noted:

Tier 1:

- The model code should be revised to describe metabolism as a function of the free liver concentration, CVL, and the metabolic parameters (e.g., Km or first order rate constants) should be re-estimated. Metabolism based upon total liver concentration, CL, is not scientifically correct.
- Evaluation of tBA dose metrics for kidney toxicity should be compared for ETBE and tBA exposures (similar to Figure 6 in Salazar et al., 2015).
- The overall presentation of the PBPK modeling should be cohesive, clear, and transparent, and should provide essential information, assumptions, results and conclusions. The text in Section 1.1.3 of the draft tBA assessment and text in Appendix should be reworded. The SAB suggests that the material in U.S. EPA (2017c) be included in Appendix B or as a separate appendix and a conclusion section added to it.

Tier 2:

- EPA should 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.

Tier 3:

- The SAB has no specific recommendations for this tier

3.2.3. Choice of dose metric.

Is the rate of ETBE metabolism an appropriate choice for the dose metric?

3.2.3.1. ETBE

The EPA's use of rate of metabolism of ETBE as the dose metric is a reasonable choice, but the SAB does not recommend its use in extrapolation from inhalation to oral routes of administration of ETBE. There is no "consistent dose-response relationship" for this dose metric dose (page B-27), when combining oral and inhalation studies to assess liver tumors. This greatly weakens the case for route to route extrapolation. The SAB considered but has not identified any other dose metrics that would work better for route extrapolation of the liver cancer endpoint for ETBE than the dose metric chosen by the Agency. A metric in liver is the appropriate dose metric when the toxicity in question happens in the liver. In practice, blood may be a useful surrogate if concentrations of ETBE in blood and liver are proportional.

The Panel discussed several investigational aspects of ETBE pharmacokinetics that could be carried out. Since tBA is a primary metabolite of ETBE, a series of simulations to compare common dose metrics (tBA area under the curve (AUC) and maximum plasma levels (Cmax)) across animals dosed with both chemicals may provide insights into better utilization of the toxicology data. Additionally, ETBE dose metrics, including AUC and Cmax, could be evaluated as an alternative hypothesis, and should not be excluded. The liver tumors at a high inhalation dose are probably not due to tBA as a metabolite, since higher internal tBA doses did not produce liver tumors in tBA bioassays.

This implicates either parent ETBE or (maybe) acetaldehyde as a first metabolite as a cause. Any dose metric selected needs to be consistent with the proposed MOA for that endpoint (see responses to subsequent charge questions), so the linkage between these two needs to be considered and explicitly described by the Agency. Concentration of parent ETBE may be a relevant dose metric. In general, as dose metrics are considered, the Agency should consider how the choice enables comparison of effects of tBA dosed directly (in tBA bioassays) and tBA as the principal metabolite of ETBE (in ETBE bioassays).

The EPA should focus on the relationship between ETBE concentration in liver and its rate of metabolism in liver, since this corresponds to the choice between ETBE as the direct actor and acetaldehyde as the direct actor. The two alternatives will give similar results as long as metabolism is not saturated, as is noted in the EPA's assessment, but the SAB recommends that the Agency further examine this aspect.

The following recommendations are noted:

Tier 1:

- The SAB recommends that route extrapolation not be implemented for the oral cancer dose-response analysis for ETBE. Therefore, the Agency does not need to select a dose metric.
- If EPA proceeds with the dose metric noted in this charge question for the route extrapolation, the EPA should make the following corrections: The text in the supplemental section of the PBPK model (page B-24 and B-27) needs to be reworded to clarify the units of this dose metric (average daily rate of ETBE metabolized per day). In Figure B-3 on ETBE page B-26, the X axis needs to

be specified with correct units.

Tier 2:

- EPA should give further consideration to undertaking a more thorough analysis of dose metrics that examines animals dosed with both ETBE and tBA. Figure B-3 on ETBE page B-26, the X axis needs to be specified with correct units. It is difficult paging through the draft tBA assessment trying to link toxicity/cancer outcomes with the modeling efforts. To be more transparent and succinct, the SAB encourages the Agency to place the information on outcomes and the information on modeling near each other.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.2.3.2. tBA

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

The SAB agrees with the EPA's approach as presented for the dose metric. The SAB notes that while the effects occur in kidney, use of blood concentration is reasonable given that it is related to kidney concentration by the partition coefficient in female rats. Since the analysis only used effects in females, any issues of modeling male rat specific protein binding do not impact these analyses.

The following recommendations are noted:

Tier 1:

- The average concentration of tBA in blood is an appropriate choice for the dose metric because there is a dose-response relationship for this dose metric and a kidney noncancer endpoint. Thus the SAB recommends use of an oral to inhalation extrapolation for tBA. The SAB recommends that the EPA state in the draft tBA assessment how the average concentration was calculated for tBA.

Tier 2:

- It is difficult paging through the draft tBA assessment trying to link toxicity outcomes with modeling efforts. To be more transparent and succinct, the SAB encourages the Agency to place these pieces of information near each other.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.3. Hazard Identification and Dose–Response Assessment: Noncancer

3.3.1. Noncancer kidney toxicity.

3.3.1.1. ETBE

The draft assessment (sections 1.2.1, 1.3.1) identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of alpha 2μ-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 SAB finds that the EPA thoroughly and systematically considered alpha 2μ-globulin effects and their role in explaining renal tumors in male rats as presented in the EPA's draft ETBE assessment. The draft ETBE assessment describes the specific criteria established by EPA regarding alpha 2μ-globulin as a male rat-specific mode of action (MOA) and concludes that ETBE does not fulfill all the established criteria for having an alpha 2μ-globulin MOA. In recognition of gaps and uncertainties in the database, EPA concluded that the database is insufficient to conclude that ETBE is an inducer of alpha 2μ-globulin nephropathy in male rats, which is a MOA that is not relevant to humans. The EPA further concludes that other MOAs operating may be relevant to human hazard assessment.

The draft ETBE assessment also discusses the role of CPN as an underlying MOA for renal dysfunction in male and female rats. EPA noted that CPN is not a specific diagnosis on its own but an aggregate term describing a spectrum of effects. These individual lesions or processes may well occur in the human kidney, and the fact that they happen to occur as a group in the aged rat kidney does not guarantee that the individual lesions are rat-specific. Importantly, these effects are exacerbated by ETBE exposure despite being common in aging male and female rats (primarily of the Fischer 344 and Sprague-Dawley strains). Thus, the EPA concludes in the assessment that exacerbation of increased absolute kidney weight, urothelial hyperplasia, and serum biomarkers by ETBE exposure are not due to a rat-specific CPN and, therefore, may be relevant to human kidney hazard assessment. (It should be noted that although both absolute and relative kidney weights are increased after ETBE exposure, the EPA's draft assessment document clearly explains why absolute organ weight is a more reliable reflection of specific effects on the kidneys in that body weight changes will impact relative organ weights and potentially obscure effects of the chemical exposure.)

The SAB is unable to reach consensus with respect to the EPA's interpretation of the ETBE database for noncancer kidney effects. Two opinions are acknowledged, one consistent with EPA's assessment and one in opposition to EPA's assessment. The principal source of disagreement was whether renal effects observed in rats could be attributed to a biological process not relevant to humans.

The rationale for agreeing with the EPA interpretation and conclusion that the non-cancer kidney effects are potentially relevant to humans includes the belief that all criteria for concluding an alpha 2μ-globulin response in male rats are not fulfilled and that certain components of the so-called rat CPN response do occur in humans, as noted by Melnick and colleagues (Melnick et al., 2012) for chronic renal fibrosis. It was also noted that the focus of the assessment should be on public health and although the EPA should not arbitrarily conclude that a MOA is relevant to human hazard, in the absence of compelling evidence to the contrary and in order to protect human health, considerations should be conservative and the potential for human relevance should not be discounted.

The rationale for opposition to EPA's assessment lies on the premise that the 1991 EPA criteria are outdated and difficult to satisfy with most toxicology study designs, noting specifically that some of the specified histological changes may not be typically recorded by pathologists, and that the spacing of histological examinations in published studies was not close enough to yield the time-course data specified by EPA criteria. For this reason, it could be recommended that EPA use the newer IARC criteria (IARC, 1999) for determining whether an alpha 2μ -globulin MOA is operational for ETBE renal effects in male rats. In this opposing opinion, findings in published studies of ETBE renal effects in male rats fulfill all of the IARC criteria, indicating that these effects were not relevant to humans.

There was also a lack of consensus among the Panel members regarding CPN. Some members agree with EPA's interpretation of the literature and rationale for concluding that ETBE renal effects could

occur independently of CPN and were therefore relevant to humans. Other members disagree, concluding that renal effects in ETBE-treated rats were manifestations of CPN (with possible contribution from alpha 2μ-globulin-mediated effects; see above). Evidence cited in support of this conclusion included: 1) the renal effects observed in rats were all consistent with CPN; 2) no effects were noted in regions of the rat kidney without CPN involvement; 3) no renal effects were noted in other species that do not develop CPN; and 4) no human relevant MOA for ETBE has been identified that would clearly lead to renal effects independent of CPN. Because there was no evidence of renal effects independent of CPN, and because CPN has no counterpart in humans, the opinion of these Panel members is that the renal effects of ETBE are not relevant to human kidney hazard assessment. Other Panel members were of the opinion that all of the noncancer kidney effects of ETBE in male rats could be explained by the alpha 2μ -globulin or CPN MOA and those in female rats by the CPN MOA, thus making all of the noncancer kidney effects of ETBE in male and female rats irrelevant for humans under the assumption that none of the components of CPN occur in humans.

There is also a concern about the use of urothelial hyperplasia as a surrogate for noncancer kidney effects. The SAB notes that although urothelial hyperplasia encompasses effects in both the papillary and bladder epithelium, there is no known mechanistic link between bladder effects such as urothelial hyperplasia and the various types of kidney injury typically observed with chemicals similar to ETBE. Nevertheless, the EPA concluded that urothelial hyperplasia is an appropriate endpoint for ETBE renal effects, stating in section 1.3.1 on page 1-109, lines 29-32: "Urothelial hyperplasia in male rats, increased severity of CPN, increased blood biomarkers in male and female rats, and increased kidney weights in male and female rats are considered the result of ETBE exposure, independent (of) α2μ-globulin, and relevant for assessing human health hazard." Opinions differed among the Panel members regarding the appropriateness of using urothelial hyperplasia as an endpoint for dose-response assessment along the same lines as the more general discussion of renal effects. Some Panel members found the use of urothelial hyperplasia as a human-relevant endpoint to be scientifically supported, while others noted that urothelial hyperplasia occurs with CPN and its human relevance is therefore questionable.

The following recommendations are noted:

Tier 1:

- The SAB is unable to reach consensus on whether noncancer kidney effects should be considered a hazard relevant to humans based on the presented information in the assessment. Justification for the EPA's choice to consider the hazard relevant to humans should be strengthened.

Tier 2:

- Although consideration of the role of alpha 2μ-globulin in ETBE-induced nephropathy in male rats is thoroughly considered according to the 1991 criteria established by the EPA, the SAB recommends that the Agency apply the more detailed criteria published by IARC in 1999.
- The EPA could consider use of another parameter, such as increases in blood (serum) biomarkers or exacerbation of nephropathy, besides urothelial hyperplasia, as a surrogate for noncancer kidney effects.
- The EPA could consider urothelial hyperplasia as separate from noncancer kidney effects in developing the human hazard assessment.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.3.1.2. tBA

The draft assessment (sections 1.2.1, 1.3.1) identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of alpha 2μ-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 SAB agrees that the EPA's draft tBA assessment thoroughly evaluated the role of alpha 2μ-globulin in male rat kidney effects of tBA in accordance with the EPA's guidance policy (EPA, 1991). Similarly, the SAB finds that the EPA's review methodically discussed the issue of CPN, and its potential role and relevance to humans was also discussed methodically. The SAB is divided on whether the EPA's draft tBA assessment is scientifically supported and clearly described. Some members find the EPA's conclusion to be clear and supported, while others find that the Agency presents no clear evidence for tBA noncancer kidney effects in rats that have any relevance for hazard assessment in humans. The bases for the split opinion regarding the relevance to humans of noncancer kidney effects in tBA-exposed rats are the same as presented for ETBE, namely disagreements about how completely effects in male rats can be explained by alpha 2μ-globulin nephropathy and whether components of CPN that occur in male and female rats can also occur in humans. A more detailed explanation was presented for the earlier section on ETBE, section 3.3.1.1.

The SAB notes the following per the draft assessment:

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

2. Any kidney effects associated with alpha 2μ-globulin nephropathy are not considered relevant for human hazard identification.

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

4. Overall, the female rat kidney effects (i.e., suppurative inflammation, transitional epithelial hyperplasia, increased severity of CPN, and increased kidney weights) are considered by the Agency to be the result of tBA exposure and relevant to human hazard characterization. The Agency therefore considers these effects as suitable for consideration for dose-response analysis and derivation of reference values.

The SAB identifies a few concerns within this portion of the EPA's draft tBA assessment. The SAB finds that suppurative inflammation and transitional epithelial hyperplasia may both have multiple, poorly defined etiologies and may not be mechanistically linked to nephropathy. The SAB also finds that suppurative inflammation and transitional epithelial hyperplasia are not mechanistically linked to

nephropathy associated with proximal tubular cell injury. The SAB recommends that the EPA consider providing additional discussion of this topic within the draft tBA assessment. The SAB notes that some Panel members formed the conclusion that kidney effects are a potential human hazard associated with tBA exposure and that this conclusion is appropriate and scientifically supported. However, the SAB also notes that some Panel members concluded that all the tBA noncancer kidney effects in rats could be explained by either alpha 2μ-globulin nephropathy or CPN and are, therefore, not scientifically supported nor relevant for hazard assessment in humans.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The EPA should provide a more thorough explanation for considering the enhancement of CPN as a kidney effect relevant to human hazard assessment.
- The EPA should consider other indicators besides suppurative inflammation and transitional epithelial hyperplasia as indicators of kidney effects or provide better justification for their choice.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.3.2. Noncancer toxicity at other sites.

3.3.2.1. ETBE

The draft assessment (sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1) 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*

The SAB notes that if the EPA chooses not to proceed with kidney effects as the endpoint for the RfD then endpoints at higher doses need to be considered, despite EPA practice not to calculate an RfD for endpoints that are at higher doses than those observed for other endpoints. The SAB agrees that nearly all of the possible effects at these sites occurred at much higher exposure levels than did effects observed on the kidney, which occurred at 170 mg/kg-d or 6,000 mg/m3. The SAB's responses regarding the three targets that the Agency considered (liver, developmental, and male and female reproductive toxicity, including the lower-dose male reproductive effects in a mutant mouse model) are discussed below.

Liver Toxicity:

The SAB reviewed the extensive database developed by the Agency on liver toxicity. The SAB agrees that there are liver weight increases in rats, but these were only significant at the highest oral (1,000

mg/kg-day) and inhalation (29,900 mg/m3) exposure levels. There were no consistent effects on liver serum enzyme markers. Histological changes (basophilic foci, centrilobular hypertrophy) were also observed at the highest doses. The basophilic foci could be of toxicological significance if they are progenitors of adenomas. The hypertrophy is consistent with the increase in total P450 (CYP) and the increase in expression of mRNAs of several CYPs measured by Kakehashi et al. (2013). These researchers also presented findings indicating that events in the male F344 rat liver are mediated through PPARα, CAR and PXR. Upon review of the EPA's draft ETBE assessment and database, the SAB concurs that the EPA provides scientific support for their conclusion that these data are an inadequate basis to conclude that ETBE causes liver tumors by these modes of action. The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that there is suggestive evidence for liver effects contributing to noncancer toxicity.

Developmental Toxicity:

The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that almost all assays show no significant developmental or neurodevelopmental toxicities with oral gavage administration of ETBE to rats and rabbits at 1,000 mg/kg-d. Minor effects (skeletal variations, postnatal deaths at a specific time point) were only observed at a high oral dose (1,000 mg/kg-d) in some studies. Since some of these effects may be associated with maternal systemic toxicity and the statistical significance depended on the method of analysis, the SAB concurs that the draft ETBE assessment provides scientific support for the EPA's conclusion that these results should not be considered for hazard assessment.

Reproductive Toxicity:

The SAB analyzed the data on male reproductive toxicity and in most cases agree that there was very little toxicity in normal (non-mutant) animal models. No male reproductive toxicity (testis weight, sperm number, morphology, and motility, histopathology, androgen-dependent accessory organs, fertility) was reported in rats after oral exposure, even at a high dose (1,000 mg/kg-d). Only one study (Bond et al., 1996, *citation by* Medinsky, 1999) observed some increase in histological damage (tubule atrophy) in rats exposed to inhaled concentrations of 7,000 and 21,000 mg/m^3, but the increase was not statistically significant, not found in another similar but longer term study (JPEC, 2010; Saito et al., 2013), and must have been minor because testis weights were unaffected. In normal mice, there were no effects by inhalation doses up to 21,000 mg/m^3 on testis weight, sperm production, overall motility, and histopathology; there was only a small effect on motility at 21,000 mg/m^3. More sensitive assays of sperm DNA damage indicated effects in normal mice by inhalation of 7,000 and 21,000 mg/m^3; however, it is difficult to evaluate the toxicological significance and consequences of this level of damage. The only clearly significant toxic effects were observed in mice deficient in aldehyde dehydrogenase 2 (ALDH2 knockout and heterozygotes, Weng et al., 2014). The effects included reductions in overall motility and sperm production and increased levels of DNA damage. The results convincingly showed that damage was produced by exposures as low as 2,100 mg/m^3 in this sensitive mutant model system.

The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that there are no female reproductive toxicities observed in studies of ETBE, even despite some systemic toxicities indicated by reduced weight gains of the dam. As adequately discussed within the draft assessment report, these studies assessed ovarian and uterine weights, counts of primordial and growing follicles, estrous cyclicity, pregnancy rates, embryo survival, and overall pup survival to weaning. These parameters are unaffected by oral administration of up to 1000 mg/kg-d for up 10 weeks plus during development and pregnancy, or by inhalation of up to 5000 ppm (20,900 mg/m3) for up to 2 years. In

rabbits, fetal implantation, viability, development, and body weight are unaffected by ETBE administered by gavage to rabbits up to 1000 mg/kg-d during pregnancy.

The following recommendations are noted:

Tier 1:

- Male reproductive toxicity: The SAB suggests describing the recommendation as "minimal effects at otherwise toxic dose levels," rather than "inadequate evidence," since the SAB concludes there is an adequate amount of evidence that shows minimal effects, at least in populations with normal ALDH2 function.
- Male reproductive toxicity: There are male reproductive effects on genetically-susceptible mice (Weng et al., 2014), which mirror large human populations, at lower doses than other toxicities. The SAB recommends that this be clearly stated, rather than just considering these results as inconsistent with the lack of effects on genetically-normal rodents.
- Female reproductive toxicity: The SAB suggests describing recommendation as "no effects even at otherwise toxic dose levels," rather than "inadequate evidence," since the SAB concludes there is an adequate amount of evidence, which shows minimal effects.

Tier 2:

- The SAB suggests that an RfC be calculated for the male reproductive effects of ETBE, as this may be the most sensitive target for a sensitive subgroup.
- Further research on mechanisms of action of ETBE on the liver, particularly those involving receptor-mediated targets and nuclear signaling pathways, is warranted to better understand their relevance and applicability to humans.

Tier 3:

- There have been no developmental toxicity studies with inhalation exposure; such studies would be necessary for a complete characterization of the potential for developmental toxicity, but in the absence of indications from gavage exposure, these are not of high priority.
- Because of profound neurological impairment in PXR and CAR knockout mice (Boussadia et al., 2016; 2017) and indications that ETBE interferes with these receptors and it is unclear that the most relevant neurological and behavioral endpoints were assessed in the older developmental studies of ETBE, and further assessment of developmental neurotoxicity potential of ETBE is warranted. Studies that examine cognition, plasticity, and behavioral outcomes would be important.
- Further studies of reproductive effects of ETBE in animals with the specific ALDH2 mutant allele that is common in human populations are needed. In addition, the association of that ALDH2 polymorphism in humans with cardiovascular and neurological disease (Zou and Wang, 2015) suggests that the effects of ETBE on these endpoints might be also analyzed in such an animal model.
- Further studies should be conducted on the consequences of the DNA damage produced by ETBE compared with those produced by known reproductive genotoxic agents (e.g., ionizing radiation) that also act primarily by producing DNA strand breaks and 8-hydroxy-deoxyguanosine.

3.3.2.2. tBA

The draft assessment (sections 1.2.3-6, and 1.3.1) finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are

scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with tert-butanol exposure, please identify them and outline the rationale for including them in the assessment.

The SAB notes that if the EPA chooses not to proceed with kidney effects as the endpoint for the RfD, then endpoints at higher doses need to be considered, despite EPA practice not to calculate the RfD for endpoints that are at higher doses than those observed for other endpoints. Nearly all of the possible effects at these sites occurred at much higher exposure levels than did effects on the rat kidney, which occurred at 180 mg/kg-d. However, the EPA's calculation of an equivalent inhalation dose by PBPK modeling, which yielded 472 mg/m^3, is open to question as no significant increases in severity of nephropathy were reported at doses of 6,400 mg/m^3 for 13-weeks and the LOAEL for increased kidney weight is 3,300 mg/m^3. The EPA's discussion of other sites within the draft assessment report is warranted, as there are some questions regarding the applicability of the kidney endpoint to humans.

Developmental Toxicity:
The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that assays show no significant developmental toxicity from tBA at oral doses of <1000 mg/kg-day or inhalation exposures of <10,600 mg/m^3. Reductions in rat and mouse fetal and pup survival and pup body weight were observed at oral tBA doses of ≥1,000 mg/kg-d or inhalation doses of ≥10,600 mg/m^3, but indirect effects related to maternal toxicities are probable.

The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that results of several studies describing neurodevelopmental effects of exposure to tBA, these studies should not be used in calculating reference values. A more detailed explanation was presented for the earlier section on ETBE, section 3.3.1.1.The SAB also agrees that the draft assessment report provides scientific support for the EPA's conclusion that effects have only been observed at high exposure levels. Behavioral deficiencies were observed in mice orally exposed to 5,000 but not 3,300 mg/kg-d during gestation. Changes in brain neurotransmitters were observed in offspring of mice exposed to 6,000 mg/m^3 by inhalation during gestation.

Reproductive Toxicity:
The SAB agrees that the draft tBA assessment provides scientific support for the EPA's conclusion that the studies performed did not find evidence of adverse reproductive effects. The only apparent statistically significant changes in males were a decline in sperm motility in rats and a possible loss in testis weight in mice. However, the decline in motility observed in rats at 1000 mg/kg-d was only marginal, from 94% to 91% (Huntingdon Life Sciences, 2004), and within historical control ranges. The possible (see Comments below) testis weight loss (NTP, 1995) at a dose of 3900 mg/kg-d could be an indirect effect, as there was 60% mortality at this dose. The only reported significant effect in females in the draft tBA assessment was an extension of the mouse estrous cycle from 4 to 5 days after oral exposure to 11,600 mg/kg-d of tBA for 13 weeks. However, as indicated in the recommendations below, EPA should also have analyzed the percentages of females that did not show clear estrous cycles. Then, as none of the rats exposed to 3,620 mg/kg-d showed clear estrous cycles, a significant effect in the rat would have been shown. Nevertheless, the SAB agrees that the draft tBA assessment provides scientific support for the EPA's conclusion that neither of these effects should be considered to be a result of direct action on the reproductive system as animal weight gains were significantly reduced and mortality was 40% and 60% in the rats and mice, respectively.

The following recommendations are noted:

Tier 1:

- The SAB recommends that the Agency include contact dermatitis (Edwards, 1982) in hazard identification as dermal exposure is a relevant route of exposure.
- The SAB suggests changing the description to "minimal effects at otherwise toxic dose levels," rather than "inadequate information to assess," since the SAB believes there is an adequate amount of information, and only minimal effects have been shown, even at toxic dose levels.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- Studies of neurobehavioral effects were conducted more than 20 years ago. Additional studies with more modern sensitive methods are warranted. Detailed behavioral testing in the cognitive, social, anxiety, and hyperexcitability domains are needed.
- Research on non-mammalian systems (e.g., zebrafish) to determine whether or not there are developmental targets of tBA could be pursued.
- Maternal toxicity has effects on offspring development, particularly on neural and behavioral development, and on female reproductive performance. Therefore, the LOAEL for lethargy and ataxia should be considered in the reference dose analysis. More specific information on metabolic and sedative actions of tBA on the exposed dam is needed, since it impacts reproductive function and development of the offspring. Therefore, the LOAEL for lethargy and ataxia should be considered in the reference dose analysis.

3.3.3. Oral reference dose for noncancer outcomes.

3.3.3.1. ETBE

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 responses to this question are premised on overall acceptance of the support of kidney effects of ETBE as an appropriate endpoint. The SAB has not reached consensus to support acceptance of kidney effects. The differing views are based on the extent of confidence in a CPN-based mechanism for these effects and the findings in rats but not in mice. However, if urothelial hyperplasia in male rats (Suzuki et al., 2012) is used for risk assessment, then the derivation of an oral reference dose of $5x10^{-1}$ mg/kg-day is considered to be scientifically supported and its derivation clearly described in the text. Several recommendations emerged from the SAB's deliberations.

The following recommendations are noted:

Tier 1:

- The EPA should carefully examine the question of the validity and applicability of the endpoints chosen and analyzed for the oral RfD, including the potential for CPN to serve as the mechanism of the kidney effects.

Tier 2:

- If urothelial hyperplasia is deemed an inappropriate endpoint for derivation of the oral reference dose, then the SAB encourages the Agency to consider use of liver hypertrophy instead as the basis of the oral reference dose for ETBE.
- Because the assessment report notes that ETBE and tBA appear to have caused a similar set of kidney responses, and because tBA as a metabolite of ETBE is implicated as a cause, the SAB encourages the Agency to examine the degree to which patterns and response levels are similar across the two chemicals, and whether a common response to tBA (either as a metabolite of dosed ETBE or as the tested material itself) can be discerned.
- The tables within this section need to include units for completeness and interpretability. The EPA should consider a more integrated presentation of the current text, tables and graph; as is, it is difficult to track information and the text often requires much page-flipping.

Tier 3:

- Lack of consensus on the role of CPN as a mechanism of kidney effects included disagreement as to whether CPN constituted a set of manifestations or whether individual components seen in CPN could also occur separate from CPN. An updated assessment of CPN including criteria for its definition, manifestation as a group of outcomes vs. individual outcomes would be helpful. It would be informative for EPA to include outcomes of statistical analyses and their rationale in study selection choice.
- EPA should consider evaluation of rat-human differences in ETBE metabolic activity so as to assess rat to human extrapolation that could then be used to assess effects relative to internal dose of tBA as they appear in both profiles.

3.3.3.2. tBA

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

The SAB's response to this question is premised on overall acceptance of the support of nephropathy effects in female rats as an appropriate endpoint for the oral reference dose for tBA. However, there is not a consensus among members as to the appropriateness of the selection of nephropathy effects. The differing views are based on the extent of confidence in CPN- and/or alpha 2μ-globulin-based mechanisms for these effects as a mechanism of tBA effects. However, if the EPA's selection of increases in severity of nephropathy in female rats in response to tBA administration in drinking water remains the basis of the oral reference dose, then the SAB considers the derivation of the oral reference dose of $4x10^{-1}$ mg/kg-day to be scientifically supported and its derivation clearly described.

The following recommendations are noted:

Tier 1:

- The validity and applicability of the endpoints chosen and analyzed for the oral RfD for tBA should be carefully reexamined, including the potential for CPN and/or alpha 2μ-globulin to serve as mechanism(s) of the kidney effects of tBA, in light of SAB advice regarding consideration of the criteria for definition of CPN.
- The units need to be added to the tables in this section for completeness and interpretability.

- It would be useful to attempt a more integrated presentation of the current text, tables and graphs. As currently laid out, the reader is forced to engage in a lot of page flipping in order to read the draft tBA assessment, making it difficult to track information

Tier 2:

- If nephropathy in females is ultimately deemed to be an inappropriate endpoint for derivation of the oral reference dose for tBA, then the SAB encourages the EPA to use liver hypertrophy for the endpoint derivation based on evidence of tBA effects.

Tier 3:

- The role of CPN as a mechanism of kidney effects include disagreement as to whether CPN constituted a set of manifestations or whether individual components seen in CPN could also occur separate from CPN. An updated assessment of CPN including, e.g., criteria for its definition, manifestation as a group of outcomes vs. individual outcomes would clarify these issues.
- The outcomes of statistical analyses and their rationale in study selection choice should be included in the EPA's draft tBA assessment.
- The Agency should consider evaluation of rat-human differences in ETBE metabolic activity so as to assess rat to human extrapolation that could then be used to assess effects relative to internal dose of tBA as they appear in both profiles.

3.3.4. Inhalation reference concentration for noncancer outcomes.

3.3.4.1. ETBE

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.

The question of the appropriate toxicological interpretation of the male rat urothelial hyperplasia, as seen in Saito et al. (2013), including whether these are actually renal pelvis transitional hyperplasia and hence part of CPN and whether such responses are relevant to humans, received much discussion. The SAB did not reach consensus on the appropriate interpretation of the kidney endpoints reported in the rat or on their relevance to humans. The SAB concludes that if these endpoints are indeed used, the derivation of the RfC candidate values is described clearly. Contingent on accepting that urothelial hyperplasia in male rats (Saito et al., 2013) is a separable and human-relevant endpoint, the EPA's derivation of a RfC of 9 x 10^0 mg/m^3 is scientifically supported. Beyond this issue, some members suggested alternative approaches to derivation of the RfC, including selection of alternative endpoints.

As is the case with previous charge questions, there was concern expressed about the limited 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 noncancer 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, this perspective should be more strongly supported by 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.

For noncancer outcomes, evidence found to be consistent relied upon kidney effects of organ weight changes, and histopathology (urothelial hyperplasia). Organ weight changes for chronic and sub-chronic exposures by the inhalation route were clearly reproducible across studies in the rat. For urothelial hyperplasia, chronic 2-year studies using inhalation exposure established that this response increased with treatment in male rats. The urothelial hyperplasia data were the only endpoint from chronic exposure (2-year) studies, and organ weight changes were the only endpoint from sub-chronic (13-week) studies that were considered for dose-response analysis (Saito et al., 2013; JPEC, 2010). The SAB agrees that the Saito et al. (2013) study, upon which the RfC is based, was well conducted, and adhered strictly to GLP guidelines, including evaluations of sufficient numbers of animals per group (both sexes), and a broad collection and assessment of appropriate kidney tissue samples, and relevant outcome measures (organ weight, histopathology).

The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that if urothelial hyperplasia is deemed a separable and human-relevant endpoint, it is, in principle, a more specific indicator of kidney toxicity than the relatively nonspecific endpoint of kidney weight change. However, the SAB notes that data of urothelial hyperplasia from the 2-year male rat study by Saito et al. (2013) and increased absolute kidney weight in male rats from the 13-week study by JPEC (2008) suggest that increased absolute kidney weight appeared as a more sensitive index of kidney toxicity (Table 2-5, Pg. 2-16). Absolute kidney weight increases correlate with histopathological changes in the kidney observed in subchronic and chronic studies (Craig et al., 2014). If the EPA utilized increases in absolute kidney weight in male rats as the candidate value for the RfC, a more health-protective RfC value would be determined. Further, it appears that what the EPA refers to as urothelial hyperplasia is actually renal pelvis transitional hyperplasia, which is part of CPN. Thus, the SAB recommends that the Agency describe this endpoint as "increases in severity of nephropathy" to more appropriately correspond with the characterization in the draft tBA assessment.

The SAB also notes that the absence of ETBE effects on most reproductive and developmental endpoints at doses of 20,900 mg/m^3 (5000 ppm) [compared to a LOEL of 1500 ppm (6270 mg/m^3) for kidney endpoints] suggests such effects are not important for setting an RfC. However, sperm DNA damage (DNA breaks, 8-oxo-deoxyguanine) and minor histopathological changes in sperm of ALDH2 knockout C57BL/6 mice were observed at lower exposure levels than those reported in rat kidneys. These effects were also observed in wild type C57BL/6 mice at the higher exposure concentrations. Therefore, male reproductive toxicity could also be considered in the setting of an RfC because of the known ALDH2 polymorphisms present in human populations. See Section 3.3.2.1. of this report for further discussion.

As described previously, in the view of many but not all Panel members, exacerbation of CPN, which is apparently augmented by alpha 2μ-globulin in male rat kidneys, is not relevant to humans. The Agency could consider utilizing the exacerbation of CPN in female rats as the toxic endpoint for RfC derivation.

The SAB largely agrees that benchmark dose (BMD) modeling for kidney effects was performed appropriately by the EPA. The EPA adjusted intermittent concentrations, derived human equivalent concentrations and performed benchmark modeling or extrapolation for deriving points of departure (PODs) in accordance with EPA guidelines. It was noted that the use of PBPK modeling be reconsidered for cross-species extrapolation to replace the body weight ¾ default.

Also in accordance with EPA guidelines, the Agency applied uncertainty factors to the PODs and the SAB finds that this application is reasonable and consistent with EPA guidelines. Page 2-18, line 8 of the assessment notes the range among candidate values for an inhalation RfC varied over a 100-fold range. The SAB's examination of Table 2-5 suggests that much of this range is actually a consequence of differing choices of uncertainty factors (UFs) [depending on what is done for UFs] as well as of the male-female differences. The PODs themselves are rather similar (for shorter and longer durations) within the same sex, though somewhat different between males and females.

The SAB finds that the assessment clearly describes the limitations of the estimates. All the candidate values except for the estimated RfCs 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, are within the same order of magnitude. Therefore, the draft assessment report provides scientific support that there is relative consistency in the various estimates.

The kidney responses in the rat are similar for inhalation and oral exposures to ETBE and are, presumably, attributable to metabolic generation of tBA. Considering that the exposure to tBA is systemic from metabolism occurring elsewhere (the liver), the SAB recommends that the Agency assess how similar the apparent potencies are for inhalation and oral exposure if rendered in terms of projected tissue concentrations for tBA.

Although there is copious tabulation of experimental results, it is difficult to trace the information on any one study from its discussion in the draft assessment to the tabulations in the Supplemental Information, and to the dose-response analysis elsewhere in the Supplemental Information. A complicating factor is that there are different analyses of the same data (e.g., absolute and relative organ weights), different durations of exposure for otherwise similar experiments, and lack of uniqueness of "author (date)" designations, making it challenging to be sure that one is examining corresponding data in the different places where the data are discussed or presented.

Critically, there does not seem to be any reporting of statistical analysis of individual studies (trend tests or pair wise significance tests, and other statistical tests determined to be appropriate), and this omission hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role of such statistical analysis in identifying results to report and the decisions to include or exclude them from analysis should be clearly stated within the assessment report.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The SAB suggests the following alternatives for deriving the RfC, including:
  - DNA damage (DNA breaks, 8-oxo-deoxyguanine) and minor histopathological changes in C57BL/6 mouse sperm which could be indicative of male reproductive effects in susceptible human subpopulations.
  - Exacerbation of CPN in female rats.
  - Basing the RfC on increased absolute kidney weights in male rats in the 13-week study because this endpoint appears to be more sensitive than urothelial hyperplasia.

- Assess specific potencies and cross-route evaluation by comparing estimated tissue doses of the metabolite tBA.
- EPA should provide statistical analysis to help elucidate differences in response based on sex and to make clear the rationale for including or excluding studies.
- Sex differences in response appear more marked for inhalation than for oral exposures. An evaluation of possible reasons for this (including mere statistical fluctuation which, if responsible, would suggest averaging endpoint values across sexes) would be informative.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.3.4.2. tBA

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

The SAB agrees that if the Agency accepts that the severity of tBA-induced nephropathy in the female rat is relevant to humans, the estimated 5 x 10^0 mg/m^3 RfC is scientifically defensible. The derivation of the RfC is mostly described clearly, except for issues that require further clarification as described below.

As with ETBE, the SAB expresses concern that EPA did 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, in principle is reasonable, but the draft tBA assessment should provide the line of scientific evidence of comparative histological changes in rodent and human nephropathies to support this statement.

The SAB agrees that the EPA's evaluation of the literature for noncancer effects supports the selection of kidney effects for establishing the overall RfC. The SAB also agrees with EPA's rationale for selecting dose-response data from female rats as a more appropriate basis for calculating BMDs for kidney effects than dose-response data from male rats.

The SAB agrees that the tBA draft assessment provides scientific support for the EPA's conclusion that no route-specific chronic studies are available for derivation of candidate RfC values for tBA. EPA derived PODs from the female rat dose-response data from the NTP (1995) 2-year oral study with route-to-route extrapolation, and dose-response data for the same strain of rat as the oral study from the 13-week NTP (1997) study adjusted for exposure duration. The SAB considers that the use of route-to-route extrapolation based on the Borghoff et al. (2016) PBPK model as modified by U.S.EPA (2017) is reasonable. However, the use of route-to-route extrapolation for deriving RfC's is a deviation from typical practice. Route-to-route extrapolation leads to considerable added uncertainty and the contingency of the estimates on the validity of the chosen extrapolation method. Given the availability of some inhalation data (though not for full lifetime), the discussion to use route-to-route extrapolation ought to explain in more detail why preference was given to an RfC derived from the extrapolated oral

doses over inhalation results. More specific details need to be provided about the application of the PBPK model for route-to-route extrapolation, which are not presented in sufficient detail on page 2-12 of the draft tBA assessment or in Appendix B of the Supplemental Information. In particular, the choice of the dose metric to represent "internal dose" (page 2-12, lines 12-24 of the draft tBA assessment) requires further explanation and additional justification. The caveats introduced by the uncertainty inherent in the use of the PBPK model for route-to-route extrapolation should be more explicitly stated when summarizing the findings in this section and in the Executive Summary.

Aside from issues of route-to-route extrapolation, derivation of PODs from the NTP (1997) 13-week inhalation study (endpoint: absolute kidney weight), and from the NTP (1995) oral study with route-to-route extrapolation (endpoints: increased absolute kidney weight, kidney inflammation, kidney transitional epithelial hyperplasia, and increases in severity of nephropathy) are performed following straightforward methods according to EPA guidelines. The PODs for candidate RfC values are adjusted by uncertainty factors also according to established guidelines. The SAB agrees that the range of candidate values for the RfC is not excessively large (i.e., within a factor of 7), which adds some measure of reliability to the derivation of candidate values. In particular, the difference in the candidate RfCs derived from the subchronic inhalation study based on increases in kidney weight is approximately only 20% lower than the selected RfC.

There are additional areas that require clarification by EPA (similar to concerns in section 3.3.3.1 and 3.3.4.1 for ETBE, and section 3.3.3.2 for tBA). Although there is extensive tabulation of experimental results, it is difficult to trace the information provided for any one study from its discussion in the text to the tabulations in the Supplemental Information, and to the dose-response analysis elsewhere in the Supplemental Information. This is complicated by the multiple different analyses of the same primary data (e.g., absolute and relative organ weights), different durations of exposure for otherwise similar experiments, and lack of uniqueness of "author (date)" designations, making it challenging to the reader to be certain that data under examination correspond as presented and discussed in different locations of the draft tBA and ETBE assessments.

Critically, there seems to be little reporting of statistical analysis of individual studies (trend tests or pairwise significance tests, and other statistical tests determined to be appropriate) and this omission hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role of such statistical analysis in identifying results to report and decisions to include or exclude them from analysis are not very clearly stated.

There is no reporting of units for the responses (as opposed to the exposures) in the Supplemental Information tables, and this leads to difficulty in interpretation.

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

The following recommendations are noted:

Tier 1:

- More detailed information should be provided about the specific application of the Borghoff et al. (2106)/U.S. EPA (2017e) PBPK model used for route-to-route extrapolation to derive the inhalation RfC.

Tier 2:

- It would be valuable to provide estimates of route-specific potencies for inhalation and oral exposure in terms of projected tissue concentrations for tBA, including estimates for tBA as the principal metabolite in studies of exposures to ETBE.
- EPA should provide more reporting of statistical analysis of individual studies to help clarify the appropriateness of inclusion/exclusion and use of studies.
- Additional statistical and cross-route analyses may help elucidate sex difference in response, and could potentially permit averaging responses across sexes.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.4. Hazard Identification and Dose–Response Assessment: Cancer

3.4.1. Cancer modes-of-action in the liver.

3.4.1.1. ETBE

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 SAB finds that there is scientific support for the EPA's conclusion that liver tumors in male rats are relevant to human hazard identification. According to EPA Cancer Guidelines, a conclusion that carcinogenic effects in animals are not relevant to humans requires "convincing and extensive experimental evidence." (U.S. EPA, 2005). For example, for a PPARα agonist, evidence must be sufficient to show that the liver tumors are the result of a PPARα MOA, and other potential MOAs have been examined and found to be inoperative (U.S. EPA, 2003). The draft assessment examines in some detail evidence that male rat liver tumors from ETBE might be the result of a PPARα, PXR, or CAR MOA, each of which has been postulated to produce liver tumors in a manner not relevant to humans. The draft ETBE assessment also considers other possible MOAs, including acetaldehyde-mediated liver effects, genotoxicity, and oxidative stress. A study by Kakehashi et al. (2013) found evidence of PPAR-, PXR-, and CAR-mediated events in rats treated with ETBE, but activation of these receptors alone is insufficient to establish a MOA. The EPA found that data to support the existence of other key events necessary to establish one or more of these nuclear receptor-mediated MOAs are weak or absent. Further, the draft assessment points out that other plausible, potentially human-relevant MOAs cannot be ruled out, in particular one mediated through metabolism of ETBE to acetaldehyde. The SAB agrees that experimental evidence for a PPAR, PXR, or CAR MOA for ETBE does not rise to the "convincing and extensive" threshold as described in the EPA cancer guidelines. The SAB also agrees that the MOA for the rat liver tumors remains at this point undetermined. Under circumstances such as this, tumor responses in animals are assumed to be relevant to human hazard identification.

While supporting the EPA's decision regarding human relevance of the male rat liver tumors, the SAB finds that improvement is needed for aspects of the discussion of MOA for hepatic effects of ETBE in Section 1.2.2 of the draft assessment. Specifically:

1. The draft assessment lacks clarity on specific information needed to conclude that a PPARα, CAR, or PXR MOA is operative. Key events for each of the MOAs are outlined, followed by a narrative regarding the nature and existence of data available regarding these key events. However, there is no articulation of a framework or set of criteria to determine what data would be sufficient to conclude that one of these MOAs is operative. In other words, the draft assessment provides no sense of where the bar is set for establishing a PPARα, CAR, or PXR MOA for ETBE rat liver tumors. As a result, the MOA analysis for receptor-mediated events appears more subjective than it should. Further, some of the EPA criticisms of data regarding key events are seen as inconsequential or in error, which further detracted from this section.

2. Evidence for other, human-relevant, MOAs is not clearly presented. The draft assessment provides a summary table (Table 1-13) with evidence regarding each of 10 potential carcinogen mechanisms, or "key characteristics." Examples, include genotoxicity, oxidative stress, immunosuppression, altered cell proliferation, cell death, or nutrient supply. For half of these, the evidence is summarized as "No positive studies identified." Regarding this presentation, the Agency should clarify whether data exist for these key characteristics but are negative, or that no evidence for or against exists.

3. Evidence for an acetaldehyde MOA is not well developed. The draft assessment states (pg. 1-55) "Evidence suggests that metabolism of ETBE to acetaldehyde could contribute to ETBE-induced liver carcinogenesis." This evidence is summarized on pages 1-53 and 1-54, and is based primarily upon previously demonstrated carcinogenic effects of acetaldehyde, which is a metabolite of ETBE. The EPA appears to consider this a strong candidate MOA, and it is the basis for most of the discussion of Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes in Section 1.3.3. However, the SAB finds that to advance this as a potential MOA for ETBE rat liver tumors, additional critical analysis of the literature is needed. This should include more detailed comparisons with acetaldehyde tumor data, including dose (from ETBE versus acetaldehyde given directly) and tumor site concordance.

Although the charge question asks about human relevance based upon MOA, the SAB raises concern regarding the human relevance of the ETBE rat liver tumors because they were only observed in one sex and at an excessively high dose (as defined in the EPA Cancer Guidelines).

The following recommendations are noted:

Tier 1:

- EPA should clarify the evidence needed to conclude that a PPARα, CAR, and/or PXR MOA is operative and indicative that liver tumors may not be relevant to humans. Examples may be helpful to illustrate the types of studies/information needed to satisfy each criterion.
- EPA should revisit the evaluation of information available for ETBE using these criteria. The EPA may specifically want to reconsider statements about transient hypertrophy.
- EPA should revise Table 1-13 and accompanying narrative to be more descriptive regarding availability of information for each MOA. Instead of saying "No positive studies identified" indicate whether studies relevant to the MOA exist and where results are positive or negative.

- Acetaldehyde is proposed as a strong candidate MOA for male rat liver tumors, but the plausibility of this MOA is not well explored. Evidence for this MOA should be developed and presented more thoroughly; or, alternatively, the Agency is encouraged to reduce emphasis on this MOA in the final assessment.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.4.1.2. tBA

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 alpha 2μ-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

The SAB has not reached consensus regarding the EPA's conclusion that male rat kidney tumors are relevant to human hazard identification and is scientifically supported. The draft assessment concludes that evidence for a MOA involving alpha 2μ-globulin or CPN is incomplete or not coherent, respectively. While some tumors might be attributable to alpha 2μ-globulin nephropathy augmented by CPN, others could be due to other unspecified processes that are assumed to be relevant to humans. The SAB has not reached consensus because some members agree with the assessment and some members conclude that renal tumors could be explained by CPN, and are therefore not relevant to humans. Additional discussion of this issue is provided in the response on noncancer kidney effects of ETBE (Section 3.3.1.1).

The following recommendations are noted:

Tier 1:

- Consistent with responses to previous charge questions related to noncancer kidney effects of ETBE and tBA, the SAB recommends that the EPA provide additional justification for the assumption that kidney tumors in male rats exposed to tBA are relevant to humans.

Tier 2:

- The suggested workshop on interpretation of human relevance of kidney effects in rats with CPN in Section 3.3.1.1 should include cancer as well as non-cancer endpoints.

Tier 3:

- The SAB has no specific recommendations for this tier.

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 SAB concurs that mode of action for follicular cell tumors in male and female mice treated with tBA is unknown. Per EPA science policy, these tumor responses are considered relevant to humans.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.4.2. Cancer characterization.

3.4.2.1. ETBE

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.

The SAB considered whether evidence for ETBE's carcinogenic potential technically meets requirements under EPA's 2015 Guidelines for Carcinogen Risk Assessment for the descriptor "Suggestive Evidence of Carcinogenic Potential." There is concern that the quality of data supporting this designation is weak, even for the relatively low threshold for evidence needed for that designation. The SAB notes that there are conflicting cancer bioassay results (adenomas + 1 carcinoma) in 1 organ of 1 sex of 1 species in 1 of 3 bioassays, at an exceedingly high carcinogenic dose (5,000 ppm vapor = 4.2 g/kg/day), lack of genotoxicity (the preponderance of evidence indicates a lack of genotoxicity), and lack of concordance of effect with different exposure routes. Nevertheless, the SAB agrees that the "Suggestive Evidence" descriptor should be retained for inhaled ETBE, as evidence of the carcinogenic potential of ETBE met minimal criteria for that designation as described in EPA's 2015 Cancer Guidelines.

The SAB notes that inhaled ETBE has been found to produce liver tumors in male rats (Saito et al., 2013). Ingestion of ETBE, however, was neither carcinogenic to the liver of rats in two 2-year bioassays (Maltoni et al., 1999; Suzuki et al., 2012) nor in a 23-week gavage study (Hagiwara et al., 2011). The EPA Guidelines for Cancer Risk Assessment (2015) state, "When tumors occur at a site other than the

point of initial contact, the descriptor 'Suggestive Evidence' generally applies to all exposure routes that have not been adequately tested at sufficient doses." ETBE, as noted above, has been consistently negative in oral bioassays.

There were differences of opinion on the relevance, justification and scientific validity of the initiation-promotion assay described in the Cancer Hazard Characterization section, and therefore the SAB does not reach consensus in its response to this question. One opinion was that the inclusion of the assay findings is warranted, because they could have relevance to ETBE's carcinogenic potential or provide information about the possible risks of chemical interactions involving ETBE. Other members did not attach scientific value to the assay's results because they felt that the assay was not relevant to humans due to its use of high doses of multiple potent initiators, only reflected ETBE-induced metabolic activation of the initiators, or believed the assay had no value in risk assessment.

The SAB also observed that initiation-promotion assays had been used in the past by the EPA as supportive evidence for conclusions based on more traditional cancer bioassays, but felt that it was not appropriate to use such as assay as key evidence to support a conclusion of carcinogenic potential for a given route of exposure.

The following recommendations are noted:

Tier 1:

- The SAB supports the use of the descriptor "Inadequate Information" for oral ETBE, and "Suggestive Evidence" for inhaled ETBE.
- The SAB recommends that that EPA not use the initiation-promotion assay as key evidence to support a conclusion of carcinogenic potential.

Tier 2:

- The SAB maintains that EPA should explain within the assessment that the assigned cancer classifications are an EPA Cancer Guidelines policy-based decision.

Tier 3:

- The SAB observes that it would be useful for the EPA in future assessments to devote more attention to assessing assays' design, relevance, interpretation, limitations and utility in characterizing chemicals' cancer potential.

3.4.2.2. tBA

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.

The SAB agrees that there is scientific support for the EPA's decision to select "Suggestive Evidence of Carcinogenic Potential" as the proper descriptor for tBA, because tBA was found to cause renal tubule adenomas in male F344 rats and thyroid follicular adenomas in female B6C3F1 mice. This cancer

descriptor is scientifically supported for oral exposure, though there have apparently been no inhalation bioassays of tBA. The EPA Guidelines for Cancer Risk Assessment (2015) state: "When tumors occur at a site other than the point of initial contact, the descriptor generally applies to all exposure routes that have not been adequately tested at sufficient doses." While there was a suggestion that the correct descriptor for tBA is "Likely to be Carcinogenic to Humans", since tBA produced tumors in two species, the SAB, however, recommends that the "Suggestive Evidence" be applied to both oral and inhalation tBA exposure.

The SAB has not reached consensus regarding the mode(s) of action by which tBA caused renal tubule tumors in male rats. One opinion expressed was an agreement with the assessment in that it is difficult to disentangle other potential mechanisms from alpha 2μ-globulin and CPN. A second opinion expressed centered on the fact that some of the manifestations of CPN could occur in the human kidney due to tBA exposure. A third opinion expressed that pathological findings in the kidneys of tBA-dosed male rats were consistent with alpha 2μ-globulin and CPN acting as co-MOAs to cause the renal tubule adenomas. EPA's guidance on renal tumors in male rats (U.S. EPA, 1991) states that unless the relative contribution of alpha 2μ-globulin nephropathy and other processes can be determined, dose-response analysis should not be performed. The SAB, therefore, notes that the cancer risk assessment of tBA must be based upon thyroid follicular tumors because renal tumor findings were less convincing.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The SAB recommends that EPA expand the scope and breadth of its discussion of potential modes and sites of action of tBA on the thyroid.

Tier 3:

- The SAB suggests that EPA consider organizing a scientific forum in the future to address MOAs and relevance of chemically-induced rodent thyroid tumors to humans.

3.4.3. Cancer toxicity values.

3.4.3.1. ETBE

Section 3 of EPA's cancer guidelines (2005) states:

> *"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."*

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

The SAB has observed that there does not appear to be a rationale for performing a quantitative analysis for ETBE liver cancer in Sections 2.3 or 2.4. These Sections simply refer to the Section 1.3.2, which

describes the basis for the selection of the "Suggestive Evidence" descriptor, and cite the EPA Guidelines for Carcinogen Risk Assessment (2005) to support the fact that the EPA guidelines provide the option of performing a quantitative analysis when this descriptor is selected. The EPA guidelines, however, indicate that when such a determination is made, a rationale for the quantitative analysis should explain how a quantitative analysis based on the available evidence "may be useful for some purposes," when "considering the uncertainty in the data and the suggestive nature of the weight of evidence." No such rationale is provided in the EPA's draft ETBE assessment for the decision to perform a quantitative analysis in the case of ETBE. A rationale based on potential worker and consumer exposures was suggested. The Agency could clarify the rationale within the draft assessment. The SAB concludes it is unlikely that performing a quantitative assessment of the data on ETBE liver carcinogenicity would be useful for "providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities" (*Section 3 of EPA's cancer guidelines; 2005).* In Section 1.3.2 (p. 1-112, lines 9-11), the EPA summarizes the limited evidence for ETBE carcinogenicity, stating: "The results for ETBE raise a concern for cancer, but the effects are 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 supporting evidence from initiation/promotion studies and genotoxicity studies that ETBE is carcinogenic. Any quantitative analysis based on this limited evidence would be uncertain and non-instructive.

In addition, the SAB expresses concern about the ability of dose-response modeling (in this case, benchmark dose modeling) to provide instructive information when there is a flat, unresponsive dose response at all doses except the high dose. Because EPA's policy (EPA, 2005) is to use only the multistage model for benchmark dose modeling of cancer dose-response, only a single estimate of the BMDL is produced. However, the rationale for this policy is uncertain and had other models been investigated, it would have been seen that many different models could adequately fit these data, and would likely yield widely divergent BMDL values. This is because these data provide minimal meaningful information for dose-response analysis. Given this, it is difficult to see how any, or all, possible models could provide realistic estimates of the true cancer risk, or even relative risk compared to other carcinogens whose cancer potency was derived from more robust data. Nevertheless, several members favored conducting a quantitative analysis to provide some sense of the magnitude of potential risks.

The SAB agrees that the Saito et al. (2013) study is well-conducted and well-reported, but the data for neoplastic liver lesions from inhalation exposure, by themselves, are not suitable for a quantitative analysis because tumors were only observed at the highest concentration. The SAB noted that the highest concentration is also where centrilobular hypertrophy, nuclear receptor activation, and induction of metabolism may have contributed to the outcome. With a statistically significant increase in tumors at the high dose only, the Saito et al. (2013) data are not sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for ETBE.

The following recommendations are noted:

Tier 1:

- EPA should refrain from conducting a quantitative analysis for ETBE carcinogenicity or explain the limitations of the analysis and clearly state the intended purpose is to simply provide some sense of the magnitude of potential risks.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- EPA should reconsider its policy (U.S. EPA, 2005) of limiting benchmark dose modeling of cancer dose response to the multistage model as this model is not a biologically-based model and does not provide a unique description of cancer dose-response.

3.4.3.2. tBA

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

The SAB has observed that there does not appear to be a rationale for performing a quantitative analysis for tBA thyroid cancer in Sections 2.3. This Section simply refers to the Section 1.3.2, which describes the basis for the selection of the "Suggestive Evidence" descriptor, and cites the EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) to support the fact that the EPA guidelines provide the option of performing a quantitative analysis when this descriptor is selected. The EPA guidelines, however, indicate that when such a determination is made, a rationale for the quantitative analysis should explain how a quantitative analysis based on the available evidence "may be useful for some purposes," when "considering the uncertainty in the data and the suggestive nature of the weight of evidence." No such rationale is provided in the draft tBA assessment for the decision to perform a quantitative analysis in the case of tBA. One rationale to consider potential worker and consumer exposures was suggested.

The SAB deems it unlikely that performing a quantitative assessment of the data on tBA thyroid carcinogenicity would be useful for "providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." In Section 1.3.2 (p. 1-112, lines 9-11), the EPA summarizes the limited evidence for tBA carcinogenicity, stating: "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." In addition, the SAB concludes that there is serious concern about the ability of dose-response modeling (in this case, benchmark dose modeling) to provide instructive information when there is a flat, unresponsive dose response at all doses except the high dose. Because

EPA's policy (U.S. EPA, 2005) is to use only the multistage model for benchmark dose modeling of cancer dose-response, only a single estimate of the BMDL is produced. However, had other models been investigated, it would have been seen that many different models could adequately fit these data, and would likely yield widely divergent BMDL values. This is because these data provide minimal meaningful information for dose-response analysis. Given this, it is difficult to see how any, or all possible models could provide realistic estimates of the true cancer risk, or even relative risk compared to other carcinogens whose cancer potency was derived from more robust data. Thus, any quantitative analysis based on this limited evidence would entail significant uncertainty and have the potential to be misleading. Nevertheless, several members favor conducting a quantitative analysis to provide some sense of the magnitude of potential risks.

The SAB agrees that the NTP (1995) study is well-conducted and well-reported, but the data for neoplastic thyroid lesions from drinking water exposure, by themselves, are not a suitable basis for a quantitative analysis because a tumor response was only observed at the highest concentration. With a statistically significant increase in tumors at the high dose only, and evidence from other studies supporting a potentially nonlinear mode of action, the NTP (1995) data are not sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for tBA.

The following recommendations are noted:

Tier 1:

- EPA should refrain from conducting a quantitative analysis for tBA carcinogenicity or explain the limitations of the analysis and clearly state the intended purpose is to simply provide some sense of the magnitude of potential risks.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- EPA should reconsider its policy of limiting benchmark dose modeling of cancer dose response to the multistage model as this model is not a biologically based model and does not provide a unique description of cancer dose-response.

3.4.4. Oral slope factor for cancer.

3.4.4.1. ETBE

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

The Saito et al. (2013) study used three concentrations of ETBE and observed significant increases in liver tumors in male but not female rats at the highest inhaled concentration only. The oral cancer slope factor was obtained by converting the inhalation point of departure to an oral dose using the rate of ETBE metabolism in the liver, which was derived from a PBPK model.

The SAB is concerned that the Saito et al. (2013) ETBE inhalation study is not suitable for developing an oral cancer slope factor, due to the issues associated with developing an oral cancer slope factor using the Saito et al. (2013) study described below:

• Unlike the Saito et al. (2013) ETBE inhalation study, well-conducted contemporary ingestion cancer studies up to the limits of ETBE solubility did not demonstrate cancer. The route differences could be due to pharmacokinetic or toxicodynamic processes, but either would require quantification to demonstrate cross-route consistency of the tumor observations.
• As EPA's analyses indicated, combining oral and inhalation studies did not result in a consistent dose response relationship using the dose metric of average daily rate of ETBE metabolism at periodicity. This argues against route extrapolation using this dose metric; no other dose metric was identified that provided consistent results between oral and inhalation exposures.

The SAB has no alternative approach suggestion for developing an oral cancer slope factor, and noted that the oral slope factor derivation was well described. The SAB also agrees that the modeling was performed in accord with standard EPA principles.

The following recommendations are noted:

Tier 1:

- Since the Saito et al. (2013) ETBE inhalation study is not suitable for developing an oral cancer slope factor, EPA should not derive an oral slope factor by route extrapolation absent pharmacokinetic/pharmacodynamic modeling that demonstrates consistency between the oral and inhalation study results.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.4.4.2. tBA

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 NTP (1995) tBA drinking water study used three doses of tBA and observed significant increases in thyroid follicular cell tumors in female mice at the high dose and non-significant increases at the low dose. The dose-metric for the dose-response analysis used to develop the oral cancer slope factor was exposed dose.

The SAB was not able to reach consensus on the suitability of the NTP (1995) tBA drinking water study for developing an oral cancer slope factor. Some members were concerned about the potential lack of biological relevance due to the magnitude of the high dose and the possibility of nonlinear metabolism kinetics at that dose. However, some members conclude the EPA's choice for oral slope factor for tBA was scientifically supported. Reasons supporting this position include:

- The lack of supporting data for a mouse anti-thyroid MOA, indicating that there is no reason to conclude that the female mouse thyroid follicular cell tumor data are not relevant to human cancer risk assessment.
- The tBA dose producing female mouse thyroid follicular cell tumors in the 1995 NTP study did not cause excessive treatment-related mortality or otherwise exceed the Maximum Tolerated Dose (MTD) in females although increased mortality is present in males at this dose. This indicates the tBA high dose was not excessive.
- The issue of high dose non-linear metabolism kinetics was speculative, as there is no mouse tBA TK model available.

The SAB has no recommendations to alternative approaches for developing an oral cancer slope factor and there are no comments to indicate that the oral slope factor derivation is poorly described or was not performed in accord with standard EPA principles.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier. However, the SAB suggests that the EPA revisit their policy to use the Multi Stage Cancer model as the preferred cancer dose-response model. The SAB noted that many different models could fit these data with equally good statistics of fit, but with widely different dose-response functions in the dose range of interest. Therefore, EPA should consider a wider choice of models when performing cancer dose-response analyses.

3.4.5. Inhalation unit risk for cancer.

3.4.5.1. ETBE

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

The Saito et al. (2013) study used three concentrations of ETBE and observed significant increases in liver tumors in male but not female rats at the high dose only. The dose-metric for the dose-response analysis used to develop the oral cancer slope factor was exposure concentration, with continuous exposure and Human Equivalent Concentration adjustments applied.

The SAB has not reached consensus on this question. Some members find that the Saito et al. (2013) ETBE inhalation study is not suitable for developing a cancer inhalation unit risk (IUR), given a potential lack of biological relevance. These members concluded that the ETBE concentration which induced liver tumors to be excessively high and only one concentration significantly increased tumor

incidence in male rats, leading to questions among these members regarding whether modeling a single positive concentration would produce a meaningful IUR.

Other members expressed that the Saito et al. (2013) study is appropriate for dose-response analysis, and indicated that there is scientific support for the EPA's ETBE IUR derivation. Supporting reasons for this position included the quality of the Saito et al. (2013) study, which is well designed, conducted and reported, and also the liver metabolism of ETBE to acetaldehyde, a genotoxic carcinogen. These members raised caveats regarding the biological relevance but did not alter their position that EPA should develop an IUR for ETBE. These members also noted that EPA provides guidance for deriving IURs in such cases and that this guidance is followed in the ETBE IUR derivation.
The SAB has no recommendations for alternative approaches to developing an IUR, and has no comments indicating that the IUR derivation was done incorrectly or was poorly described. Some members however did provide comments to indicate the modeling was performed correctly.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.4.5.2. tBA

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 alpha 2μ-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.

EPA decided to not develop an inhalation unit risk for tBA from the mouse thyroid tumor data because of the lack of a mouse toxicokinetic model, and from the male rat kidney tumor data because of the inability to determine the relative contribution of alpha 2μ -globulin nephropathy to tumor formation. The SAB concurs with EPA's decision.

Some members raised additional concerns about the study, including the lack of biological relevance due to the magnitude of the high dose, and the possibility of nonlinear metabolism kinetics at that dose. Other members believed that 1) the high dose of tBA in the NTP (1995) drinking water study did not exceed the MTD, and thus there is no reason to conclude that the dose was excessive, and 2) the issue of high dose non-linear metabolism kinetics was speculative, as there is no mouse tBA TK model available.

The following recommendations are noted:

Tier 1:

- The SAB has no specific recommendations for this tier.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

3.5. Question on Susceptible Populations and Lifestages

3.5.1. ETBE

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.

The SAB agrees with the position that EPA has taken regarding the "plausible evidence" for a vulnerable subgroup. Specifically, EPA specifies that individuals with an inactive form of ALDH2*2 may be a susceptible population. EPA suggests that individuals with inactive ALDH2*2 variants may be a potentially important subpopulation that may have susceptibility to health effects from ETBE exposure. This statement is based on literature documenting that metabolism of ETBE yields acetaldehyde, a documented genotoxic compound that in this subgroup could lead to prolonged exposure due to slow metabolism of this compound. The EPA has importantly noted that an anticipated 50% of individuals of Chinese, Japanese, and Korean descent are carriers of this variant. However, the EPA does not note the possibility of other potentially vulnerable population subgroups, such as individuals that have non-coding region variants in ALDH2. Individuals with these non-coding region variants are noted in the literature (Edenberg, 2007). Specifically, these non-coding variants could possibly affect gene expression, with implications on metabolism of acetaldehyde. As such, other vulnerable subgroups may exist, including individuals of European and African descent (Edenberg, 2007). The SAB includes Edenberg (2007) as a reference to articles by Dickson et al. (2006) and others noting not only ALDH2 non-coding region variants, but also other variants in alcohol metabolism that may be relevant to ETBE exposure in humans and could be important for identification of susceptible populations. The SAB recommends the incorporation of this issue into the ETBE assessment to improve the scientific concepts of the assessment. In addition, the EPA states that there is inconclusive evidence for CYP2A6 variants. The SAB finds this conclusion appropriate with revision as recommended below.

The draft ETBE assessment states "all routes of exposure" (which is completely plausible); yet the only route of exposure that is mentioned or that appears to be cited (or evaluated in the literature) is oral exposure as it relates to potentially susceptible populations. Since inhalation is likely an important route of exposure, as might be other routes, it is important to note that the literature is limited in this area and that oral exposure is likely to yield different responses than an inhalation (or other types of) exposure route(s). The lack of information on this point raises uncertainties about the degree of susceptibility from other routes of exposure.

The ETBE assessment does not mention vulnerable life stages. This suggests that there may not be any vulnerable life stage with respect to ETBE exposure. Yet, data exist showing that pregnancy may be a sensitive time period for alcohol metabolism due to the presence of estrogen, which inhibits alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities. In addition to pregnant women,

fetuses may also be a vulnerable subpopulation, due to their limited ability to metabolize alcohol, despite the presence of ADH. Specifically, the amount of ALDH2 expressed in the fetus is about half of that expressed in adults. Information regarding life stages should be included in the assessment.

The following recommendations are noted:

Tier 1:

- The SAB recommends that the Agency clearly describe the uncertainty between oral exposure and other routes of exposure in the ETBE assessment and provide relevant positions with respect to differences in expected outcomes (i.e., gastrointestinal vs. nasal endpoints).

- The SAB recommends the identification and incorporation of information on susceptible populations into the ETBE assessment to improve the scientific concepts of the assessment.

- The SAB recommends that information regarding life stages should be included in the assessment.

Tier 2:

- The SAB encourages the Agency to provide more supportive evidence from human studies on acetaldehyde metabolism throughout the report.

- The SAB encourages the Agency to consider mentioning differences relevant to sex and possible vulnerabilities that may be related to sex differences in exposures and outcomes.

Tier 3:

- The ETBE assessment should note that evidence pertaining to these inactive ALDH2*2 variants will be incorporated into future considerations.

3.5.2. tBA

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.

The draft tBA assessment states that there is no identified susceptible population. The SAB finds that this statement may be partly attributed to the implication, from in vitro studies, suggesting that CYP450 system may be involved in the metabolism of tBA; it is unknown which specific CYPs are involved in the biotransformation. To this end, it is unclear as a metabolite of ETBE why other populations mentioned in the draft ETBE assessment are not considered. Most of the studies mentioned focused on non-human studies. However, there are human data to support altered metabolism with respect to xenobiotic metabolism during pregnancy for the maternal-fetal unit.

It is unclear why vulnerable lifestages are highlighted here and not in the draft ETBE assessment. That said, the SAB agrees with the EPA that regarding tBA the evidence is minimal for identifying vulnerable populations and life stages.

Finally, the SAB disagrees with certain findings presented by the Agency in Table 1-12 of the EPA's draft tBA assessment. The actual body weight for the treated group was not double that of the control group, as implied by Table 1-12. Rather, treated dams gained twice as much weight as the dams in the control group during a specific interval. This difference in body weight gain is reasonable given other characteristics noted within the EPA's report and the EPA's final draft tBA assessment does not need to include additional reasoning on this topic.

The following recommendations are noted:

Tier 1:

- The EPA should correct the actual body weight for the treated group in Table 1-12 of the EPA's draft tBA assessment.

Tier 2:

- The SAB recommends adding some of this supporting evidence from ETBE to the argument proposed, given the limited data for tBA and the fact that it is a metabolite of ETBE.

Tier 3:

- The tBA assessment should note that evidence pertaining to inactive ALDH2*2 will be incorporated into future considerations.

3.6. Question on the Executive Summary

3.6.1. ETBE

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.

Generally, the Executive Summary is clear and presents the major conclusions of the draft assessment. As the Agency makes revisions to the body of the draft, the Executive Summary will need to be changed accordingly. Comments here are therefore on overarching aspects of the draft Executive Summary and the depth of details as they are presented.

The draft Executive Summary offers statements about the questions considered and summarizes the findings that are in the end chosen.

The "Key Issues" section of the Executive Summary should highlight findings as to the interpretation and relevance of key toxicity endpoints. EPA should succinctly summarize the nature of interpretation questions, the basis for the proffered resolutions/decisions, and the consequent influence on the outcome of the toxicity assessment.

The following recommendations are noted:

Tier 1:

- The SAB advises that it will be important for the final Executive Summary to highlight the consequences of alternative choices for the final assessment, especially when these hinge on decisions made about the interpretation and relevance of key toxicity endpoints that have been contested (based on the history of public comment on the draft assessment).

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- Current understanding in neurobehavioral effects is insufficient. The needs for additional investigation in this area and for other data gaps should be noted.

3.6.2. tBA

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.

Generally, the Executive Summary is clear and presents the major conclusions of the draft assessment. As the Agency makes revisions to the body of the draft, the Executive Summary will need to be changed accordingly. Comments here are therefore on overarching aspects of the draft Executive Summary and the depth of details as they are presented.

The draft Executive Summary offers statements about the questions considered and summarizes the findings that are in the end chosen.

The "Key Issues" section of the Executive Summary should highlight findings as to the interpretation and relevance of key toxicity endpoints. EPA should succinctly summarize the nature of interpretation questions, the basis for the proffered resolutions/decisions, and the consequent influence on the outcome of the toxicity assessment.

The following recommendations are noted:

Tier 1:

- The SAB advises that it will be important for the final Executive Summary to highlight the consequences of alternative choices for the final assessment, especially when these hinge on decisions made about the interpretation and relevance of key toxicity endpoints that have been contested (based on the history of public comment on the draft assessment).
- Page xiii, Line 14: Reference HSDB (2007) is cited for tBA 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. U.S. EPA-560/13-80-029 (1980). These two articles do not provide evidence for the presence of tBA in milk, although the presence of 1-butanol was demonstrated. This statement needs clarification.

Tier 2:

- The SAB has no specific recommendations for this tier.

Tier 3:

- The SAB has no specific recommendations for this tier.

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report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

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ADDITIONAL PEER-REVIEWED STUDIES

The SAB recommends the following peer-reviewed studies for considered in the assessment of noncancer and cancer health effects of ETBE and tBA:

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APPENDIX A: EPA'S CHARGE QUESTIONS

Revised 7/26/17 by the CAAC-ETBE/tBA committee per discussion during the July 11, 2017, teleconference. Changes are presented as underlined text.

Charge to the Science Advisory Board for the IRIS Toxicological Review of Ethyl *tert*-Butyl Ether (ETBE) June 2017

Introduction

EPA thanks the expert scientists on the augmented SAB Chemical Assessment Advisory Committee for reviewing this draft assessment.

This draft assessment reviews publicly available studies on ETBE to identify adverse health outcomes and to characterize exposure–response relationships. Peer review is essential to the quality and integrity of IRIS assessments, which provide scientific information that supports EPA's actions to protect public health. The draft assessment was reviewed by scientists across EPA and other federal agencies. EPA also solicited public comment and convened a public science meeting to discuss major science issues. Experts identified by the National Academy of Sciences participated in the public discussions. Responses to major public comments appear as supplemental material to the draft assessment.

EPA is seeking SAB advice on the clarity and scientific underpinnings of the overall assessment. The peer review should consider whether the conclusions presented in the draft assessment are clearly presented and scientifically supported. Below, a set of charge questions for each major analysis are presented. The SAB is expected to consider questions and issues raised during public comment as part of its deliberations. The advice will be most useful when prioritized to indicate its relative importance during revision:

Tier 1: Recommended Revisions – Key recommendations that are necessary in order to improve the critical scientific concepts, issues and/or narrative within the assessment.
Tier 2: Suggestions – Recommendations that are encouraged for EPA to adopt in order to strengthen the scientific concepts, issues and/or narrative within the assessment, but other factors (e.g., Agency need) should be considered by EPA before undertaking these revisions.
Tier 3: Future Considerations – Useful and informative scientific exploration that may inform future evaluations of key science issues and/or the development of future assessments. These recommendations are likely outside the immediate scope and/or needs of the current assessment under review.

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?

Hazard Identification and Dose-Response Analysis
Chapter 1 (Hazard Identification) and the supplemental materials summarize the chemical properties, toxicokinetics, and health effects associated exposure to ETBE. Chapter 2 (Dose Response Analysis)

uses this information to derive an oral reference dose and inhalation reference concentration for noncancer outcomes, in addition to an oral slope factor and inhalation unit risk for cancer.

2. Chemical Properties and Toxicokinetics

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

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?

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

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 alpha 2μ - 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.

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

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.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 9×10^{0} mg/m^3, based on urothelial hyperplasia in male rats (Saito et al., 2013). Please comment on whether this value is scientifically supported and its

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

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.

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

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states:

"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data usually would not support one. However, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

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

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 1×10^{-3} per mg/kg–day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is

scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

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.

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.

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.

Revised 7/26/17 by the CAAC-ETBE/tBA committee per discussion during the July 11, 2017, teleconference. Changes are presented as underlined text.

Charge to the Science Advisory Board for the IRIS Toxicological Review of *tert*-Butanol
June 2017

Introduction

EPA thanks the expert scientists on the augmented SAB Chemical Assessment Advisory Committee for reviewing this draft assessment.

This draft assessment reviews publicly available studies on *tert*-butanol to identify adverse health outcomes and to characterize exposure–response relationships. Peer review is essential to the quality and integrity of IRIS assessments, which provide scientific information that supports EPA's actions to protect public health. The draft assessment was reviewed by scientists across EPA and other federal agencies. EPA also solicited public comment and convened a public science meeting to discuss major science issues. Experts identified by the National Academy of Sciences participated in the public discussions. Responses to major public comments appear as supplemental material to the draft assessment.

EPA is seeking SAB advice on the clarity and scientific underpinnings of the overall assessment. The peer review should consider whether the conclusions presented in the draft assessment are clearly presented and scientifically supported. Below, a set of charge questions for each major analysis are presented. The SAB is expected to consider questions and issues raised during public comment as part of its deliberations. The advice will be most useful when prioritized to indicate its relative importance during revision:

Tier 1: Recommended Revisions – Key recommendations that are necessary in order to improve the critical scientific concepts, issues and/or narrative within the assessment.

Tier 2: Suggestions – Recommendations that are encouraged for EPA to adopt in order to strengthen the scientific concepts, issues and/or narrative within the assessment, but other factors (e.g., Agency need) should be considered by EPA before undertaking these revisions.

Tier 3: Future Considerations – Useful and informative scientific exploration that may inform future evaluations of key science issues and/or the development of future assessments. These recommendations are likely outside the immediate scope and/or needs of the current assessment under review.

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?

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

2. Chemical Properties and Toxicokinetics

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

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?

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

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 alpha 2µ-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.

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.

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.

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

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 alpha 2µ-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.

(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.

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.

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states:

"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data generally would not support one, however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence."

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

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

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 alpha 2μ -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.

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.

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.

APPENDIX B: EDITIORIAL CORRECTIONS

ETBE:

1. On page 1-5, lines 35-36: It is stated that single mouse inhalation study showed “weak increases in kidney weight.” I think the text should be more specific as the descriptor “weak” is vague and not really informative.

2. Page 1-35, lines 20-21: Delete "renal" from the phrase "renal nephrotoxicity" as it is redundant.

3. Comments about presentation in draft ETBE assessment and Charges:

4. Delete the word "purposes" in the first sentence of Charge Question 3b.

5. Page 1-37, line 12. This sentence is unclear. Does “from adult exposure” mean that the fetuses were exposed from the parental oral exposure, or that the effects on the F1 liver weights were similar to those of exposed adults? It needs clarification.

6. Throughout male reproductive text; ALDH2+/- mice are referred to as "heterogeneous"; the correct term is "heterozygous".

7. Table 1-14; Page 1-60: The dose for inhalation studies is mistakenly given here as "mg/kg-d"; it should be "mg/m3".

8. Table 1-14; Figures 1-11, 1-12 (page 1-65 to 1-75): Absolute testis weight is a much better and more precise measure of toxicity. Testis weight does not vary with body weight changes. Wherever available, only absolute testis weights should be presented to avoid unnecessary confusion.

9. Table 1-14: This table says that the testis weight data were not shown in Bond et al. 1996b. The data on rat testis weights are found in Table 23; page 76.

10. Page 1-80, lines 32-33: The statement that the findings in the F1 adults were similar to the P (F0) adults should be modified to include the observation that in the F1, pituitary weights of males were significantly increased at the 1,000 mg/kg-d dose (Gaoua, 2004b).

11. Page 1-90, Line 23: Delete the words "or biological". Missing right atrioventricular valve is of biological significance.

12. Table 1-16, Page 1-95: Although there were no significant differences in postnatal day 21 weights in the F0 or F1 pups, Tables 25 and 26 of Gaoua (2004b) show significant increases in terminal body weights of these pups. That should be noted as a footnote in Table 1-16 of the draft ETBE assessment.

13. Throughout the male reproductive effects text; ALDH2+/- mice are referred to as "heterogeneous"; the correct term is "heterozygous".

14. Table 1-14; Page 1-60: The dose for inhalation studies is mistakenly given here as "mg/kg-d", it should be "mg/m3".

15. 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 change.

16. 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.

17. Recommended modification, P. 2-15, Line 23-24, "... the ETBE inhalation 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 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.

18. There is no reporting of units for the responses (as opposed to the exposures) in the Supplemental Information tables, and this also leads to difficulty in interpretation. Units should be added where appropriate.

19. Correct the following statements:
    - p. 2-6, lines 9-10, "...does not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD"
    - p. 2-10 line 3, "For ETBE, only kidney effects were identified as a hazard".

20. Electronic link to US EPA (2002) on p. R-10 does not lead to the correct draft ETBE assessment.

<u>tBA</u>

1. Tert-Butyl Alcohol is repeated twice in the list of passwords for the Toxline database in Table LS-1.

2. The description of the dose metrics calculated with the model needs to clear and correct throughout the draft tBA assessment. On page B-24, "daily amount of ETBE metabolized in liver", presumably means "daily average rate of ETBE metabolized in liver" and similar changes in language are needed for tBA. Figure B-3 the y-axes are indicated as "ETBE metabolized (mg/hr)" and "tert-butanol metabolized (mg/hr)"; either y-axis legends or figure legend text needs to clarify that this is "daily average rate metabolized". The SAB recommends that the assessment should always use the complete description wherever the dose metric is defined, e.g., ETBE: "average rate of metabolism of ETBE in the liver after periodicity is achieved" tBA: "the average concentration of tBA in the blood at steady state (for continuous inhalation) or after periodicity is achieved (for oral exposure)". Other modifications to text in response to comments above would be desirable.

3. Descriptions of dose metrics in text and figures (e.g., Appendix B) should be corrected to reflect the fact that the dose metric is average concentration of tBA in the blood after periodicity is achieved. The SAB recommends that the assessment should always use the complete description wherever the dose metric is defined, e.g., ETBE: "average rate of metabolism of ETBE in the liver after periodicity is achieved" tBA: "the average concentration of tBA in the blood at steady state (for continuous inhalation) or after periodicity is achieved (for oral exposure)".

4. On page 2-16, lines 10-11: The draft tBA assessment describes, in this paragraph, the derivation of an RfC value based on increased kidney weights and other kidney effects from a chronic oral exposure study in female rats, and states that the effects occurred "spanning a range from 44 × 100 to 3 × 101 mg/m3, for an overall 7-fold range." This is not a 7-fold range, but is only about 1.5-fold.

5. Table 1-14, Figure 1-13: The EPA should state that in the NTP (1995) publication there appeared to be a significant loss of testis weight after 13 weeks' exposure mice to tBA at 8210 mg/kg-d from 0.115 mg to 0.096 mg (Table F3), but the draft tBA assessment was self-contradictory as in

Table H2 they indicate that this tBA exposure only decreased testis weights, non-significantly, from 0.115 to 0.101 mg.

6. Table 1-14, Figure 1-13: The draft tBA assessment is incorrect when it indicated that the NTP (1995) publication showed no effect of oral doses of tBA at 1,560 or 3,620 mg/kg-d on female estrous cycle in rats (Page 1-59, line 16). While it is true that there was not any change in estrous cycle length in rats with measurable estrous cycles, 2/10 and 4/4 surviving rats had cycles that were longer than 7 days, were unclear, or showed no evidence of cyclicity (Table H1, NTP, 1995). This loss of clear cycles is a much more serious effect than a slight change in cycle length in rats that showed somewhat normal cycles. It seems clear that there are significant effects of high doses of tBA resulting in loss of cyclicity, and this endpoint should be analyzed in the draft tBA assessment. The statistical significance of the slight increase in lack of cyclicity at 1,560 should be analyzed. Since the 1,560 mg/kg-d dose did not cause any lethality or effects on body weight in the female rats, effects at this dose cannot be attributed to general toxicity.

7. Table 1-14, Figure 1-13, Page 1-59, lines 14-17: Data and results of percentages of mice showing no measurable estrous cyclicity should be added since this is an endpoint that has more serious consequences than the increase in estrous cycle length. There is a definite effect on this endpoint at an oral dose of 8,210 mg/kg-d at which 4/6 mice failed to show clear evidence of estrous cyclicity (Table H2, NTP, 1995). This addition does not change the conclusion since lethality and effects on body weight were observed indicating that the loss of estrous cyclicity could have been an indirect effect. In addition, the data on the incidence of mice showing no measurable cyclicity after inhalation administration needs to be analyzed. In the control group 0/10 shown no clear cyclicity, but in the various treatment groups 2/10, 1/10, and 3/10 showed loss of measurable estrous cyclicity. Analysis of whether or not this is a significant effect needs to be performed and if the effect is significant, changes in the draft tBA assessment need to be made.

8. Table 1-12 on page 1-50: The column of body weight gain PND1-21 as a percentage of the gain in the control for the Huntingdon 2004 study should be deleted. It cannot be readily interpreted and particularly the number of 100% gain during PND 1-21 in 1000 mg/kg-d females compared to control has led to confusion in the SAB and was misinterpreted as suggesting a basic metabolic alteration. This is not a surprising finding when the actual weights are examined. During gestation (PND0-GD0) the treated dams only gained 27g compared to 50g in the control. Lower weight gain is a typical effect of a toxicant. During lactation, the control dams gained 31g but the treated dams gained 62g. That is readily explained by two factors: the treated dams were only nursing 10.2 pups per litter while the control dams were nursing 15.2 pups and the treated dams were making up for their reduced weight gain during gestation.

9. EPA should review p. 2-16, Line 10. “44” is likely a mistake, should it be “4”?