NOTICE: **Do Not Cite or Quote.** Revised comments submitted by individual members are for review and to assist meeting deliberations by the CAAC-ETBE/tBA committee.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

Revised individual comments on ETBE by Karen Chou 9/19/17

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

Yes, the strategies are clearly described.

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

Hazard Identification and Dose-Response Analysis.
2. Chemical Properties and Toxicokinetics
2a. Chemical properties. Is the information on chemical properties accurate?

Tier 1: Please provide temperature for water solubility.

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

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

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

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

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

The conclusion and supporting rationales are described. Some modifications can be made to increase clarity. Following are a few suggested modifications:

Tier 2, suggested consideration: P. 1-21, Line 5-6: please provide reference(s) for this statement, "Acetaldehyde is metabolized further in the liver and is not thought to play a role in extrahepatic toxicity". Is there a reference to support this statement? Acetaldehyde is present in the circulation after exposure to ETBE.

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

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

Tier 2: Suggested addition, p. 1-35, Line 19-20: ... be entirely explained by the **male rat-specific** alpha2u-globulin or **rat-specific** CPN processes.

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

3b. Noncancer toxicity at other sites. The draft assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with ETBE exposure, please identify them and outline the rationale for including them in the assessment.
-Liver effects: Suggestive evidence
-Developmental toxicity: Inadequate evidence
-Male and female reproductive toxicity: Inadequate evidence

Tier 2, suggested clarification, P. 1-37, Line 12: ... from "parental and" adult "oral" exposure... . Please clarify that the F-1 were exposed to ETBE and metabolites through F-0 parental exposure during premating, mating, gestation and lactation.

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

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

Tier 1, recommended modification: p. 1-76, Line 11-14: Please provide the following clarification in the discussion. Animal's age is a major difference between the studies by JPEC (2010b) and Medinsky et al. (1999). The reproductive organs in JPEC's study were examined in animals older than 2 yr, while the animals in the study by Medinsky et al. (1999) were much younger. These two studies are not comparable in terms of the stages of reproduction organs.

Tier 1: Please include testicular effects, such as mineralization and arteritis in 500 ppm and 1500 ppm treatment groups in the discussion. There are additional observed effects in Table L 1-6 of the report by JPEC (2010b), but omitted in the publication by Saito et al. (2013).

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

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

Tier 1, suggestion, relevant endpoint for quantitative assessment: Number of fetuses is a relevant endpoint for quantitative analysis, which decrease is observed in two difference species in two different studies.

Tier 1, recommended addition: Parental male pituitary weight, both absolute and relative weights, increased (14%) in 1000 mg/kg treatment group. See section 3.6.2.1.1 on P. 71 of the report by Gaoua, (2004b).

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

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

Tier 1: P. 2-6, Line 9-10, "... the ETBE oral database adequately covers ...reproductive and developmental effects": The statement is made without supporting evidence. Please conduct dose response analyses (reference doses) for reproductive and developmental effects to support this statement.

Tier 1, recommended modification, P. 2-6, Line 9-10, "... the ETBE oral database...does not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD": Please modify or delete this statement. This statement deviates from the logic of scientific investigation: additional studies will always carry the possibility of identifying a more sensitive target tissue or a more sensitive species.

Tier 1: recommended deletion, P. 2-10, Line 3, "only kidney effects were identified as hazard": This is statement is not true; many other effects are observed.

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

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 (p. 2-11 to p. 2-20) presents an inhalation reference concentration of 9×10^{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.

Tier 1: When reproductive and developmental endpoints are relevant to human health and when the data are sufficient for reference dose derivation, reference doses should be developed to support the statement on P.15, Line 21- 23, "The ETBE inhalation database... adequately covers all major systemic effects, including reproductive, developmental, ...effects."

Tier 1: recommended modification, P. 2-15, Line 23-24, "... the ETBE inhalation database...dose not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD": Please modify or delete this statement. This deviates from the logic of scientific investigation: additional studies will always have the possibility of identifying a more sensitive target tissue or a more sensitive species.

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

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

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

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

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

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

Tier 2, Suggested addition, P. 1-112, Line 26: An increase in total oncologically relevant pathologies of the forestomach was observed in male Sprague-Dawley rats of the 250 mg/kg treatment group (Maltoni et al., 1999).

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

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

Tier 2, the reviewer supports the decision of conducting a quantitative analysis. US EPA's Guidelines for Carcinogen Risk Assessment does not prevent or prohibit the derivation of

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

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

The value is scientifically supported and its derivation clearly described.

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

The value is scientifically supported and its derivation clearly described.

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

Suggested additions:

Tier 1: Placental transfer of acetaldehyde has been demonstrated in both humans and animals (Blakley and Scott 1984; Karl et al. 1988). In addition, fetuses have less capacity to detoxify acetaldehyde. In humans, regardless of the genotype, the amount of ALDH2 expressed at fetal stage is about half of the adult level (Yoshida et al. 1990). When both the pregnant woman and the fetus carry the homozygous ALDH2 of the variant genotype, the fetus would belong to the highest risk group among all genetically identified susceptible populations.

Tier 1: Additional susceptible population include chronic alcohol users, and individuals who are also exposed to MTBE and tBA.

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

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

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

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

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

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

Tier 2, suggested addition, P. xxii, Line 11-13: Please add descriptions of the findings from existing reproductive and developmental studies to the Executive Summary. These may include degenerative spermatocytes, sperm cell DNA breaks, and decrease in sperm numbers from inhalation exposure, and increases in plasma estradiol concentration in male rats, decreases in number of fetuses, missing missing right atrioventricular valve in the offspring in two studies with two species (rabbits and rats) from oral exposure.

Tier 2, P. XXV, Line 18-19: Susceptible population include individuals carrying ALDH2 and ALDH1b1 variants with low catalytic activity toward acetaldehyde, fetuses, alcohol users, and individuals who are also exposed to MTBE and tBA. Please see comments for Question 5.

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References:

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- de Peyster A, Stanard B, Westover C. 2009. Effect of ETBE on reproductive steroids in male rats and rat Leydig cell cultures. Toxicol Lett 190(1): 74-80.
- Glassock RJ, Denic A, Rule AD. 2017. The conundrums of chronic kidney disease and aging. Journal of Nephrology 30(4): 477-483.
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- Karl P, Gordon B, Lieber C, Fisher S. 1988. Acetaldehyde production and transfer by the perfused human placental cotyledon. Science 242(4876): 273-275.
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