

NOTICE: 8/17/2017: CAAC Augmented for ETBE/tBA Summary to Assist Meeting Deliberations-- Do Not Cite or Quote -- This work is draft and does not reflect consensus advice or recommendations, has not been reviewed or approved by the chartered SAB and does not represent EPA policy.

# CAAC Augmented for ETBE/tBA Committee

## Report Out Summary

# Tier Ranking

- **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.
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# **Q1: Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods**

ETBE - Q1: Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section.

- **Overall, the panel considered that the search strategies, criteria for study inclusion/exclusion and evaluation and for study quality are described clearly. The panel also agreed that search strategy likely capture all the relevant key studies.**
- **The panel noted that the approach to the search did not adhere strictly to the NRC (2011) recommendations and EPA should provide a clarification (Tier 1).**
- **The panel also identified some inconsistencies in terms of dates for search updates across sources, as well as source limitations in the manual searches for additional relevant citations (Tier 2).**

ETBE - Q1: Were the strategies clearly described and objectively applied?

- **The panel agreed that the search strategies were described clearly.**
- **The panel members were not able to determine how studies were objectively judged for quality based on the classification and quality criteria.**
- **There is no documentation on decision making for each of the studies. EPA should provide documentation about this process, perhaps as part of the HERO database (Tier 1).**

tBA - Q1: Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section.

- **Overall, the panel considered that the search strategies, criteria for study inclusion/exclusion and evaluation and for study quality are described clearly. The panel also agreed that search strategies likely capture all the relevant key studies.**
- **The panel noted that the approach to the search did not adhere strictly to the NRC (2011) recommendations and EPA should provide a clarification (Tier 1).**
- **The panel also identified source limitations in the manual searches for additional relevant citations (Tier 2).**

tBA - Q1: Were the strategies clearly described and objectively applied?

- **The panel agreed that the search strategies were described clearly.**
- **The panel members were not able to determine how studies were objectively judged for quality based on the classification and quality criteria.**
- **There is no documentation on decision making for each of the studies. EPA should provide documentation about this process, perhaps as part of the HERO database (Tier 1).**

## **Q2: Chemical Properties and Toxicokinetics**

**2a. Chemical properties**

**2b. Toxicokinetic modeling**

**2c. Choice of dose metric**

## ETBE– Q2a: Chemical properties. Is the information on chemical properties accurate?

- **There were several inconsistencies between the chemical properties data provided in Table 1-1 and values actually reported in the cited source or found in other sources.**
- **Tier 2 – Suggestions for improvement**
  - **Suggest EPA have a template and focus on listing only properties relevant to the compound and the specific assessment.**
  - **Multiple values for a given parameter (ie, water solubility) could be found in the literature.**
    - **References used should be QC checked to ensure the accurate citation is presented and that the data in tables match those in the reference.**
    - **Suggest using primary data sources, not reviews or other government documents.**
    - **If more than one value is found in primary peer reviewed sources, suggest EPA provide a rationale for the choice of the one presented.**
    - **If secondary sources or reviews are used , suggest EPA cross check values with original sources**
    - **If results from published studies are used, the quality of the experiment may be reviewed to see if the design/data are sound.**
    - **If values presented are estimates, that should be identified.**
    - **Units and conditions under which the data were generated should be identified (solubility in water at what temperature, etc).**

### **Tier 1 – correct inconsistencies**

- **Examples of inconsistencies in Table 1-1.**
  - **Water solubility**
  - **Log octanol:water partition coefficient**
  - **Odor recognition in water**

ETBE – Q2b: 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?

- EPA’s application of the PBPK model for ETBE, including the TBAsubmodel, in dose-response characterization of ETBE is an appropriate way to incorporate science using state-of-the-art methods that is strongly supported by the committee.
- Tier 1 Recommended Revisions: It is misleading to say “A more detailed summary of the toxicokinetic models is provided in Appendix B.1.5 (US EPA 2017)” on page 1.3 lines 11-12. The text should indicate that some additional information is available in the Supplement, as well as in the cited EPA draft report (US EPA 2017) and the original literature references. Alternatively, the text of the draft report could be included in the Supplement, in which case it would benefit from adding a conclusions section. 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. Other modifications to text in response to comments above would be desirable.
- Tier 1 Recommended Revisions: 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 1 Recommended Revisions: Revise model code to describe metabolism as a function of the free liver concentration, CVL, and re-estimate metabolic parameters (e.g., Km or first order rate constants).
- Tier 3 Future Considerations: For purposes of using PBPK models in IRIS assessments, EPA needs to establish a consistent practice for documentation of both the model itself and the review of the model (and any modifications made by EPA to implement it). It is not desirable for EPA to write long descriptions of the model it is using that would repeat much of what is in published literature, but on the other hand providing more summary information is desirable. In light of the limited role of the PBPK model in this analysis, the assessment should not be delayed while establishing such best practices.

ETBE – Q2c: Choice of dose metric. Is the rate of ETBE metabolism an appropriate choice for the dose metric?

- The dose metric, rate of metabolism of ETBE, should not be used in extrapolation from inhalation to oral routes of administration of ETBE. There was no “consistent dose-response relationship” for this dose metric dose (B-27), when combining oral and inhalation studies to assess liver tumors. No other dose metrics have been identified that would work better for route extrapolation of the liver cancer endpoint.
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- **Tier 1 Recommended Revisions:** It is recommended that route extrapolation not be implemented for the oral cancer dose-response analysis, so selection of a dose metric will not be necessary.

## tBA – Q2a: Chemical properties. Is the information on chemical properties accurate?

- **There were some inconsistencies between the chemical properties data provided in Table 1-1 and values actually reported in the cited source or found in other sources.**
- **Tier 2 - suggestions**
  - **Suggest EPA have a template and focus on listing only properties relevant to the compound and the specific assessment.**
  - **Multiple values for a given parameter (ie, water solubility) could be found in the literature.**
    - **References used should be QC checked to ensure the accurate citation is presented and that the data in tables match those in the reference.**
    - **Suggest using primary data sources, not reviews or other government documents.**
    - **If more than one value is found in primary peer reviewed sources, suggest EPA provide a rationale for the choice of the one presented.**
    - **If secondary sources or reviews are used, suggest EPA cross check values with original sources**
    - **If results from published studies are used, the quality of the experiment may be reviewed to see if the design/data are sound.**
    - **If values presented are estimates, that should be identified.**
    - **Units and conditions under which the data were generated should be identified (solubility in water at what temperature, etc).**
- **Tier 1 – items needing corrections**
  - **Examples of inconsistencies in Table 1-1.**
    - **Log octanol:water partition coefficient**
    - **Melting and boiling points**
    - **Density/specific gravity**
    - **Odor detection and recognition in air/water not presented as for ETBE**

tBA – Q2b: Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

- Tier 1 Recommended Revisions: Reword text in Section 1.1.3 of the Toxicological Profile. 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 committee suggests that the material in US EPA (2017) be included in Appendix B or as a separate appendix.
- Tier 1 Recommended Revisions: 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 1 Recommended Revisions: 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).

tBA – Q2c: Choice of dose metric. Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?

- **Tier I recommended revisions:** 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 non-cancer endpoint. Thus an oral to inhalation extrapolation is recommended.
- Inter-species extrapolation can be conducted based on human inhalation PBPK model for ETBE and TBA (Nihlen and Johanson 1999) thereby avoiding a default HED calculation.

## **Q3 – Noncancer**

- **3a. Noncancer kidney toxicity**
- **3b. Noncancer toxicity at other sites**
- **3c. Oral reference dose for noncancer outcomes**
- **3d. Inhalation reference concentration for noncancer outcomes**

ETBE - Q3a: The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of  $\alpha$ 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

- 1. The panel agreed that the conclusion was clearly described but disagreed about scientific support.**
- 2. The explanation that absolute kidney weight is a more reliable reflection of specific effects on the kidneys is clearly explained and scientifically supported.**
- 3. Consideration of alpha-2u effects and their role in explaining renal tumors in male rats is presented in a thorough and systematic manner according to established EPA Guidelines. The IRIS document concludes that ETBE does not fulfill all the established criteria for having an alpha-2u MOA and that the database is insufficient to conclude that ETBE is an inducer of alpha-2u-globulin nephropathy in male rats. It was further concluded that other MOAs operate that may be relevant to human hazard assessment.**

ETBE - Q3a: The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of  $\alpha_2$ -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.

**4. The EPA noted that CPN also encompasses individual lesions or processes that also occur in the human kidney. The fact they happen to occur as a group in the aged rat kidney does not assure 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).**

**5. The EPA concludes that exacerbation of increased absolute kidney weight, urothelial hyperplasia, and serum biomarkers by ETBE exposure are not due to a rat-specific CPN and are, therefore, relevant to human kidney hazard assessment.**

ETBE - Q3a: The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of  $\alpha$ 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

**6. Considerable discussion and disagreement occurred with respect to how the ETBE database for noncancer kidney effects are interpreted, with no clear consensus being reached.**

**a. Public comments: Disagreed with the conclusion that noncancer kidney effects of ETBE are relevant to humans. Concluded that all of the noncancer kidney effects of ETBE in male rats could be explained by the alpha-2u or CPN MOA and those in female rats by the CPN MOA. Based on this, concluded that noncancer kidney effects should not be considered a hazard for humans.**

ETBE - Q3a: The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of  $\alpha$ 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

## **6. Continued:**

**b. Assigned committee members: There was disagreement, with some members agreeing with the overall interpretation and conclusion of the EPA presented in the IRIS document and others who concluded that there was little evidence to support the human relevance of noncancer kidney effects in rats.**

**c. An additional committee member noted that the focus of the IRIS document should be on public health; considerations should be conservative and consider potential human relevance to protect human health. Thus, that committee member believed that the conclusions of the EPA were reasonable and appropriate.**

ETBE - Q3a: The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of  $\alpha$ 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

## **Tier 1 Recommendation:**

**1) There was considerable disagreement among committee members about whether noncancer kidney effects should be considered a hazard relevant to humans. Justification for the choice should be strengthened.**

ETBE - Q3a: The draft assessment identifies kidney effects as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of  $\alpha$ 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

## **Tier 2 Recommendations:**

- 1) Although consideration of the role of alpha-2u globulin in ETBE-induced nephropathy in male rats is thoroughly considered according to the 1991 criteria established by the EPA, we recommend application of the more detailed criteria published by IARC in 1999.**
- 2) 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.**
- 3) Consider urothelial hyperplasia as mechanistically distinct from tubular epithelial toxicants and as a separate indicator in developing the human hazard assessment.**

ETBE - Q3b. 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.

- General agreement to support use of kidney (urothelial hyperplasia observed at 170 mg/kg-d, 6,000 mg/m<sup>3</sup>) as opposed to following endpoints for deriving reference doses.
- Liver
- Relative liver weight in rat shows increases after high oral (1,000 mg/kg-d) but not inhalation doses
- Weight increases are non-specific; no consistent effects on liver enzyme markers
- Pathological lesions might be precancerous; unlikely to directly indicate liver dysfunction
- Further research on mechanisms of action, particularly involving nuclear signaling pathways is warranted to better understand effects (Tier 3)
- Agree that there is suggestive evidence for liver effects contributing to noncancer toxicity

ETBE - Q3b. 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.

- Developmental Toxicity

- Minor effects (skeletal variations, early postnatal deaths) only observed at high oral dose (1,000 mg/kg-d in some studies; some effect may be associated with maternal toxicity)
- Suggest describing recommendation as "minimal effects at otherwise toxic dose levels", rather than "inadequate evidence" (Tier 1)
- Research on non-mammalian systems (e.g. zebrafish) that have detected developmental targets of ETBE should be included (Tier 2)
- Developmental neurotoxicity needs to be evaluated for ETBE (Tier 3)

ETBE - Q3b. 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.

- Reproductive Toxicity (1)

- No male reproductive toxicity (testis weight, sperm number, morphology, and motility, histopathology, androgen dependent accessory organs, fertility) in rats even at high oral dose (1,000 mg/kg-d).
- Only testicular effect observed in rats at inhalation doses  $\geq 7,000$  mg/m<sup>3</sup> was some histological damage (tubule atrophy) but was not reproducible and must have been minor because testis weights were unaffected
- Testis weight, sperm production, sperm motility unaffected up to inhalation doses of  $\geq 21,000$  mg/m<sup>3</sup> in wild-type animals.
- Only damage observed in wild-type mice at inhalation doses  $\geq 7,000$  mg/m<sup>3</sup> at was DNA damage in sperm. Difficult to evaluate magnitude of that damage.

ETBE - Q3b. 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.

- Reproductive Toxicity (2)

- Effects on sperm production and motility were observed at inhalation doses as low as 2,000 mg/m<sup>3</sup> in *Aldh2* knockout and heterozygous mice.
- No female reproductive toxicities observed at maximal doses used
- Suggest describing recommendation as "minimal effects at otherwise toxic dose levels", rather than "inadequate evidence" (Tier 1)
- Recommendation: Probable male reproductive effects in genetically susceptible populations (Tier 1).
- Suggestion: Further studies in animal with *Aldh2* mutant alleles (Tier 3)

ETBE - 3c. Section 2.1 presents an oral reference dose of  $5 \times 10^{-1}$  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.

- **Responses are premised on overall acceptance of the support of kidney effects of ETBE as an appropriate endpoint (3a) for which there was not a consensus.**
- **When considered an appropriate endpoint, the derivation was considered to be appropriate and clearly described**
- **The difference in consensus was based on the extent of confidence in a CNP-based mechanism for these effects**
- **Tier 1: The question of the validity and applicability of the endpoints analyzed for the oral RfD needs to be carefully examined, including the potential for CNP as a mechanism of effect.**
- **Tier 1: Include outcomes of statistical analyses and their rationale in study selection choice**

ETBE - 3c. Section 2.1 presents an oral reference dose of  $5 \times 10^{-1}$  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: Include units in Tables**
- **Tier 2: If urothelial hyperplasia is deemed an inappropriate endpoint for derivation of the oral reference dose, consider the use of liver hypertrophy**
- **Tier 2: Consider a more integrated presentation of the current text, tables and graph; as is, it is difficult to track information.**
- **Tier 2: Consider evaluation of rat-human differences in ETBE metabolic activity to assess rat to human extrapolation to look at effects relative to internal dose of tBa as they appear in both profiles**
- **Tier 3: Corrections are needed to p. 2-6, lines 9-10, p. 2-10 line 3 and p. R-10.**

ETBE - 3d. Section 2.2 presents an inhalation reference concentration of  $9 \times 10^0$  mg/m<sup>3</sup>, 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 panel reached consensus that, if the relevance of urothelial hyperplasia on the rat kidney to the human is accepted, the derivation of the RfC is clearly described and scientifically supported.**
- **A panel member suggested that sperm damage in the mouse model could also be considered (Tier 3).**
- **It would be valuable to assess route-specific potencies and cross-route evaluations by comparing estimated tissue doses of the metabolite tBA (Tier 2).**
- **More careful statistical analyses may help elucidate sex differences and help in the decisions to include or exclude studies (Tier 2).**
- **EPA needs to strengthen their arguments for the relevance of ETBE effects on the rat kidney to the human kidney (Tier 1).**

tBA - Q3a: The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of  $\alpha_2$ -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.

- 1. The panel agreed that the conclusions were clearly described but did not reach a consensus about scientific support.**
- 2. Just as with ETBE, the EPA thoroughly evaluated the role of alpha-2u globulin in male rat kidney effects of tBA according to the well-established criteria. Similarly, the issue of CPN and its potential role and relevance to humans was also discussed methodically.**
- 3. Major conclusions of the IRIS document were as follows:**
  - a) Kidney effects were 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.**

tBA - Q3a: The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of  $\alpha_2$ -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.

### **3. Major conclusions (continued):**

**b. Any kidney effects associated with  $\alpha_2$ -globulin nephropathy are not considered relevant for human hazard identification.**

**c. Effects associated with such CPN in female rats are considered relevant for human hazard identification and suitable for derivation of reference values.**

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

tBA - Q3a: The draft assessment identifies kidney effects as a potential human hazard of tert-butanol. EPA evaluated the evidence, including the role of  $\alpha_2$ -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.

## **Recommendations:**

### **Tier 2:**

- 1) Provide more thorough explanation for consideration of enhancement of CPN as a kidney effect relevant to human hazard assessment.**
- 2) Consider other indicators besides suppurative inflammation and transitional epithelial hyperplasia as indicators of kidney effects or provide better justification for their choice.**

tBA - Q3b. 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.

- Use of rat kidney (severity of nephropathy increases at 180 mg/kg-d calculated by PBPK to be equivalent to 470 mg/m<sup>3</sup>) as opposed to following endpoints for deriving reference doses.
- Developmental Toxicity
- Reductions in rat and mouse fetal and pup survival and pup body weight were observed at an oral gavage tBA doses of  $\geq 1,000$  mg/kg-d; may be related to slight maternal toxicities
- Suggest describing recommendation as "minimal effects at otherwise toxic dose levels", rather than "inadequate evidence" (Tier 1)
- Research on non-mammalian systems (e.g. zebrafish) to determine whether or not there are developmental targets of tBA should be pursued (Tier 3)

tBa - 3c. Section 2.1 presents an oral reference dose of  $4 \times 10^{-1}$  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.

- **Responses are premised on overall acceptance of the support of nephropathy effects of tBa as an appropriate endpoint (3a) for which there was not a uniform opinion.**
- **When considered an appropriate endpoint, the derivation was considered to be appropriate and clearly described**
- **The difference in consensus was based on the extent of confidence in a CNP and/or alpha2  $\mu$ globulin based mechanisms for these effects**
- **Tier 1: The question of the validity and applicability of the endpoints analyzed for the oral RfD needs to be carefully examined including the potential for CNP and/or alpha2  $\mu$ globulin as mechanism(s) of effect.**
- **Tier 1: Include outcomes of statistical analyses and their rationale in study selection choice**

tBA - 3c. Section 2.1 presents an oral reference dose of  $4 \times 10^{-1}$  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.

- **Tier 1: Include units in Tables**
- **Tier 2: If urothelial hyperplasia is deemed an inappropriate endpoint for derivation of the oral reference dose, consider the use of liver hypertrophy**
- **Tier 2: Consider a more integrated presentation of the current text, tables and graph; as is, it is difficult to track information.**
- **Tier 2: Consider evaluation of rat-human differences in ETBE metabolic activity to assess rat to human extrapolation**
- **Tier 3: p. 2-16, Line 10: “44” is likely a mistake, should it be “4”?**

tBA - 3d. Section 2.2 presents an inhalation reference concentration of  $5 \times 10^0 \text{ mg/m}^3$ , 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 panel considers that, if the relevance of increases in the severity of nephropathy in the female rat is relevant to humans, the RfC derivation is clearly described and generally supported scientifically.**
- **The panel had some reservations about the untypical application of route to route extrapolation from an oral dose to an inhalation exposure. EPA should provide more details and justification for the application of the PBPK model (Tier 1).**
- **It would be valuable to assess route-specific potencies by a comparative evaluation of tissue doses of the metabolite tBA (Tier 2).**
- **More careful statistical analyses may help elucidate sex differences and help in the decisions to include or exclude studies (Tier 2).**
- **EPA needs to strengthen their arguments for the relevance of tBA effects on the rat kidney to the human kidney (Tier 1).**

## Q4 – Cancer

- **4a. Cancer modes-of-action**
  - ETBE: Liver
  - tBA: Kidney , Thyroid
- **4b. Cancer characterization**
- **4c. Cancer toxicity values**
- **4d. Oral slope factor for cancer**
- **4e. Inhalation unit risk for cancer**

ETBE – Q4a: As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPAR $\alpha$ , 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 Panel finds the conclusion that liver tumors in male rats are relevant to human hazard identification to be scientifically supported.**
- **According to EPA Cancer Guidelines, a conclusion that carcinogenic effects in animals are not relevant to humans requires “convincing and extensive experimental evidence.” For example, for a PPAR $\alpha$  agonist, evidence must be sufficient to show that the liver tumors are the result of a PPAR $\alpha$  MOA, and other potential MOAs have been examined and found to be inoperative (USEPA, 2003).**
- **Liver tumors in ETBE-exposed rats could not be clearly shown to be produced by a PPAR $\alpha$ , CAR, or PXR MOA, and other potential MOAs could not be ruled out.**

ETBE – Q4a: As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPAR $\alpha$ , 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 Panel notes:**

- **Evidence for lack of human relevance is stronger for the PPAR $\alpha$  MOA than CAR or PXR MOAs**
- **The report lacks clarity on specific information needed to conclude that a PPAR $\alpha$ , CAR, or PXR MOA is operative**
- **Some criticisms of evidence regarding a potential PPAR $\alpha$ , CAR, or PXR MOA appear to be in error or inconsequential**
- **Evidence for other [human relevant] MOAs are not clearly presented**
- **Evidence presented for an acetaldehyde MOA is not well developed**
- **Although the CQ asks about human relevance based upon MOA, some panel members expressed concern regarding the human relevance of the ETBE rat liver tumors because they were only observed at an excessively high dose (as defined in the EPA Cancer Guidelines)**

ETBE – Q4a: As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPAR $\alpha$ , 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.

- **Tier 2 recommendations**

- **Clarify the evidence needed to conclude that a PPAR $\alpha$ , CAR, and/or PXR MOA is operative and indicative that liver tumors may not be relevant to humans. (Where is the bar set?). Examples may be helpful to illustrate the types of studies/information needed to satisfy each criterion.**
- **Revisit the evaluation of information available for ETBE using these criteria. May specifically want to reconsider statements about transient hypertrophy, etc.**
- **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, but evidence for this MOA should be thought through and presented more thoroughly, including more detailed comparisons with acetaldehyde tumor data in terms of dose (from ETBE versus acetaldehyde given directly), tumor site concordance, etc.**

ETBE – Q4b. 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.

- Numerous committee members felt that “Inadequate Information to Assess” rather than “Suggestive Evidence of Carcinogenicity” was a more appropriate descriptor for ETBE, due to conflicting bioassay results, limited high-dose only benign tumors, non-genotoxicity, etc.
- A number of members noted that: 1 inhalation bioassay was positive, that the exposure route should be considered “Suggestive”. 2 or 3 oral bioassay was negative, so that route of exposure should be considered “Inadequate.”
- Several (a minority of) member believed that descriptor “Suggestive Evidence” should be retained for all routes of exposure.

ETBE – Q4b. As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA’s cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral exposure. Please comment on whether the decision to include 2-stage initiation-promotion studies in the human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the initiation promotion data in the cancer hazard characterization is scientifically supported. Please comment on whether the “suggestive evidence” cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

- There was substantial differences of opinions on the relevance and scientific validity of the initiation-promotion assay.
- A number of members felt the assay could forewarn the public of potential risks of chemical interaction involving ETBE. Some members pointed out the assay had been used as supportive evidence but that it was not appropriate to support a conclusion of carcinogenic potential.
- Other members would not attach too much weight to the assay results, felt the assay was not relevant to human due to its design, believed it was not scientifically valid, and believed the assay had no value in risk assessment.
- It was suggested that the EPA devote more attention to assessing and describing the assays’ design, interpretation limitations, relevance, etc.

ETBE - 4c. 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.

- 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 Section 1.3.2, which describes the basis for the selection of the "suggestive evidence" descriptor, and cite the EPA (2005) Guidelines for Carcinogen Risk Assessment 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 document for the decision to perform a quantitative analysis in the case of ETBE.
  - One panel member provided a suggested rationale based on potential worker and consumer exposures.

## ETBE - 4c. (Cont.)

- The majority of the committee feels that performing a quantitative assessment of the data on ETBE liver carcinogenicity would not 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 agency summarizes the limited evidence for ETBE carcinogenicity: “The results for ETBE raise a concern for cancer, but the effects were limited primarily to one tissue (liver), at one dose (highest), and in one sex/species combination (male rats), which were almost entirely benign.” There is also supporting evidence from initiation/promotion studies and genotoxicity studies that if ETBE is carcinogenic, the mode of action is that of a promoter, which complicates dose/response analysis.
- The majority of the committee do not support performing a quantitative analysis of ETBE carcinogenicity because of the high uncertainty and potential for providing misleading risk estimates; however, several members of the committee favored conducting a quantitative analysis to provide some sense of the magnitude of potential risks.
- The committee agrees that the Saito et al 2013 study is well-conducted and well-reported, but the majority of the members do not feel that the data for neoplastic liver lesions from inhalation exposure, by themselves, are a suitable basis for a quantitative analysis.
  - Tumors were only observed at the highest concentration, which was in the range where centrilobular hypertrophy, nuclear receptor activation, and induction of metabolism may have contributed to the outcome.
  - In addition, there is serious concern about the ability of dose-response modeling (in this case, benchmark dose modeling) to provide meaningful and useful information when there is a flat, unresponsive dose response at all doses except the high dose.

## ETBE - 4c. (Cont.)

- Tier 1 Recommended Revisions: A majority of the committee recommends that EPA refrain from conducting a quantitative analysis for ETBE carcinogenicity.
- Tier 3 Recommendation: 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.

ETBE - 4d. Section 2.3 presents an oral slope factor of  $1 \times 10^{-3}$  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.

## Tier I

- **Most panel members commenting believed that the Saito et al. (2013) ETBE inhalation study was not suitable for developing an oral slope factor (see responses to charge question 4c).**
- **Reasons included lack of biological relevance (excessive high dose, only one dose significantly increased).**
- **One panel member believed the oral slope factor was scientifically supported.**
- **No alternative approach was suggested.**
- **No panel comments indicated that the oral slope factor derivation was done incorrectly or was poorly described, some comments indicated the modeling was done correctly.**

ETBE – 4e. Section 2.4 presents an inhalation unit risk of  $8 \times 10^{-5}$  per mg/m<sup>3</sup>, 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.

## Tier I

- **No consensus was reached on this question.**
- **Some panel members believed that the Saito et al. (2013) ETBE inhalation study was not suitable for developing an inhalation unit risk (IUR) (see responses to charge question 4c).**
- **Reasons included lack of biological relevance (excessive high dose, only one dose significantly increased tumor incidence).**
- **Some panel members believed Saito (2013) was appropriate for dose-response analysis, indicating the IUR was scientifically supported.**
- **Some of those panel members also had caveats regarding excessive high dose, only one dose significantly increased.**
- **No alternative approach was suggested.**
- **No panel comments indicated that the inhalation unit risk derivation was done incorrectly or was poorly described, one comment indicated the modeling was done correctly**

tBA – Q4a(i): As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving  $\alpha$ 2u-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

- **The Panel finds the conclusion that male rat kidney tumors are relevant to human hazard identification to be scientifically supported.**
- **The draft assessment concludes that evidence for a MOA involving  $\alpha$ 2u-globulin or chronic progressive nephropathy (CPN) is incomplete or not coherent (respectively). While some tumors might be attributable to  $\alpha$ 2u-globulin nephropathy augmented by CPN, others could be due to other unspecified processes. These processes are assumed to be relevant to humans.**
- **The Panel concurred with these conclusions.**

**[No recommendations]**

tBA – Q4a(ii): 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 Panel finds the conclusion that thyroid follicular cell tumors mice are relevant to humans to be scientifically supported.**
- **Some Panel members expressed concern whether an increase in thyroid follicular cell tumors was in fact demonstrated in male mice. This is discussed further in the response to CQ 4c.**
- **[No recommendations]**

tBA – Q4b. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA’s cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether the “suggestive evidence” cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

- There was a consensus that “Suggestive Evidence of Carcinogenic Potential” was the proper descriptors for TBA, as it caused renal tubule adenomas in male rats and follicular adenomas in female mice.
- This cancer descriptors is scientifically supported for oral exposure. No inhalation cancer bioassay data was found for TBA.
- A majority of the committee agreed the renal tumors was attributable for alpha2u-globulin and chronic progressive nephropathy (CPN) and thus were not relevant for human hazard evaluation.
- EPA policy dictates that does-response analyses should not be performed, if the relative contribution of alpha2u to tumorigenesis is not known.

tBA – Q4b. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA’s cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether the “suggestive evidence” cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

- If kidney tumors “drop-out” from consideration, cancer risks assessment of TBA must be based on the thyroid tumors.
- It is suggested that the EPA expand upon potential modes and sites of action of TBA in causing thyroid follicular adenomas.
- It is recommended that EPA state it is agency policy that when a chemical is found to be carcinogenic by one route of exposure, it is assumed to be carcinogenic by other routes in the absence of evidence to the contrary.

tBA - 4c. 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.

- 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 (2005) Guidelines for Carcinogen Risk Assessment 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 document for the decision to perform a quantitative analysis in the case of TBA.
  - One panel member provided a suggested rationale based on potential worker and consumer exposures.

- The majority of the committee feels that performing a quantitative assessment of the data on tBA thyroid carcinogenicity would not 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 agency summarizes the limited evidence for TBA carcinogenicity: “In B6C3F1 mice, administration of tert-butanol in drinking water increased the incidence of thyroid follicular cell adenomas in females and adenomas or carcinomas (only one carcinoma observed) in males (NTP, 1995), as discussed in Section 1.2.2”.
- The majority of the committee do not support performing a quantitative analysis of tBA carcinogenicity because of the high uncertainty and potential for providing misleading risk estimates; however, several members of the committee favored conducting a quantitative analysis to provide some sense of the magnitude of potential risks.
- The committee agrees that the NTP 1995 study is well-conducted and well-reported, but the majority of the members do not feel that the data for neoplastic thyroid lesions from drinking water exposure, by themselves, are a suitable basis for a quantitative analysis because a single tumor was observed, and only 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.
  - In addition, there is serious concern about the ability of dose-response modeling (in this case, benchmark dose modeling) to provide meaningful and useful information when there is a flat, unresponsive dose response at all doses except the high dose.

- Tier 1 Recommended Revisions: A majority of the committee recommends that EPA refrain from conducting a quantitative analysis for TBA carcinogenicity.
- Tier 3 Recommendation: 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.

tBA - 4d. Section 2.3 presents an oral slope factor of  $5 \times 10^{-4}$  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.

## Tier I

- **Most panel members commenting believed that the NTP (1995) TBA drinking water study was not suitable for developing an oral slope factor (see responses to charge question 4c).**
- **Reasons included lack of biological relevance (only one dose significantly increased).**
- **One panel member believed the oral slope factor was scientifically supported by the NTP (1995) data.**
- **No alternative approach was suggested.**
- **No panel comments indicated that the oral slope factor derivation was done incorrectly or was poorly described, some comments indicated the modeling was done correctly.**

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

## Tier I

- **Most panel members commenting believed that the NTP (1995) TBA drinking water study was not suitable for developing an inhalation unit risk (IUR) (see responses to charge question 4c).**
- **Reasons included lack of biological relevance (excessive high dose, only one dose significantly increased).**
- **One panel member believed that a cross-route extrapolation could be done using default physiological parameters (70 kg body weight, 20 m<sup>3</sup>/day inspiration rate) and appropriate absorption fractions.**
- **No alternative approach was suggested.**
- **No panel comments indicated that the IUR derivation was done incorrectly or was poorly described, some comments indicated the modeling was done correctly.**

## **Q5: Susceptible Populations and Lifestages**

ETBE – Q5. 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.

- We are in agreement with EPA’s position that there is “plausible evidence” for a vulnerable subgroup, specifically:
  - Individuals with the inactive form of ALDH2\*2 variants--relevant to individuals in certain Asian subgroups.
- We are also in agreement with inconclusive evidence for CYP2A6 variants due to lack of studies
- That said, we raised the following concerns:
  - Note that the cited studies are from oral exposure routes only. No studies involved inhalation-based exposures [Tier 3]
  - Report does not mention or address variants in non-coding regions of ALDH, nor does the report consider other allelic variations that may be more common in subpopulations (Dickson et al 2006) [Tier 1]
  - Report does not mention the maternal-fetal unit as a possible susceptible population, which may be relevant due to changes in metabolism. [Tier 1]
  - Sex specificity should also be noted, as this is a recurring observation throughout several of the sections and has potential relevance to susceptible populations [Tier 3]

tBA – Q5. 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.

- We are in agreement with the EPA's position that states "there is no identified susceptible population."
- The following concerns were noted:
  - There is no human data reported; however, human data exist to support altered metabolism with respect to xenobiotic-metabolism during pregnancy for the maternal-fetal unit. This information should be cited in the report. (example: Hakkola et al, 1998)
  - Table 1-12 has a finding of 100% increase in body weight gain in PND 1-21 paired with a 33% fetal loss. If true, needs to be clearly stated in draft.

## Q6: Executive Summary

ETBE – Q6. 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 changes are made to the body of the draft, the Executive Summary will need to be changed accordingly.
- However the questions of hazard and potency are resolved, the Executive Summary needs to capture the scientific pursue that is vigorous exercised in this assessment, and specifically articulated for the broad audience. [Tier 1]
- Acknowledge the degree of uncertainty stemming from the relative lack of significant dose response at low and intermediate doses. [Tier 2]

ETBE – Q6. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

- The Executive Summary must acknowledge that the conclusions drawn are critically dependent on best scientific judgments about the relevance of observed toxicity endpoints and relate these to relevant health risks in humans. The issues about how available animal results are interpreted for human health risk assessment need to be summarized. [Tier 1]
- All Potential effects identified in Hazard Identification should be stated in the Executive Summary, including reproductive, developmental, and neurobehavioral effects. [Tier 1]
- Add statements linking ETBE assessment to tBA assessment [Tier 1]
- For Key Issues, specify contingency and knowledge gaps in the decision-making, and clearly describe uncertainties, assumptions, and the strength and weakness of the decisions. [Tier 1]
- Additional key issues: cancer characterization, PBPK models, dose metrics, BMD model selection [Tier 1]
- State all endpoints used in dose-response analysis before the selection of final toxicity values [Tier 1]

tBA – Q6. 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 changes are made to the body of the draft, the Executive Summary will need to be changed accordingly.
- However questions of hazard and potency are resolved, the Executive Summary needs to capture the scientific pursue that is vigorous exercised in this assessment, and specifically articulated for the broad audience. [Tier 1]
- Acknowledge the degree of uncertainty stemming from the relative lack of significant dose response at low and intermediate doses. [Tier 2]

tBA – Q6. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

- The Executive Summary should acknowledge that the conclusions drawn are critically dependent on best scientific judgments about the relevance of observed toxicity endpoints to the potential for risk in humans. The issues about how available animal results are interpreted for human health risk assessment need to be summarized. [Tier 1]
- All Potential effects identified in Hazard Identification should be stated in the Executive Summary, including reproductive, developmental and neurobehavioral effects. [Tier 1]
- Specify the chosen dose metrics, explaining the basis for calculation, the role of metabolism in activation and clearance, major assumptions or use of alternatives to defaults, and the basis for cross-species dose equivalency. [Tier 1]
- Add justification for the use of route-to-route extrapolation. Describe the strength and weakness of the decision, as well as major knowledge gaps. [Tier 1]
- State all endpoints used in dose-response analysis before the selection of final toxicity values [Tier 1]