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Compilation of the SAB Chemical Assessment Advisory Committee augmented for the review of ETBE and tBA (SAB CAAC-ETBE/tBA Committee)
Member Response to Comments on the ETBE/tBA Draft Report

The following comments from committee members were submitted in response to comments received on the draft report available at https://yosemite.epa.gov/sab/sabproduct.nsf/MeetingCal/46495425F4649F7E85258227003EC276?OpenDocument. This compilation contains all comments received as of March 26, 2018.

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Dr. Deborah Cory-Slechta

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.

1. page 28: The second and third bullets of Tier 1 recommendations for ETBE are very similar to the third and fourth bullets of Tier 3 recommendations for *tert*-Butanol, on page 29. "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." EPA is seeking clarification on whether these recommendations were indeed seen as being a much higher priority for ETBE than for *tert*-Butanol. Or would the SAB CAAC consider these recommendations to be of similar tier for both assessments (and if so what would that tier be)?

   Response: The intent was that these recommendations were for both ETBE and for tert-butanol, with a similar tier for both assessments, and this would be considered a second tier recommendation.

2. Throughout the SAB report: The statements about the uncertainties whether or not to use rat kidney effect data (particularly the male rat) leave open raised questions of EPA Draft report results. It was striking how little mention of the negative results in mice was in the EPA Draft Report. It should be recommended (Tier 1, Line 28, Page 23) that these studies in mice, which show little or no kidney effects of these agents (e.g. chronic drinking water administration of tBA in NTP, 1995), be given more mention in the EPA report; they provide support that for the argument that the rat data may not be applicable to other species.

   Response: Not sure what to say for this because it isn't specific to this question, it states "throughout the SAB report". I did add a change in the context paragraph

3. P 29, line 31. Might be helpful to state why liver hypertrophy should be used as an alternative to nephropathy.

   Response: Added a phrase on evidence of its effects.

Proposed revisions

The responses to question 3c. 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 their 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.

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.

- ~~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 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.

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.

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.

Dr. Maria Morandi

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.

1. Page 31. Line 1-2: I have a concern about the relevance of increased kidney weight for setting reference concentration/dose. Especially when underlying pathology is not identified.

Response: The relevance of absolute kidney weight for deriving the reference concentration should be discussed by the panel during the teleconference because there are diverse opinions among Panel members. The 2-year and 13-week studies show decreases in absolute kidney weight in both male and female rats. Candidate RfC's based on this outcome for the subchronic studies are 0.4, 1, 2 and 2 mg/m^3, compared to 40 mg/m^3 based on increased CPN severity in female rats and 9 mg/m^3 based on increases in urothelial hyperplasia in male rats.

2. Page 31 line 10: include ppm conversion for 21,000 mg/m3 in parentheses for completeness.
   Response: Text on Page 31, line 10 will be revised as follows: "...endpoints at doses of 5000ppm (20,900 mg/m^3) [compared to a LOEL of 1500 ppm (6270 mg/m^3) for kidney...". The concentration of 21,000 mg/m^3 was apparently rounded off so it is changed to the exact level reported in the reference and the ETBE report.

3. Page 31 line 12: indicate exact strain of mouse. C57B6C3F1? Other?
   Response: Text in Page 31, line 12 will be revised as follows: "... (DNA breaks, 8-oxo-deoxyguanine) and minor histopathological changes in C57BL/6 mice..."

4. Page 31, line 38: Approximately 4 times higher than the selected RfC *(based on what parameter?).*
   Response: Agreed. Lines 37-39 in Page 31 will be removed.

5. Page 32 line 23: indicate exact strain of mouse. C57B6C3F1? Other?
   Response: Text in Page 32, lines 22-23will be revised as follows: "...histopathological changes in C57BL/6 mine...".

6. Tier 2 Recommendations
   pg. 32, lines 21-23 - Endpoints such as DNA breaks, and 8-oxo-deoxyguanine have rarely, if ever been used as the basis for RfD/RfC derivation. This is probably because the significance in downstream toxicity (e.g., tumor development) is not clear in any given case. Furthermore, "minor histopathological changes" are difficult to characterize in the abstract with respect to their relevance for RfD/RfC development. As a rule of thumb such changes tend to be considered if they can be demonstrated to be on a pathway of progression to more clearly adverse effects, but not if such a pathway cannot be, at least reasonably, conjectured. I suggest deleting these recommendations.
   Response: This issue should be discussed during the teleconference. These are changes in rat sperm, so they could be indicative of reproductive effects. I agree that more explanation is need regarding links with reproductive effects.

7. P. 32, line 20-3: Suggest these Tier 2's be Tier 1's.
   Response: The work group judged that the recommendations did not meet the criteria for Level 1 recommendations. This issue should be discussed during the teleconference.

Dr. John Budroe

Most of the comments below stem from the Report not adequately reflecting the lack of consensus for Sections 3.4.2 (Cancer characterization), 3.4.3 (Cancer toxicity values), and including some Tier I recommendations in those sections that lead to ambiguity which has consequences for Sections 3.4.4. and 3.4.5. This, plus the lack of consensus regarding the Section 3.4.4.1 and 3.4.4.2 issues results in having text at cross-purposes in those sections. I've attached some comments and examples on that, which also has some suggested section rewrites for Sections 3.4.4.1, 3.4.4.2 and 3.4.5.2.

Charge Question 4b:

3.4.2.2. tBA

The Report states: "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 Report recommendation to apply the "Suggestive Evidence" descriptor to tBA oral exposure is completely opaque. Since tBA produces tumors in two species/sexes (male rats and female mice), this recommendation goes against the EPA's 2005 Guidelines for Carcinogen Risk Assessment for the descriptor "Likely to be Carcinogenic to Humans" without providing any justification for doing so. This recommendation also understates the lack of consensus on this issue.

Charge Question 4c:

3.4.3.1. ETBE

The Report states "The SAB concludes it is highly 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", and "Any quantitative analysis based on this limited evidence would be highly uncertain and potentially misleading.", but also states "The SAB agrees that the Saito et al. (2013) study is well-conducted and well-Reported", and "several members favored conducting a quantitative analysis to provide some sense of the magnitude of potential risks." The Report also makes a Tier 1 recommendation that "EPA should refrain from conducting a quantitative analysis for ETBE carcinogenicity **or** [bolding added] explain the limitations of the analysis and clearly state the intended purpose is to simply provide some sense of the magnitude of potential risks."

This section of the Report ignores the SAB-reviewed 2005 Guidelines for Carcinogen Risk Assessment policy described above, and makes a Tier I recommendation that would allow EPA to perform a dose-response assessment if the results of that analysis are qualified as to its limitations. This introduces ambiguity as to the impact of this Report section on Section 3.4.5.1. Additionally, the statement that "Any quantitative analysis based on this limited evidence would be highly uncertain and potentially misleading" goes beyond the discussion of this charge

question at the CAAC meeting, ignores the lack of consensus on this issue and is therefore unjustified.

Finally, the discussion of modeling the Saito *et al.* liver tumor data set was inaccurate – all ETBE concentrations induced increased tumor incidences compared to controls. The data points which did not reach statistical significance still provide useful information for dose-response modeling purposes. Unfortunately, the ETBE/tBA Expanded CAAC did not include any biostatisticians that could have provided appropriate expertise to the discussion of this issue. In light of this information, the Tier III recommendation is unjustified.

3.4.3.2. tBA

The Report does not state that the NTP (1995) tBA drinking water study was not well-conducted and reported. In fact, that study was very well conducted and reported, and because of the positive tumor results in two species/sex, would justify a "Likely to be Carcinogenic to Humans" designator. Therefore, the comments for Section 3.4.3.1 also apply to this Report section - this section of the Report ignores the SAB-reviewed 2005 Guidelines for Carcinogen Risk Assessment policy described above, and makes a Tier I recommendation that would allow EPA to perform a dose-response assessment if the results of that analysis are qualified as to its limitations. This introduces ambiguity as to the impact of this Report section on Section 3.4.4.2.

Additionally, the statement that "The SAB deems it highly 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." goes beyond the discussion of this charge question at the CAAC meeting, ignores the lack of consensus on this issue and is therefore unjustified.

Finally, as with the Section 3.4.3.1 discussion on modeling, the discussion of modeling the NTP 1995 drinking water study female mouse thyroid tumor data set was inaccurate – one other tBA concentration induced increased tumor incidences compared to controls. The data points which did not reach statistical significance still provide useful information for dose-response modeling purposes. Unfortunately, the ETBE/tBA Expanded CAAC did not include any biostatisticians that could have provided appropriate expertise to the discussion of this issue. In light of this information, the Tier III recommendation is unjustified.

Charge Question 4d:

3.4.4.1. ETBE

The charge question: *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.*

Lines 6 through 23 of Report page 44 appear to state that consensus was reached for the issue of the suitability of the Saito et al. (2013) study for developing an oral cancer slope factor. Lines 31 through 36 of page 44 indicate that no consensus was reached, especially for bullets 2 and 3 of lines 6 through 23. Those bullets should be struck, as should lines 25 through 29, where again, no consensus was reached on this suggested policy change.

A suggested rewrite for section 3.4.4.1 is listed below:

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 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 oral cancer studies up to the limits of 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:
EPA should not derive an oral ETBE slope factor by route extrapolation from the Saito et al. (2013) ETBE inhalation study absent pharmacokinetic/pharmacodynamics modeling that demonstrates consistency between the oral and inhalation study results.

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 statement in lines 17 – 19 posits a consensus that does not exist, and lines 21 – 32 are inappropriate for the same reasons provided in the comments regarding sections 3.4.3.1. and 3.4.3.2. **The following section rewrite is suggested:**

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: No recommendation due to lack of SAB consensus.

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 α2μ-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.

As stated in the comment on section 3.4.4.2, the high dose of tBA in the NTP 1995 drinking water study did not exceed the MTD. There is therefore no reason to conclude that dose was excessive. Additionally, the issue of high dose non-linear metabolism kinetics is speculative, as there is no mouse TK model available. These issues were raised in the comments following the meeting discussion, indicating a lack of consensus for these issues.

The following section rewrite is suggested:

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 α2u-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 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 supports EPA's decision to not present an inhalation unit risk for tBA.