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**SAB Chemical Assessment Advisory Committee augmented for the review of
ETBE and tBA (SAB CAAC-ETBE/tBA Committee)
Individual Member Response to Comments on the ETBE/tBA Draft Report
March 27, 2018.**

The following member comments were submitted in response to comments received on the draft report available at
<https://yosemite.epa.gov/sab/sabproduct.nsf/MeetingCal/46495425F4649F7E85258227003EC276?OpenDocument>.

Dr. Stephen Roberts

Question 4a

3.4 Hazard Identification and Dose–Response Assessment: Cancer

3.4.1 Cancer modes-of-action in the liver.

3.4.1.1 ETBE

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

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

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

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

some of the EPA criticisms of data regarding key events are seen as inconsequential or in error, which further detracted from this section.

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

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

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

The following recommendations are noted:

Tier 1:

- EPA should clarify the evidence needed to conclude that a PPAR α , CAR, and/or PXR MOA is operative and indicative that liver tumors may not be relevant to humans. Examples may be helpful to illustrate the types of studies/information needed to satisfy each criterion.
- EPA should revisit the evaluation of information available for ETBE using these criteria. The EPA may specifically want to reconsider statements about transient hypertrophy.
- EPA should revise Table 1-13 and accompanying narrative to be more descriptive regarding availability of information for each MOA. Instead of saying “No positive studies identified” indicate whether studies relevant to the MOA exist and where results are positive or negative.
- Acetaldehyde is proposed as a strong candidate MOA for male rat liver tumors, but the plausibility of this MOA is not well explored. Evidence for this MOA should be developed and

presented more thoroughly; or, alternatively, the agency is encouraged to reduce emphasis on this MOA in the final assessment.

• ~~None.~~

Tier 2:

None.

- ~~EPA should clarify the evidence needed to conclude that a PPAR α , CAR, and/or PXR MOA is operative and indicative that liver tumors may not be relevant to humans. Examples may be helpful to illustrate the types of studies/information needed to satisfy each criterion.~~
- ~~EPA should revisit the evaluation of information available for ETBE using these criteria. The EPA may specifically want to reconsider statements about transient hypertrophy.~~
- ~~EPA should revise Table 1-13 and accompanying narrative to be more descriptive regarding availability of information for each MOA. Instead of saying "No positive studies identified" indicate whether studies relevant to the MOA exist and where results are positive or negative.~~
- ~~Acetaldehyde is proposed as a strong candidate MOA for male rat liver tumors, but the plausibility of this MOA is not well explored. Evidence for this MOA should be developed and presented more thoroughly; or, alternatively, the agency is encouraged to reduce emphasis on this MOA in the final assessment.~~

Tier 3:

- None.

3.4.1.2 tBA

Cancer modes-of-action in the kidney. As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving $\alpha_2\mu$ -globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

The SAB has not reached consensus regarding the EPA's conclusion that male rat kidney tumors are relevant to human hazard identification and is scientifically supported. The draft assessment concludes that evidence for a MOA involving $\alpha_2\mu$ -globulin or CPN is incomplete or not coherent, respectively. While some tumors might be attributable to $\alpha_2\mu$ -globulin nephropathy augmented by CPN, others could be due to other unspecified processes that are assumed to be relevant to humans.

The SAB has not reached consensus because some members agree with the assessment and some members conclude that renal tumors could be explained by CPN, and are therefore not relevant to humans. [Additional discussion of this issue is provided in the response to Charge Question 3a. in the context of noncancer kidney effects of ETBE \(Section 3.3.1.1\).](#)

The following recommendations are noted:

Tier 1:

- ~~None~~ [Consistent with responses to previous charge questions related to noncancer kidney effects of ETBA and tBA, the panel recommends that the EPA provide additional justification for the assumption that kidney tumors in male rats exposed to tBA are relevant to humans.](#) -

Tier 2:

- ~~None~~ [The suggested workshop on interpretation of human relevance of kidney effects in rats with CPN in Section 3.3.1.1 should include cancer as well as non-cancer endpoints.](#)

Tier 3:

- None.

Cancer modes-of-action in the thyroid. As described in section 1.2.2, thyroid tumors were observed in male and female mice following tert-butanol exposure, and an anti-thyroid mode-of-action was evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid follicular cell tumors in rodents (U.S. EPA, 1998), found the information inadequate to determine whether an anti-thyroid mode-of-action was operating and considered the thyroid follicular cell tumors in male and female mice to be relevant to humans. Please comment on whether this conclusion is scientifically supported.

~~The SAB finds that there is scientific support for the EPA's conclusion that thyroid follicular cell tumors in mice are relevant to humans for tBA.~~ [The SAB concurs that mode of action for follicular cell tumors in male and female mice treated with tBA is unknown. Per EPA science policy, these tumor responses are considered relevant to humans.](#) ~~However, the SAB finds that there is uncertainty as to whether an increase in thyroid follicular cell tumors is demonstrated in male mice.~~

The following recommendations are noted:

Tier 1:

- None.

Tier 2:

- None.

Tier 3:

Comments by individual member of the SAB CAAC-ETBE/tBA Committee.
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- None.