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Subject: TMBs: points of clarification in the draft peer review report
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Attachments: [EPA Request for Clarification in the SAB Draft TMBs Report 1-29-15.docx](#)
Importance: High

Hi Tom,

We pulled together some questions on clarifications relating to various sections in the draft peer review report on TMBs. Please distribute to the panel for their consideration. Vince will briefly walk through these today during the time we have on the Agenda. We also understand that this would be added to the SAB's website.

Thanks,
Samantha

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EPA Request for Clarifications in the SAB Revised Draft Report (12-22-14) on TMBs

Overall the revisions to the report have been helpful to EPA and the Agency's understanding of the panel's recommendations and suggestions. EPA has a few questions for clarification, detailed below.

LETTER TO THE EPA ADMINISTRATOR

- 1) Page 2, lines 24-28: The panel seems to be stating that they agree with the EPA's choice to use the PBPK model and extrapolation approach, but it would be helpful for EPA if the report is more clear and definitive on whether the panel agrees (or disagrees) with the PBPK-related conclusions made by EPA.
- 2) Page 2, lines 30-39: The panel seems to agree with choices of study, endpoint, and extrapolation made by EPA, but recommend looking at other studies and deriving reference doses and concentrations from those studies for comparison. It would be helpful if the panel could be explicit in how EPA should compare these, for example, is the panel asking EPA to present the information and additional reference values in a revised draft assessment and perform a comparison for illustrative purposes, and further supporting the decision to use the PBPK model?

EXECUTIVE SUMMARY

- 1) Page 2, lines 28-30: Similar to #2 above: The panel recommends developing reference values and comparing them to the reference values calculated from the PBPK extrapolation approach. Can the panel be more explicit in how EPA should make this comparison and for what purposes (e.g., to replace the original reference values or for illustrative purposes)?

DRAFT REPORT

- 1) In several excerpts from the draft report (emphasis added in bold), the panel provides statements related to the potential reversibility of the nervous system effects. It would be helpful for EPA if the panel could provide explicit recommendations regarding whether the panel agrees or disagrees with the overall lack of reversibility of neurotoxicity in general, and the pain sensitivity measures in particular, and the expected impact of these recommendations on the assessment.

Section 3.1.4, page 13, lines 33-42: "There was also disagreement among the TMB Panelists related to the interpretation of the pain sensitivity data, with **some members questioning whether the document adequately examined the question of reversibility following termination of exposure**, which further bears on whether ongoing or repeated exposures to TMBs should be deemed to have accumulating toxicity beyond effects evident in shorter-term exposure; **other panel members believed that the data were consistent with cumulative toxicity and lack of reversibility**....The full discussion of these issues and their treatment in the TMBs assessment is covered in the responses to the specific charge Questions in Section 3.2 of this report."

Section 3.2.5, page 23, lines 41-41 and page 24, lines 1-9: "The text, where applicable, could include **additional qualifications as to "reversibility of effects"** at the 2-week post-exposure time-point as this. This assessment of reversible effects of failures on the rotarod is based on the finding of lack of statistical rather than biological significance, with difference between treated and control groups at one week post-exposure following a 13-week exposure period for one of two isomers. However, this represented a reduction from 40 percent rotarod failure during the final week of exposure compared to

35 percent one week post-exposure, as compared to 0 percent rates for controls. There was no such statistical reversal for one but not the other isomer, and for both isomers, the magnitude of the reduction post exposure was minimal difference in values from the last time point during exposure. Further, it is not clear that the statistical analyses of these data incorporated a repeated measures component that would be required by the experimental design. **Thus, while a case was stated for a statistically significant reversal, it was not consistent nor did it appear to be biologically meaningful.**"

Section 3.2.5, page 24, lines 33-38: "The SAB agrees that the observation of prolonged latency in the hot plate test 24 hour post-footshock delivery that was observed in studies by Gralewicz and colleagues (1997, 2001) **also constitutes an adverse effect [EPA note: these tests were conducted multiple weeks after the cessation of exposure, making them applicable to discussions of reversibility]**. The administration of footshock immediately after the hot plate test trial essentially maximizes the capabilities of the nervous system and, thus, provides a type of nervous system probe that then unmasks a prolonged latency to a hot plate stimulus 24 hours later. It shows that when the nervous system is maximally stressed, it cannot respond/recover in a normal timeframe."

- 2) Section 3.2.5, page 26, lines 21-23 on the UF_H: The panel agrees with the UF of 10 and describes for several lines the rationale for a 3 (provided by one reviewer) ending with the rationale for why the other members of the panel supported the 10. It would be helpful to EPA for the panel to conclude the discussion of this uncertainty factor with a clear statement that the SAB panel agrees with the selection of an UF_H of 10.
- 3) Section 3.2.5, page 27, lines 15-34 on the UF_D: The report states that the panel was divided and describes the rationale various members had for a database uncertainty factor of 3 or a 10. However, a recommendation to the Agency on this uncertainty factor is not provided.
- 4) Two sections of the report, 3.2.3 and 3.2.5, seem to contradict each other in terms of the panel's views on the nervous system effects for TMBs. As shown below with extracted statements from the draft report (emphasis added in bold), EPA is unclear of the panel's recommendations regarding statistical and biological significance, if the panel considers the neurotoxicity assays to be widely-used behavioral assays that were carefully carried out, or that the neurotoxicity endpoints are uncertain and somewhat unconventional with an unclear functional significance and unclear extrapolation to humans? Clarification from the panel would be helpful to EPA in implementing the recommendations.

Statistical and biological significance

- Section 3.2.3, page 17, line 20: "In light of the **uncertainty involving the neurotoxicity** endpoints using the draft TMB assessment..."
- Section 3.2.3, page 17, lines 29-31: "The SAB recommend that descriptions of results more **closely adhere to the rule that statistical significance** provides the criterion of whether an effect has occurred (although data trends can be cautiously noted)".
- Section 3.2.5, page 23, lines 37-40: "Given the commonality of even the trends in data across these studies, some mention of the **biological significance in the absence of statistical significance**...should be mentioned."

Unconventional or widely-accepted endpoints

- Section 3.2.3, page 17, lines 39-40: "...because the neurological effect data that were presented are largely from a single laboratory, with somewhat **unconventional endpoints**."
- Section 3.2.3, page 17, line 41: "Although the quality of the studies seems very high, the **functional significance of the observed effects is not as clear**..."
- Section 3.2.5, page 23, lines 20-26: "The SAB generally agrees with the choice of the Korsak and Rydzynski (1996) study...it was based on **widely-used behavioral assays**. An examination of the study indicates these behavioral studies were **carefully carried out** and data from control animals were **consistent with previously published observations**."
- Section 3.2.5, page 24, lines 24-31: "The SAB agrees that the reduction in pain sensitivity as indicated by an increased latency to pawlick response in a hotplate test is a **valid adverse nervous system effect and appropriately selected** as a critical effect for the derivation of the RfC. This effect was variously seen in response to short-term, 4- week, and 90-day studies. The associated U-shaped dose-effect curves seen with these isomers, moreover, are **highly consistent with the effects of various other pharmacological agents (e.g., opioids) on this response** and likely reflective of the mechanisms by which these isomers act. This **assay is widely used** in the behavioral pharmacology literature and particularly in the study of pain nociception and opioid pharmacology."
- Section 3.2.5, page 24, lines 33-38: "The SAB agrees that the observation of prolonged latency in the hot plate test 24 hour post-footshock delivery that was observe in studies by Gralewicz and colleagues (1997, 2001) **also constitutes an adverse effect**. The administration of footshock immediately after the hot plate test trial essentially maximizes the capabilities of the nervous system and, thus, provides a type of nervous system probe that then unmasks a prolonged latency to a hot plate stimulus 24 hours later. It shows that when the nervous system is maximally stressed, it cannot respond/recover in a normal timeframe."

Human relevance

- Section 3.2.3, page 17, lines 41-42: "Although the quality of the studies seems very high, the functional significance of the observed effects is not **as clear, nor is the extrapolation to humans**."
- Section 3.2.5, page 24, lines 14-15: "...include **more specific descriptions of the similarity** of the animal behavioral endpoints to what has been observed in humans."

5) There are several sections throughout the draft report (extracted below with emphasis added in bold) that relate to the use of the C-9 studies and the other related compounds (i.e., toluene, xylene, and ethylbenzene) in which the panel suggests that EPA describe these data in more detail. The Agency is unclear how exactly and to what extent the panel is recommending that EPA use these data. There are a few general examples for uses of these data provided in the current draft report, such as qualitative support for hazard identification regarding possible MOAs (i.e., "qualitative and mechanistic interpretations of TMB toxicity"), assumed chronicity of health effects, and also possibly for use in filling in some data gaps in the UFD. There seems to be some disagreement amongst the reviewers on the use of these data. It would be very helpful if the panel can provide consensus recommendations on how the C-9 studies and, separately, related chemicals should be used in the assessment (something explicit: e.g., "the C-9 studies should be considered for their potential to reduce database uncertainty by addressing potential data gaps in the set of studies available for individual TMB isomers, while information on related chemicals (i.e., xylenes; ethylbenzenes; toluene) should be discussed in the context of its potential to describe mechanistic events or modes of action or vulnerable populations/lifestages that might be conserved across all aromatic compounds, including TMB isomers").

Executive Summary, page 2, lines 27-31: “The **breadth of the literature review and discussion should be expanded to include other closely related aromatic solvents and possibly mixtures**. The SAB concludes that because human exposures to the TMBs generally involve complex mixtures, the **available studies on mixtures – including the C-9 fraction and white spirit studies – deserve further discussion** to transparently describe the EPA’s considerations of these data.

Section 3.1.4, page 13, lines 28-33: “The TMB Review Panel was divided, however, on the adequacy of the responses and the advisability of the dispositions that were made as presented in the summary. In particular, **there were a variety of views on the role that testing of the C-9 fraction should have in the assessment, with some panelists accepting the reasons for omission of this from the main evaluation and others feeling that these results had a role that had not been adequately explored**. The discussion of the C-9 fraction in the August 2013 draft of the TMB Assessment is further discussed in section 3.2.3 of this report.”

Section 3.1.4, page 13, lines 38-42: “The SAB recommends that the **EPA provide a more robust discussion of the data and studies considered in the TMB assessment including the C-9 fraction and mixtures**. The full discussion of these issues and their treatment in the TMBs assessment is covered in the responses to the charge questions in Section 3.2 of this report directed.”

Section 3.2.2, page 15, lines 26-36: “The SAB noted that the description of the search strategy did not mention xylenes or ethylbenzene. **Because of the close similarity of xylenes to TMBs and the very similar toxicological effects caused by xylenes, this may have resulted in important papers being excluded, thus weakening the conclusions of the assessment**. For example, the findings of Chen et al. (1999,) and Lee et al. (2005) (cited on p. 1-1) relating painters’ exposure to solvents to neurological problems have a relatively weak association to TMBs. The SAB notes that the links in these two studies are stronger to xylene and to a mixture of aromatic solvents including TMBs rather than the TMB isomers. For example, studies such as those of Ruijten et al. (1994), Qian et al. (2010), Tang et al. (2011), and El Hamid Hassan et al. (2013), are closely linked to xylene but not cited in the document. The overall association of the effects reported in these studies in painters with exposures to aromatic solvents like the TMBs is much stronger than the associations reported by Chen et al. (1999,) and Lee et al. (2005.)”

Section 3.2.3, page 16, Lines 28-36: “However, the decision to disregard or limit consideration of the studies on solvent mixtures containing, but not limited to, the TMBs appears to have affected the synthesis of evidence. **Because important toxicological observations have been made in both animal and human studies involving exposure to aromatic solvent mixtures, an important toxicological perspective was lost**. The IRIS Preamble, in Section 3.1 on identifying studies (lines 44-47) specifically states, “In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components.” The SAB concluded that the TMB assessment was prepared because human exposures to the TMBs generally involve complex solvent mixtures, the available studies on mixtures, including the C-9 fraction and white spirit, **deserve further consideration and explanation in the TMB assessment**.”

Section 3.2.3, page 16, lines 38-41 and page 17, lines 1-8: “In addition, synthesis of available data appears to have been impaired by the decision not to include literature on other closely related aromatic solvents. Toluene is briefly mentioned, but the potentially relevant literature on ethylbenzene, xylenes, and styrene is largely excluded. **This eliminated supporting toxicological information on neurological endpoints which could have helped clarify potential mechanisms of action.** Such information is clearly supported in the IRIS Preamble, section 3.1 (lines 11-15) “[s]earches for information on mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms.” This is further supported in Section 5.4, p. xxiii (lines 18-21), “Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms.” **It is therefore recommended that additional animal and human studies on related aromatic solvents be considered in the qualitative and mechanistic interpretations of TMB toxicity.** Examples of such studies are included in comments on the literature review. (See Section 3.2.2)”

Section 3.2.3, page 17, lines 10-17: “The testing of the C-9 fraction reveals another important point. Because this mixture, as tested, was about half TMBs, much of the observed effects could have been due to the TMBs. Competition for metabolic clearance would likely have increased duration of exposure to the TMBs, so the minimal observed toxicity in several C-9 studies provides important perspective to the TMB evaluation (although both positive and negative interactions are possible). Since it can be assumed that an application of the IRIS assessment for TMBs is for evaluating potential risks from exposure to the C-9 solvent and related aromatic mixtures, **The SAB suggests that the observation of effects of such mixtures is certainly relevant, and needs further discussion.**”

Section 3.2.3, page 17, lines 19-25: “More specifically, in another subchronic evaluation of the C-9 mixture, Douglas et al. (1993) found no persistent neurotoxicity. In light of the uncertainty involving the neurotoxicity endpoints used in the draft TMB assessment, this possible discrepancy needs to be addressed in detail. Such a discussion might involve considerations of the potential for chronic neurotoxicological effects of individual TMB isomers alone, versus when exposure is to TMBs in mixtures. **Data are available from many additional mixture studies to provide further perspectives on this question,** as reviewed, for example, by Richie et al. (2001).”

Section 3.2.3, page 17, lines 33-37: “For neurological effects, which are the most consistently observed, the **document clearly explains that although mechanistic data are lacking for the TMBs, there is good rationale for making analogies with toluene,** for which much more information is available. **This could have been greatly strengthened, as mentioned above, by including supportive studies on the three xylene isomers as well as ethylbenzene and other related solvents and mixtures.**”

Section 3.2.3, page 18, lines 10-20: “The SAB **recommends that the EPA expand on the discussion of the similarities of the TMBs to other methyl-substituted aromatic compounds.** The subsections in the hazard synthesis considering the similarities between the three TMB isomers are very important but would **be improved by further perspectives on related solvents.** This is critical with regard to the decision to base some of the RfDs on extrapolations among the isomers. The evidence for similar effects and endpoints among methyl-substituted aromatic compounds seems to be much stronger than what has been presented so far. The summary table (Page 1-49, Table 1-7) is very helpful in understanding the points made with regard to toxic effects. A summary table or scheme regarding toxicokinetics and

metabolism would also be useful. Section 1.1.7, which focuses on the toxicokinetic similarities among TMB isomers, would be improved by summarizing in a table or scheme both the similarities and differences among the isomers in toxicokinetics and metabolism.”

Section 3.2.5, page 27, lines 22-25: “Among those who agreed with a UFD of 3, some found the justification provided by the EPA to be satisfactory, **while others thought that toxicity data available for C-9 mixtures should contribute to the rationale to lower the value from the default of 10.** Others **disagreed with including C-9 mixture data as relevant to the database UF.** (See Section 3.2.3).”

Section 3.3.1, page 36, lines 4-15: “Based upon the available MOA information, the developmental factors which may influence toxicokinetics can be discussed. For TMBs the draft document assumes that the parent compound is responsible for toxicity with modeling assuming that a saturable Phase I oxidative Cytochrome P450 (CYP) process is responsible for decreasing parent compound levels in venous blood. This section should state whether it is known which CYP(s) are responsible for TMB saturable metabolism as different CYPs have different developmental patterns. **Analogy may be drawn with other alkylbenzenes which do have toxicokinetic modeling data in early life such as toluene.** Toluene has already been referred to in the mode of action section of the document; it is also neurotoxic and its mode of action is based upon parent compound with the level getting to the brain determined by saturable CYP metabolism. If the EPA determines these parallels to provide a useful analogy, then early life modeling papers for toluene by Pelekis et al. (2001) and Nong et al. (2006) may be useful for describing the degree of toxicokinetic uncertainty presented by early life stage exposure to TMBs.”

- 6) In two excerpts from the draft report (extracted below with emphasis added in bold), the panel provides statements regarding mixtures. EPA would like to clarify that the statement in the Preamble regarding the preference of studies on mixtures in an assessment of mixtures is a general statement conveying the importance of having data specifically related to the component(s) that are being assessed. The IRIS assessment for TMBs is not intended to be a mixtures assessment. These isomers were nominated for assessment by the offices within the Agency primarily based on their presence at Superfund sites. A mixtures assessment of TMBs (with or without other methyl-substituted aromatics) was not requested as that may not be reflective of human exposure to TMBs at these sites.

Section 3.2.3, page 16, Lines 28-36: “However, the decision to disregard or limit consideration of the studies on solvent mixtures containing, but not limited to, the TMBs appears to have affected the synthesis of evidence. Because important toxicological observations have been made in both animal and human studies involving exposure to aromatic solvent mixtures, an important toxicological perspective was lost. The IRIS Preamble, in Section 3.1 on identifying studies (lines 44-47) specifically states, **“In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components.”** The SAB concluded that the TMB assessment was prepared because human exposures to the TMBs generally involve complex solvent mixtures, the available studies on mixtures, including the C-9 fraction and white spirit, deserve further consideration and explanation in the TMB assessment.”

Section 3.2.3, page 17, lines 10-17: “The testing of the C-9 fraction reveals another important point. Because this mixture, as tested, was about half TMBs, much of the observed effects could have been due to the TMBs. Competition for metabolic clearance would likely have increased duration of exposure to the TMBs, so the minimal observed toxicity in several C-9 studies provides important perspective to the TMB evaluation (although both positive and negative interactions are possible). **Since it can be assumed that an application of the IRIS assessment for TMBs is for evaluating potential risks from exposure to the C-9 solvent and related aromatic mixtures**, The SAB suggests that the observation of effects of such mixtures is certainly relevant, and needs further discussion.”

- 7) In several excerpts from the draft report (extracted below with emphasis added in bold), the panel provides statements regarding the derivation of additional candidate reference values using available inhalation and oral studies and compare to the values derived using the PBPK approach. EPA would like to clarify that the Saillenfait study was used to develop candidate RfCs for 1,3,5-TMB, and that the PBPK model was not used in the RfC derivation for 1,3,5-TMB. Is the panel requesting the derivation of further isomer-specific RfCs in addition to the ones currently derived in the draft assessment?

Letter to the EPA Administrator, page 2, lines 28-34: “There are inhalation and oral toxicology studies for 1,3,5-TMB and the **analyses of these studies should be expanded to develop candidate reference values for other endpoints than the critical effect the EPA selected**. The SAB notes that the endpoints for these studies are not the same neurotoxicological effects used in the PBPK approach for 1,2,4-TMB. **The SAB recommends that the agency derive a reference concentration and reference dose for 1,3,5-TMB using available toxicology studies for 1,3,5-TMB** and compare those results to the reference concentrations and reference doses developed for 1,3,5-TMB using the PBPK approach extrapolating from 1,2,4-TMB.”

Executive Summary, page 2, lines 18-24: “**The inhalation study conducted by Saillenfait** and the oral study by Koch and later by Adenuga provide additional insights in the toxicology of these isomers. The SAB notes that the endpoints for these studies are not the same neurotoxicological effects used in the PBPK approach used for 1,2,4-TMB. **The SAB recommends that the agency derive a candidate RfC and RfD for 1,3,5-TMB using available inhalation and oral dosing toxicology studies for 1,3,5-TMB and compare those results to the approach the EPA used to develop the RfCs and RfDs for 1,3,5-TMB** using the PBPK approach extrapolating from 1,2,4-TMB.”

Section 3.2.7, page 29, lines 33-35: “...the SAB **recommends that the EPA revise and expand the discussion section to calculate fetal and maternal endpoint-based candidate gestational RfCs** for a comparison to the neurotoxicological-based RfC consistent with IRIS policy.”