

**Science Advisory Board (SAB) Draft Report (December 22, 2014) to Assist  
Meeting Deliberations -- Do Not Cite or Quote –**

This draft is a work in progress, does not reflect consensus advice or recommendations, has not been reviewed or approved by the chartered SAB and does not represent EPA policy.

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EPA-SAB-15-xxx

The Honorable Gina McCarthy  
Administrator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, D.C. 20460

Subject: Science Advisory Board Review of the IRIS Draft Toxicological Review of  
Trimethylbenzenes

Dear Administrator McCarthy:

The U.S. Environmental Protection Agency's National Center for Environmental Assessment requested a peer review of the scientific basis for the draft *Toxicological Review of Trimethylbenzenes* developed for the agency's Integrated Risk Information System. The assessment is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to three trimethylbenzene isomers: 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB.

In April 2011, the National Research Council released its *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde* and included comments and recommendations for improving the development of IRIS assessments in general. The *Toxicological Review of Trimethylbenzenes* is one of the first IRIS assessments to address the NRC recommendations for improving the development of IRIS assessments. The SAB was asked to review the scientific and technical analyses used to develop reference concentrations and reference doses for the three trimethylbenzene isomers and to comment on the agency's enhancements to the IRIS Program in response to the implemented to address the NRC recommendations. The SAB Chemical Assessment Advisory Committee was augmented with additional toxicological experts to conduct this review.

The SAB is aware that the agency is taking a phased approach to address the NRC recommendations for several assessments near the end of their development focusing on streamlining the documents, increasing the transparency and clarity of the assessment, and better presenting the data and information considered through the use of standard tables, editing and formatting. The SAB acknowledges the improvement in the new format for IRIS assessments and commends the agency for its progress in addressing the NRC recommendations. The SAB recognizes that the TMB assessment was under development and implements the first phase of the agency's efforts to enhance the IRIS process and looks forward to reviewing future IRIS assessments with additional enhancements. The SAB used the *Toxicological Review of Trimethylbenzenes* as a case study to provide advice and comments on

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1 improving IRIS toxicological assessments. Specific comments on developing the preamble and  
2 executive summary are provided in the enclosed report. The SAB also found that the tables and  
3 presentation of data and information considered are an improvement and provided specific suggestions  
4 to improve the presentations for hazard identification and dose-response analyses. The SAB anticipates  
5 that after several IRIS reviews are completed, the Chemical Assessment Advisory Committee will  
6 compare the reviews to provide the agency, through the Chartered SAB, with advice and comments on  
7 the agency's progress to enhance IRIS assessments.

8  
9 The SAB agrees with the agency that physiologically based pharmacokinetic (PBPK) modeling is an  
10 appropriate approach to developing reference concentrations and reference doses. When implementing a  
11 PBPK modeling approach the SAB strongly recommends that the EPA provide a transparent and  
12 detailed discussion of the rationale for selecting this approach. The discussion should include the  
13 available studies, data, and information considered by the agency, how these data were compared and  
14 considered, and why these analyses led the agency to use a PBPK approach rather than chemical-  
15 specific studies.

16  
17 The EPA should conduct independent peer review of the PBPK model and modeling results if it is a new  
18 version, previously unpublished or is a modification of a published model. In the enclosed report the  
19 SAB conducts a review of the PBPK model and provides specific recommendations to improve the use  
20 of modeling for trimethylbenzenes.

21  
22 The SAB finds that the physiologically based pharmacokinetic modeling approach and extrapolating  
23 inhalation data to an oral exposure is appropriate for the reference concentration and reference dose for  
24 1,2,3-TMB 1,2,4-TMB and 1,3,5-TMB. However, the presentation of the analysis should be expanded to  
25 better describe the inhalation and oral toxicology studies considered and rationale for using the PBPK  
26 model.

27  
28 There are inhalation and oral toxicology studies for 1,3,5-TMB and the analyses of these studies should  
29 be expanded to develop candidate reference values for other endpoints than the critical effect the EPA  
30 selected. The SAB notes that the endpoints for these studies are not the same neurotoxicological effects  
31 used in the PBPK approach for 1,2,4-TMB. The SAB recommends that the agency derive a reference  
32 concentration and reference dose for 1,3,5-TMB using available toxicology studies for 1,3,5-TMB and  
33 compare those results to the reference concentrations and reference doses developed for 1,3,5-TMB  
34 using the PBPK approach extrapolating from 1,2,4-TMB.

35  
36 The SAB finds that the evidence for carcinogenicity of trimethylbenzenes, although limited, was well  
37 presented by the EPA in the draft toxicological review and the SAB agrees that the EPA could not  
38 conduct a quantitative cancer assessment for any of the TMB isomers due to the lack of appropriate  
39 studies.

40  
41 The SAB also notes that there is a limited discussion in the draft TMB assessment of sensitive life stages  
42 and vulnerable populations for the TMB assessment due to lack of data on the toxicological responses in  
43 these populations. The SAB encourages the agency to expand the description and importance of these  
44 analyses in future assessments.

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1  
2 Regulatory agencies are frequently required to address risks associated with short-term exposures. The  
3 principal studies used to derive the proposed reference concentrations and reference doses for the TMBs  
4 are subchronic in duration and the analysis needed to generate subchronic reference concentrations and  
5 reference doses has already been done. Given the usefulness of subchronic toxicity values and the small  
6 amount of additional work need to add them to the Toxicological Review the SAB recommends that the  
7 review be expanded to include the presentation of subchronic reference concentrations and reference  
8 doses.

9  
10 The SAB appreciates the opportunity to provide the EPA with advice and looks forward to the agency's  
11 response.

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14 Sincerely,

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20  
21 Enclosure  
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**NOTICE**

This report has been written as part of the activities of the EPA Science Advisory Board (SAB), a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The SAB is structured to provide balanced, expert assessment of scientific matters related to problems facing the agency. This report has not been reviewed for approval by the agency and, hence, the contents of this report do not represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names of commercial products constitute a recommendation for use. Reports of the SAB are posted on the EPA website at <http://www.epa.gov/sab>

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**U.S. Environmental Protection Agency  
Science Advisory Board  
Chemical Assessment Advisory Committee Augmented for  
Review of the Draft IRIS Trimethylbenzene Assessment**

**CHAIR**

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12 Mr. Thomas Carpenter, Designated Federal Officer, U.S. Environmental Protection Agency,  
13 Washington, DC

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<sup>1</sup> Dr. Krishnan was unable to participate on the Chemical Assessment Advisory Committee Augmented for Review of the Draft IRIS Trimethylbenzene Assessment after the June 2014 Meeting.

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**Acronyms and Abbreviations**

|    |           |   |
|----|-----------|---|
| 1  |           |   |
| 2  |           |   |
| 3  | ADME      | absorption, distribution, metabolism, and excretion                         |
| 4  | BMD       | benchmark dose  |
| 5  | C-9       | aromatic hydrocarbon fraction including ethyltoluenes and trimethylbenzenes |
| 6  | CNS       | central nervous system  |
| 7  | CYP450    | cytochrome P450   |
| 8  | EPA       | U.S. Environmental Protection Agency  |
| 9  | GD        | gestational day   |
| 10 | HEC       | human equivalent concentration  |
| 11 | HERO      | Health and Environmental Research Online                                    |
| 12 | IRIS      | Integrated Risk Information System  |
| 13 | $K_m$     | Michaelis-Menten constant   |
| 14 | LOAEL     | lowest-observed-adverse-effect level  |
| 15 | MOA       | mode of action  |
| 16 | NCEA      | National Center for Environmental Assessment                                |
| 17 | NOAEL     | no-observed-adverse-effect level  |
| 18 | NRC       | National Research Council   |
| 19 | ORD       | Office of Research and Development  |
| 20 | PBPK      | physiologically based pharmacokinetic                                       |
| 21 | PC        | partition coefficients  |
| 22 | POD       | point of departure  |
| 23 | ppm       | parts per million   |
| 24 | RfC       | reference concentration   |
| 25 | RfD       | reference dose  |
| 26 | SAB       | Science Advisory Board  |
| 27 | SD        | standard deviation  |
| 28 | TMB       | trimethylbenzene  |
| 29 | UF        | uncertainty factor  |
| 30 | $UF_A$    | interspecies uncertainty factor   |
| 31 | $UF_H$    | intraspecies uncertainty factor   |
| 32 | $UF_S$    | subchronic-to-chronic uncertainty factor                                    |
| 33 | $UF_L$    | LOAEL-to-NOAEL uncertainty factor   |
| 34 | $UF_D$    | database deficiency uncertainty factor                                      |
| 35 | $V_{max}$ | maximum rate of uptake/conversion   |
| 36 |           |   |

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**1. EXECUTIVE SUMMARY**

The Environmental Protection Agency’s (EPA) National Center for Environmental Assessment (NCEA) requested the Science Advisory Board to conduct a peer review of the draft *Toxicological Review for Trimethylbenzenes* (August 2013) developed by the Integrated Risk Information System (IRIS) program hereafter referred to as the TMB assessment. This assessment reviews the publicly available studies on the three isomers of trimethylbenzene (i.e., 1,2,3-trimethylbenzene [1,2,3-TMB], 1,2,4-trimethylbenzene [1,2,4-TMB], and 1,3,5-trimethylbenzene [1,3,5-TMB]) and identifies the adverse health effects to characterize inhalation and oral exposure-response relationships for each isomer. This assessment was prepared because of the presence of trimethylbenzenes (TMBs) at Superfund sites. Of the 38 sites on the EPA’s National Priorities List that report TMB isomer contamination (38 sites), 93 percent report 1,3,5-TMB contamination, 85 percent report 1,2,4-TMB contamination, 12 percent report 1,2,3-TMB contamination, and 17 percent report contamination by unspecified TMB isomers.

The National Research Council (NRC), in its *Review of the Environmental Protection Agency’s Draft IRIS Assessment of Formaldehyde*, also provided recommendations for improving the development of IRIS assessments in general. The *Draft Toxicological Review of Trimethylbenzenes* is one of the first IRIS assessments to address the NRC recommendations.

For the current review, the SAB was asked to review the scientific and technical analyses used to develop reference concentrations (RfC) and reference doses (RfD) for the three TMB isomers and to comment on the agency’s enhancements made to the IRIS Program in response to the NRC recommendations. The SAB Chemical Assessment Advisory Committee was augmented with additional toxicological experts to conduct the review.

***Enhancements to the IRIS Program***

The agency implemented a phased approach to address the NRC recommendations for several assessments near the end of their development focusing on streamlining the documents, increasing the transparency and clarity of the assessment, and better presenting the data and information considered through the use of standard tables, editing and formatting. The SAB acknowledges the improvement in the new format for IRIS assessments and commends the agency on the progress it has made in addressing the NRC recommendations. The SAB recognizes that the TMB assessment was developed “mid-stream” in the EPA’s efforts to enhance the IRIS process and looks forward to reviewing future IRIS assessments with additional enhancements. Based on its review of the draft TMB assessment, the SAB provides recommendations on ways to further enhance IRIS toxicological assessments. For example, comments are provided on developing the preamble and executive summary. The SAB also found that the tables and presentation of data and information considered are an improvement and provided specific suggestions to improve the presentations for hazard identification and dose-response analyses. The SAB anticipates that after several IRIS reviews are completed the Chemical Assessment Advisory Committee will compare the reviews to provide the agency with advice and comments through the Chartered SAB, on the agency’s progress to enhance the IRIS program’s assessments.

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***Chronic Hazard Assessment of Trimethylbenzenes***

The SAB agrees with the agency that physiologically based pharmacokinetic (PBPK) modeling is an appropriate approach to developing RfCs and RfDs. When implementing a PBPK approach, the SAB strongly recommends that the EPA clearly discuss the available studies, data, and information considered by the agency, how these were considered, and why these analyses led the agency to use a PBPK approach rather than specific studies for the TMBs. Whenever the agency uses a PBPK model that involves a new or modified PBPK model, the agency should commission an independent peer review of the model, assumptions made in the modeling, the model's fit to PK datasets, model predictions and the model's application in the risk assessment. In the enclosed report the SAB conducts a review of the PBPK model and provides specific recommendations to improve the use of modeling for trimethylbenzenes. A review the PBPK modeling used to develop is provided in Appendix B of this report. The SAB report provides specific recommendations to improve the use of modeling for TMBs.

The SAB finds that the PBPK modeling approach, which extrapolates inhalation data to an oral exposure, is appropriate for the RfC and RfD for 1,2,3-TMB, 1,2,4-TMB and 1,3,5-TMB. However, the SAB notes that the presentation of the analysis should be expanded to better describe the inhalation and oral toxicology studies considered and the rationale for using the PBPK model and extrapolation approach over the studies considered. 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.

The SAB finds that while the search strategy and rationale to select studies was clearly articulated the exclusion criteria and implementation of those criteria was not as transparent. 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.

The SAB concludes that the EPA's hazard assessment of the carcinogenicity of TMBs integrates all available scientific evidence and agrees with the EPA that there is "inadequate information to assess the carcinogenic potential" of TMBs. The carcinogenicity of 1,2,4-TMB has been assessed in only a single study. The EPA found that there were a number of deficiencies concerning this bioassay and the SAB agrees that there are not sufficient data to conduct a quantitative assessment. The SAB also notes that no carcinogenicity bioassays have been conducted with 1,2,3-TMB or 1,3,5-TMB.

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1 ***Sensitive Life Stages and Subchronic Reference values***

2 In addition to responding to the EPA Charge Questions, the SAB identified two additional topics that  
3 warrant further consideration by the agency: (1) an expanded discussion of sensitive life stages and  
4 vulnerable populations; and (2) the benefit of publishing the subchronic RfC and RfD that were  
5 developed using the analysis for the TMB isomers.

6  
7 There is a limited discussion of sensitive life stages and vulnerable populations in the preamble. The  
8 SAB recognizes that there is limited information available for TMBs. However, the SAB encourages the  
9 agency to expand the description and importance of these analyses in the hazard identification and dose-  
10 response for sensitive life stages and vulnerable populations in future toxicological assessments.

11  
12 The SAB notes that the agency's derivation of chronic RfCs and RfDs for the TMBs is built upon  
13 subchronic values. The principal studies used to derive the proposed RfCs and RfDs are all subchronic  
14 in duration, and the analysis needed to support a robust set of subchronic toxicity values has in effect  
15 already been done. The toxic endpoints and dose-response relationships are clearly relevant for  
16 subchronic exposure, and the same points of departures would apply to the development of a set of  
17 subchronic RfCs and RfDs. Given the potential usefulness of these toxicity values for risk assessment,  
18 the importance of having the values available on IRIS, and the very small amount of additional work  
19 required to add them to the TMB Assessment, the SAB recommends that the review be expanded to  
20 include the presentation of subchronic RfCs and RfDs for 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB.

21

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**2. INTRODUCTION**

**2.1. Background**

The EPA requested a peer review of the scientific basis supporting the draft *Toxicological Review of Trimethylbenzenes (1,2,3-trimethylbenzene [1,2,3-TMB], 1,2,4-trimethylbenzene [1,2,4-TMB], and 1,3,5-trimethylbenzene [1,3,5-TMB])* that will appear on the agency's online database, the Integrated Risk Information System (IRIS). This is a new assessment; there is currently no entry in the IRIS database for any isomer of trimethylbenzene. The SAB Chemical Assessment Advisory Committee was augmented with additional toxicological experts to conduct this review.

IRIS is a human health effects assessment program that evaluates scientific information on effects that may result from exposure to specific chemical substances in the environment. IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). Through IRIS, the EPA provides science-based human health assessments to support the agency's regulatory activities and decisions to protect public health. IRIS assessments contain information for chemical substances that can be used to support the first two steps (hazard identification and dose-response assessment) of the human health risk assessment process. When supported by available data, IRIS provides health effects information and toxicity values for chronic health effects (including cancer and effects other than cancer). Governments and others combine IRIS toxicity values with exposure information to characterize public health risks of chemical substances; this information is then used to support risk management decisions designed to protect public health.

The draft *Toxicological Review of Trimethylbenzenes* (August 2013), hereafter referred to as the TMB Assessment, is based on a review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. This draft IRIS assessment includes:

- a Preamble to describe the methods used to develop IRIS assessments;
- an Executive Summary to concisely summarize the major conclusions of the assessment;
- a Literature Search Strategy and Study Selection section to describe the process for identifying and evaluating the evidence for consideration in developing the assessment;
- a Hazard Identification section to systematically synthesize and integrate the available evidence of organ/system-specific hazards; and
- a Dose-Response Analysis section to describe the selection of studies for consideration in calculating toxicity values and to provide details of the analysis and methodology in deriving and selecting toxicity values.

In addition the draft TMB Assessment includes appendices on chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and a summary of the public comments received on the May 2012 draft. The draft assessment was developed according to guidelines and technical reports published by the EPA and contains a qualitative characterization of the hazards for the

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1 TMBs, including a cancer descriptor of the isomers' human carcinogenic potential, and noncancer  
2 toxicity values, including a chronic oral RfD and a chronic inhalation RfC for all three TMB isomers. A  
3 quantitative cancer assessment for TMBs was not conducted due to inadequate data.

4 **2.2. Charge to the Science Advisory Board**

5 The draft TMB Assessment is one of the first IRIS assessments to address the NRC recommendations  
6 for improving the development of IRIS assessments. Therefore the EPA charge for this peer review was  
7 two-fold and requested: (1) a review of the scientific and technical analyses used to develop RfCs and  
8 RfDs for the three TMB isomers; and (2) advice and comment on the enhancements the IRIS Program  
9 implemented to address the NRC recommendations.

10  
11 The agency asked three general questions about the agency's progress in response to the NRC  
12 recommendations. In April 2011, the NRC released its *Review of the Environmental Protection*  
13 *Agency's Draft IRIS Assessment of Formaldehyde* (NRC 2011). In addition to offering comments  
14 specifically about the EPA's draft formaldehyde assessment, the NRC included comments and  
15 recommendations for improving the development of IRIS assessments. Generally, the IRIS Program's  
16 implementation of the NRC recommendations is following a phased approach. Phase 1 of  
17 implementation has focused on a subset of the short-term recommendations, such as editing and  
18 streamlining documents, increasing transparency and clarity, and using more tables, figures, and  
19 appendices to present information and data in assessments. The Phase 1 implementation was applied to  
20 assessments, including the draft TMB Assessment, that had been near the end of the development  
21 process. Additional NRC recommendations will be implemented in future IRIS assessments with input  
22 and feedback from the public, stakeholders, and external peer review committees. This phased approach  
23 is consistent with the NRC's Roadmap for Revision as described in Chapter 7 of the formaldehyde  
24 review report (NRC 2011).

25  
26 The EPA Charge to the SAB includes one question about the response to public comments on the May  
27 2012 TMB Assessment and specific questions about the scientific and technical approaches aspect used  
28 to develop the RfC and RfD for each of the individual isomers. Because the agency uses PBPK  
29 modeling and extrapolation of data for 1,2,4-TMB to develop the RfC and RfD for 1,2,3-TMB and  
30 1,3,5-TMB, the responses to questions often raised similar if not identical issues and advice for the  
31 agency to consider. Where appropriate this report refers the reader to issues discussed in previous  
32 sections rather than repeating the same information and advice for each isomer.

33  
34 During deliberations the SAB identified two issues that were not addressed in the EPA charge to the  
35 SAB. First, the toxicological review provided a limited discussion of health effects from exposure to  
36 vulnerable life stages. Second, the approach used to develop the RfC and RfD in this assessment built  
37 upon developing a subchronic RfC and RfD for each of the isomers and applying uncertainty factors to  
38 arrive at a chronic value for inhalation and ingestion. The SAB provides recommendations for these  
39 issues after the response to the EPA charge. Charge questions are included in italics at the beginning of  
40 each response of this report and the full charge is included as Appendix A.

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**3. RESPONSE TO CHARGE QUESTIONS**

**3.1. Enhancements to IRIS Assessments**

**3.1.1. Preamble Enhancements**

*Charge Question: NRC (2011) indicated that the introductory section of IRIS assessments needed to be expanded to describe more fully the methods of the assessment. NRC stated that they were “not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear, concise statements of criteria used to exclude, include, and advance studies for derivation of [toxicity values].” Please comment on whether the new Preamble provides a clear and concise description of the guidance and methods that EPA uses in developing IRIS assessments.*

To a substantial degree, the Preamble as currently written provides a concise and clear description of the process that is followed, its steps, the places in the process where decisions or judgments are made, the guidance that applies to making those judgments (with explanation of the main considerations and available choices), and the process by which the results of each step feed into the next. The Preamble certainly should be no longer; as it stands, it is near the limit of what can serve as an overview and explanation. The Preamble is composed of three sections: The Scope (Section 1), the overall IRIS Process for developing and reviewing assessments (Section 2), and the particulars of how an assessment is executed (Sections 3 to 7).

The description of "Scope of the IRIS Program" (Section 1) is brief and clearly describes the IRIS program, but the SAB notes that it lacks any overarching statement about what IRIS seeks to accomplish, its ultimate purposes, and what its assessments are meant to represent to their users.

In view of the partial implementation of reforms to the overall process, the SAB presumes that the Preamble will change from one assessment to the next to reflect newly adopted procedures. The SAB recommends that it would be useful to note places where the present assessment has not yet fully implemented changes that are already planned for application to subsequent assessments. If the motivations (in terms of enhancing transparency, objectivity, and sound analysis) for future changes can be borne in mind and addressed, the overall revision of the IRIS process will be smoothed. Furthermore, assessments done before the process is complete will gain credibility and longevity.

The SAB finds that Section 2 on the IRIS Process is clear and concise in the description of the seven steps to develop assessments and the multiple levels of review and what happens at each one. It is rather vague, however, on the nature of the problem formulation step. The SAB recommends that it include some discussion (without seeking to constrain the agency's further actions) about the issues needing to be addressed, the prospects for addressing them with available data, and the uncertainties and plausible alternative interpretations that would need to be worked through. Although the section clearly indicates that the agency will prepare a summary record of response to peer review comments, it is not clear who will be the ultimate EPA decision-maker. The discussion of Step 5 notes that newly published studies

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1 that are critical to conclusions can be brought into consideration, but a more explicit reference to the  
2 stopping rule policy (and where its details can be found) would be appropriate.

3  
4 The SAB recommends that the agency take measures to ensure that the Preamble in this and future  
5 assessments be structured so that it refers the reader to the appropriate guidance and cannot be construed  
6 to contradict policy by over summarizing existing guidance.

7  
8 Sections 3 to 7 lay out the specific steps for conducting an assessment. A good deal of the material is a  
9 summary of long-extant guidance, and so will be familiar to most readers. The Preamble purpose does  
10 require setting out the processes and analyses, but perhaps this could be done somewhat more briefly.  
11 On the other hand, the aspects that are under revision need to be flagged or expressed in more general  
12 terms. In particular, the Preface notes that Phase 3 of the IRIS revision process is yet to happen, and this  
13 will include review of current methods for weight-of-evidence analysis. The methods for abstracting  
14 data, systematically considering study quality and interpretation issues, and ranking relative study  
15 impact are also in transition.

16  
17 Section 5.5 references the carcinogen classification scheme of the 2005 Carcinogenicity Assessment  
18 guidelines. The SAB notes that the same section also cites the Integrated Science Assessment criteria for  
19 causality (applied in the evaluation of criteria air pollutants) as "another example" and, further on in the  
20 Preamble it is noted that the agency is investigating what descriptors to use and may use these or others.  
21 The SAB is concerned that this may produce confusion as to what guidelines for assessment of causality  
22 apply to the current trimethylbenzenes assessment and whether the IRIS revision process is anticipating  
23 a revision or expansion of such guidance in the future. The SAB finds that, at this juncture, discussing  
24 the intent of descriptors is probably more useful than recounting definitions that may or may not be used  
25 and may or may not be seen as in keeping with the spirit of the overall revision process.

26  
27 It should be clear that the Preamble itself is not guidance; it only summarizes guidance that is set out  
28 elsewhere; an unambiguous statement to this effect should be added. This is especially critical because --  
29 being only summaries and explanations -- the treatment in the Preamble is less developed and is  
30 unaccompanied by the full guidance's discussion about motivation, meaning, interpretation, and  
31 scientific justification of the briefly described analyses, presumptions, standards, or judgments. Without  
32 reference to the fuller treatment, the SAB is concerned that there is danger that an oversimplified version  
33 may be mistaken for policy. In some places where existing guidance is described and explained, there is  
34 a citation to the full guidance document, but in many spots, there is no such citation. The SAB finds that  
35 more care in providing citations to the operative guidance documents is necessary if the Preamble is to  
36 adhere to the distinction between established policy and explanation. Citations also give readers an  
37 indication that there is a fuller description of the issue to be found and where to find it.

38  
39 Some precepts articulated in the Preamble appear to the SAB as not consistent with existing EPA  
40 guidance or announced policy. This raises questions about whether the agency is changing policy from  
41 established guidance and whether such changes have been appropriately vetted, and implemented.  
42 Several statements seem to be outside of existing guidance and are provided as examples:

- 43 • p. xxii, line 67 that negative genetic toxicity studies carry less weight than positive ones;
- 44 • p. xxiii, line 78 that funding source can downgrade the credibility of studies;

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- the organ-specific reference values on p. xxx, line 32;
- and the dismissal of specificity as an aspect of causal analysis on p. xx, line 81.

The SAB finds that all of these issues are important and should be discussed. However, they should be supported with citations to existing policy or guidance. If these are not existing policy, then they should be flagged as matters under discussion. In view of the incremental alterations that are expected to occur in IRIS assessments over a series of assessment documents, it is important that any changes to the Preamble from assessment to assessment – especially those that could be construed as altered guidance or standards for future data interpretation – be considered carefully and called out for attention in document reviews.

In summary the SAB recommends that:

- the document should point out places where the course of its analysis touches on aspects of the more general IRIS review process that have not yet been implemented, but for which further development is planned; and
- the Preamble should make clear that it's summary of relevant guidance does not supersede that guidance, and it should provide adequate citation to that guidance.

### **3.1.2. Presenting Assessment Steps and Outcomes**

*Charge Question: NRC (2011) provided comments on ways to improve the presentation of steps used to generate IRIS assessments and indicated key outcomes at each step, including systematic review of evidence, hazard identification, and dose-response assessment. Please comment on the new IRIS document structure and whether it will increase the ability for assessment to be more clear, concise and easy to follow.*

The objective should be to make it possible to read the document in three different modes:

1. quickly to get the main qualitative and quantitative conclusions and, in general terms, their bases;
2. somewhat more thoroughly, but still rapidly, to get a good picture of the kinds of data and toxicity phenomena that were considered (not just those that were chosen as critical or as bases for quantification), the main features and issues involved in the interpretation, the choices that were made (and the nature of the main alternatives) and the main rationale for the choices; and
3. in detail, to efficiently find the particulars of study features and data, their analysis and the detailed reasoning behind their interpretation.

In short, the reader should easily find the (1) the conclusions, (2) the choices and reasoning applied when reaching them, and (3) the fully explained justifications of choices, respectively.

The SAB finds that the structure in the TMB document does well at the first, in the form of the Executive Summary. The leading section on "Occurrence and Health Effects" is useful as a context for the particulars that follow. A good balance between brevity and depth is struck.

The second way of reading -- for the choices and reasoning -- has also markedly improved, though some suggestions can be made, as discussed below.

The third way of reading -- the examination of particulars and the ability efficiently to find them documented with sufficient detail -- is much improved from former IRIS documents. The relegation of a

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1 lot of the details to well-structured appendices is helpful. The set of focused appendices helps a reader to  
2 find the place where particular study aspects or analyses of issues are to be found. The organization of  
3 the appendices -- and the consistency of presentation across IRIS documents -- are important in making  
4 the place to find details clear. Although the general structure of the appendix entries can be discerned,  
5 the plans for the structure and consistency have not been provided, so it will take some time and  
6 examination of other documents following the same plan for readers to find things easily. The use of  
7 appendices simultaneously allows presentation of more detail than may have been captured in earlier  
8 generations of IRIS documents and also avoids cluttering the main body of the IRIS document -- where  
9 interpretation and evaluation are considered. The appendix approach also frees the main document from  
10 seeming to need to present all the details before drawing any interpretation.

11 ***Consistent Presentation of the Studies Considered***

12 The SAB recommends that each study should be in a consistently formatted table. The table should be in  
13 an appropriate appendix and present the study-specific considerations that bear on evaluation of study  
14 quality and pertinence, including shortcomings and assumptions that are needed to interpret the study's  
15 outcomes. Consistency of format is important within each document, but it would also be a useful goal  
16 to achieve from one IRIS assessment to another.

17  
18 The SAB suggests that it would also be useful for each study to have a short overview section (also in its  
19 appendix listing, not repeating tabulated details) of the nature of the study, its examined endpoints, and  
20 relevant findings. The goal of the overview is to provide context for the tabulated details, so that the  
21 details need not be read in full to gain an idea of the general nature of the study and its importance to the  
22 assessment as a whole. This overview should not discuss interpretations.

23  
24 It is clear that the intent of this structure is to free the main document to focus on choices that were made  
25 in the analysis (selection of possible endpoints, selection of studies to represent and characterize those  
26 endpoints, and analyses and interpretations of their bearing on human risk estimation). The challenge is  
27 to bring the appropriate data and level of detail from the appendices into the main body, so that the  
28 interpretations and choices can be justified and documented, without overwhelming the interpretation  
29 discussion or leaving out potentially relevant information. Sorting this out is the essence of the  
30 systematic review process, and though clear strides have been made, more work is left to be done. The  
31 SAB suggests that, as the EPA develops its enhancements to the IRIS reviews, the key to this process is  
32 to be transparent regarding both studies chosen for inclusion and those chosen for exclusion; not just  
33 what supports an interpretation, but also what seems unexplained or even inconsistent. Studies results  
34 should be cited when they are as consistent with a hypothesized potential for human risk, and also when  
35 they have apparently contrary results with different implications for scientifically supportable inference  
36 about human risk impacts.

37  
38 The SAB finds that the overall structure of the main report provides a good framework -- with sections  
39 on literature search, hazard identification for the various candidate endpoints, and dose-response.

40 ***Describing the Literature Search***

41 The Literature Search Strategy section is brief and focuses only on identification of pertinent studies  
42 from the literature. The SAB is concerned that the general description of the process and the specific

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1 implementation for TMBs may be too exclusive, missing potentially informative ancillary studies that  
2 could help in interpretation or evaluation of those studies strictly observing toxicity outcomes of the  
3 TMBs alone in controlled settings. It should be clear that literature search is only the first step of  
4 systematic review, which needs to be followed by evaluation of each study in terms of design, quality,  
5 shortcomings, main findings (including both positive and negative findings), evaluation of the reliability  
6 of individual study results, and identification of other studies, particularly on mechanisms, that could  
7 address uncertainties in the primary database. This supports a further process of comparing results  
8 across studies to assess both the consistency of specific effects, and also the manifestation of related  
9 effects that would be expected from hypothesized underlying causative processes, both of which bear on  
10 the use of specific study results as evidence regarding the existence and nature of hazards in human  
11 target populations.

12 ***Describing the Hazard Identification Steps***

13 The individual endpoint sections of the Hazard Identification have some discussion about interpretation  
14 across studies and evaluations of bearing and relevance, though further discussion of interpretation  
15 rationales and consideration of alternatives would be beneficial. The SAB finds that it is the middle  
16 section of systematic review -- after the studies are chosen but before the interpretation of their overall  
17 bearing gets considered -- that does not have a clear home in the current document structure. As the  
18 agency develops its approach for systematic review, including defined ways for abstracting data, judging  
19 study quality, documenting factors bearing on interpretation and its limits, and considering the impact of  
20 related studies, it will be important to develop the document structure that encompasses all aspects for  
21 consideration. The SAB notes that the Preamble has a section (Section 5) on evaluation of causality,  
22 which depends on the existence of such a documented review and evaluation process, but the present  
23 document has no particular place where the Preamble's named considerations -- strength, consistency,  
24 specificity, temporal relationship, biologic plausibility, coherence, natural experiments, and analogy --  
25 are systematically considered or documented.

26  
27 The SAB recommends adding a brief summary of the main features of pharmacokinetics and  
28 metabolism before the section on Hazard Identification. The aim is not to replace the fuller treatment of  
29 these issues in the appropriate appendix, but rather to set the context for the interpretation of studies  
30 bearing on hazard, and the main presentation of pharmacokinetic details should continue to reside in the  
31 appendix. The main text's section would note such things as extent of absorption, rapidity of elimination,  
32 main metabolic processes, main means of clearance (and what part of that is by metabolism), indications  
33 whether metabolic saturation or enzyme induction might play a relevant role in toxicity studies, and any  
34 notable unusual differences between experimental animals and humans. Again, the point would not be  
35 just to list specifics (which can remain in an appendix) but to provide the basic insights that might bear  
36 on how toxicity data are interpreted or on the limits to such interpretation.

37  
38 A noteworthy change from earlier IRIS assessments is that the Hazard Identification section is separated  
39 into assessments of each endpoint, with relevant data for that endpoint being reviewed within the  
40 section. The SAB finds that this approach is a great improvement over the past practice of summarizing  
41 study by study, with all the endpoints clustered study. The endpoint-by-endpoint analysis permits the  
42 examination of consistency and sufficiency of data to draw hazard conclusions about each effect.

43

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1 This being said, there are possible overarching ties among endpoints that would help in evaluation of the  
2 hazard characterization of each (say, commonalities of dosimetry or mode-of-action) that should be  
3 discussed in an appropriate place. It would be useful to include considerations that might indicate a  
4 study as the critical study.

5  
6 The tabulation of studies is useful, and the dose levels and dose-specific responses are important details  
7 to include. The hyperlinks to the detailed description of studies in the appendices helps to make those  
8 appendices directly supportive and makes finding of relevant information more efficient. The exposure-  
9 response arrays are useful summary devices to aid communication, though they should not be read as  
10 meta-analysis forest plots or otherwise be used as the primary basis of conclusions. Nonetheless, they  
11 provide a valuable overview of the data. It is perhaps unfortunate that it is difficult to preserve the  
12 distinction between studies on a given effect (especially if the studies appear to disagree) and also that  
13 the dose-levels shown are only the extremes, the NOAEL and the LOAEL.

14  
15 ***Describing the Dose-Response Steps***

16 In the dose-response section, the tabulation of points of departure (PODs), health effects concentrations  
17 (HECs), and applied uncertainty factors (UFs) is useful, allowing endpoints to be compared and the  
18 distinction between a low POD with few UFs and a high POD with many UFs to be seen.

19  
20 It represents an important advance that the Hazard Identification sections for each endpoint have specific  
21 places for discussion of consistencies and inconsistencies among data, on the relevance of studies for  
22 human risk evaluation, on the knowledge of mode of action (even if it must say that little is known), and  
23 on alternative interpretations of the available data on potential causation. The format that addresses each  
24 of these issues in an orderly way for each endpoint is important to advancing the explanation of the basis  
25 for conclusions and enhancing transparency. However, The SAB is concerned that these interpretation  
26 passages in each Hazard Identification section are somewhat too concise, and suggests that it would be  
27 good to find a consistent way (perhaps more appendices) to document the arguments without unduly  
28 distracting from the main discussion.

29 ***Presenting the Outcomes***

30 As it stands, both the Hazard Identification and Dose-Response sections simply dive in to the first  
31 endpoint or analysis to be considered, and then have separate sections on each. There is little overview  
32 to prepare a reader for what is coming or to point to the parts that are critical versus those that are there  
33 for completeness. In general, to help enable a reader to grasp the main lines of argument and only go  
34 into detail when needed, the SAB recommends that both the Hazard Identification and the Dose-  
35 Response sections have an initial paragraph setting out the main things that will be considered and  
36 indicating which considerations (to be developed in the subsequent text) are the most notable for the  
37 larger assessment process. A parallel paragraph at the end of each of these chapters could summarize  
38 what its contents have provided to the larger assessment process. The aim of these paragraphs would be  
39 to make it possible to read the document in more detail than provided in the Executive Summary (which  
40 largely documents findings) but still quickly see the deeper structure of the report and where to focus for  
41 more information on particular aspects. That is, the initial and last paragraphs as proposed would not be  
42 justifications of choices, but only a guide to the more detailed discussion in each section.

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1       **3.1.3.       Standardized Evaluation of Critical Studies**

2       *Charge Question: NRC (2011) state that “all critical studies need to be thoroughly evaluated with*  
3       *standardized approaches that are clearly formulated” and that “strengthened, more integrative, and*  
4       *more transparent discussions of weight of evidence are needed.” NRC also indicated that the changes*  
5       *suggested would involve a multiyear process. Please comment on EPA’s success thus far in*  
6       *implementing these recommendations.*

7  
8       The SAB finds that, in general, a great deal of progress has been made in restructuring the document to  
9       focus the main body on documenting and explaining the interpretations, choices, and analyses, and  
10       relegating the supporting information to appendices. The use of links to the appendices aids in using  
11       them as support, without encumbering the flow of the main arguments. At the same time, the details of  
12       studies are important when the study results are used in constructing arguments. It will be an ongoing  
13       challenge to bring enough into the main text to document the reasoning, to avoid leaving important  
14       aspects hidden in the appendices, and still to have a readable document that fully explains the choices  
15       and conclusions made.

16  
17       The SAB notes that the process of systematic review still needs development. Documentation of the  
18       process of identifying literature has progressed, but further development is needed in establishing  
19       standard practices for abstracting relevant data, for evaluating study quality, strengths and shortcomings,  
20       and for integration of evidence across studies. This includes the phases of evaluating individual studies,  
21       of comparing the results of studies of similar objective into characterizations of their joint bearing in a  
22       way that addresses discordant results, and of the overall integration across lines of evidence to form and  
23       justify judgments about causality and appropriate dose-response analyses.

24  
25       In this development, the SAB suggests that it should be borne in mind that the process of systematic  
26       review is not solely one of identifying the “right” or the “best” data, with the interpretation and bearing  
27       on risk evaluation becoming clear once the right choices are made. The integration and weight-of-  
28       evidence evaluation process requires accepting that multiple interpretations are always possible,  
29       especially in different contexts, and that consistency of causal interpretations with available data should  
30       be considered across all applicable studies, bearing in mind the possible role of study quality limitations  
31       in generating apparent discordances. This process should consider how results of particular studies are to  
32       be generalized to apply to other situations (especially to actual human exposures); it needs to account for  
33       why other study results might disagree; and it needs to consider how other interpretations would have  
34       different consequences for risk estimation.

35  
36       The SAB recognizes that an important challenge facing the agency is that assessments must go ahead  
37       even as this further development proceeds and before all aspects are complete. It notes that a strategy of  
38       working on the structure of the assessment, and focusing the main text on documentation of the process  
39       and its choices and analytical options, is a good way to begin.

40  
41       The recommendations for revision of the IRIS process come from the NRC “Roadmap” (Chapter 7 of  
42       the Formaldehyde review) and other sources. The SAB recommends that a good principle to follow in  
43       conducting assessments during the process of revision is to consider the reasons behind the

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1 recommendations for change, and to make efforts to address the issues and to explain how the chosen  
2 approaches seek to reflect the NRC recommendations, although the methods may not yet be fully  
3 developed and agreed upon. That is, trying to address as well as one can the issues behind the  
4 recommended methodological and procedural changes is a good way to make assessments as reformed  
5 as they can be, and improve acceptance as the overall IRIS process continues to advance.

6 **3.1.4. Addressing Public Comments on the Draft *Toxicological Review of Trimethylbenzenes* (May**  
7 **2012)**

8 *Charge Question: EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes*  
9 *[May 2012] and has revised the assessment to respond to the scientific issues raised in the comments. A*  
10 *summary of the public comments and EPA's responses are provided in Appendix F of the Supplemental*  
11 *Information to the Toxicological Review of Trimethylbenzenes. Are there scientific issues that were*  
12 *raised by the public as described in Appendix F that may not have been adequately addressed by EPA?*  
13

14 Public comments on the draft IRIS *Toxicological Review of Trimethylbenzene* (as summarized in  
15 Appendix F of the assessment) focused on the standards and transparency of the draft document and  
16 several scientific areas: (1) why the EPA did not use the available data on C-9 mixtures (mostly TMB  
17 isomers and ethyltoluene isomers, which according to the comments, have similar toxicological profiles)  
18 for the IRIS evaluation; (2) why the EPA identified the critical endpoint as pain sensitivity based on a  
19 transient latency in paw lick to a hot plate stimulus following subchronic exposure, that was not evident  
20 after chronic exposure and was a reversible response after two-weeks post-exposure; and (3) why the  
21 1,3,5-TMB oral gavage toxicity study (Koch Industries, 1995), the results of which were already  
22 accepted by the EPA, was not used to reduce the identified uncertainties.  
23

24 The SAB finds that Appendix F did address issues raised in public comments and that explanations were  
25 furnished for the agency's stance on the issues and their disposition. That is, the issues were all  
26 addressed according to the agency's judgments, and those judgments were transparently discussed.  
27

28 The TMB Review Panel was divided, however, on the adequacy of the responses and the advisability of  
29 the dispositions that were made as presented in the summary. In particular, there were a variety of views  
30 on the role that testing of the C-9 fraction should have in the assessment, with some panelists accepting  
31 the reasons for omission of this from the main evaluation and others feeling that these results had a role  
32 that had not been adequately explored. The discussion of the C-9 fraction in the August 2013 draft of the  
33 TMB Assessment is further discussed in section 3.2.3 of this report. There was also disagreement among  
34 the TMB Panelists related to the interpretation of the pain sensitivity data, with some members  
35 questioning whether the document adequately examined the question of reversibility following  
36 termination of exposure, which further bears on whether ongoing or repeated exposures to TMBs should  
37 be deemed to have accumulating toxicity beyond effects evident in shorter-term exposure; other panel  
38 members believed that the data were consistent with cumulative toxicity and lack of reversibility. The  
39 SAB recommends that the EPA provide a more robust discussion of the data and studies considered in  
40 the TMB assessment including the C-9 fraction and mixtures. The full discussion of these issues and  
41 their treatment in the TMBs assessment is covered in the responses to the charge questions in Section  
42 3.2 of this report.  
43

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1 **3.2. Toxicological Review of Trimethylbenzenes**

2 **3.2.1. Executive Summary**

3 *Charge Question: The major conclusions of the assessment pertaining to the hazard identification and*  
4 *dose-response analysis have been summarized in the Executive Summary. Please comment on whether*  
5 *the conclusions have been clearly and sufficiently described for purposes of condensing the*  
6 *Toxicological Review information into a concise summary.*

7  
8 The Executive Summary condenses the large amount of information presented in the draft TMB  
9 Assessment and the Supplemental Information. Individual conclusions regarding RfC and RfD values,  
10 as well as other relevant information (e.g., carcinogenicity) for each of the TMB isomers of concern are  
11 clearly described. The SAB recognizes that there is always some tension to find the appropriate level of  
12 detail to include in the Executive Summary. The Executive Summary presents somewhat detailed  
13 information on the data used to develop the RfC and RfD for each of the three isomers and that detail  
14 may detract from the intended purpose of brevity. As discussed in Section 3.1.1, the Executive Summary  
15 should emphasize the major conclusions of the assessment and provide the specific details of the critical  
16 studies in the main text of the assessment.

17  
18 Recommendations to improve the Executive Summary include:

- 19  
20
- 21 • The Summary should be truncated to emphasize the major conclusions. Specifically, citations  
22 should be removed from the summary unless they are absolutely essential. Whole sections of the  
23 Executive Summary are devoted to elaborating on "Confidence"; for example, the last  
24 paragraphs in Sections 3 and 5 are identical except for the compound being discussed. The SAB  
25 recommends that the EPA consider treating "Confidence" as a single, very succinct section  
26 toward the end of the Executive Summary. Issues pertaining to the use and rationale for  
27 assigning confidence for each isomer should be relegated to the corresponding sections in the  
28 main text.
  - 29 • Another example where too much detail is provided is the middle paragraph on page xxxvi. The  
30 text and table both describe the calculations for the RfC, even though the details are provided in  
31 the main body of the text.
  - 32 • Much of Section 15 in the Executive Summary (Susceptible Populations and Lifestages) seemed  
33 speculative. While the concepts may be correct, they were not pertinent in the executive  
34 summary on TMBs. This section could be truncated after the first sentence, which is a clear  
35 summary of what is known. The SAB also provides more specific comments on sensitive and  
vulnerable populations in Section 3.3.1.

36 **3.2.2. Literature Search Strategy/Study Selection**

37 *Charge Question: The process for identifying and selecting pertinent studies for consideration in*  
38 *developing the assessment is detailed in the Literature Search Strategy/Study Selection section. Please*  
39 *comment on whether the literature search approach, screening, evaluation, and selection of studies for*  
40 *inclusion in the assessment are clearly described and supported. Please identify any additional peer-*

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1 *reviewed studies from the primary literature that should be considered in the assessment of noncancer*  
2 *and cancer health effects of 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB.*

3  
4 The SAB finds that the search strategy was clearly articulated. The databases were clearly defined, as  
5 were the search terms (Table LS-1). In contrast, however, the process for selecting which of the  
6 identified studies to use for the assessment was not transparent.

7  
8 A flow chart (Figure LS-1) indicates that the initial search identified approximately 4,300 papers, of  
9 which approximately 200 were used in the draft TMB Assessment. While it was clear which papers  
10 were used in the draft assessment, there were no means of determining which papers were excluded  
11 from the assessment. Thus the review does not provide sufficient documentation to determine if  
12 important papers may have been overlooked or considered and then omitted from consideration based on  
13 EPA's criteria. As such, the SAB strongly recommends that the EPA provide citations for the 4,300  
14 papers and group them according to the main reason why they were excluded. This could be  
15 accomplished in several ways, ranging from an appendix at the end of the document, to a link between  
16 the document and the Medline search that was used, to an on-line searchable data base.

17  
18 The flow chart (Figure LS-1) also indicated that 65 papers were excluded "based upon manual review of  
19 paper/abstracts." Again, there were no means of determining the identity of these papers. Furthermore,  
20 certain papers were excluded because they were "not available in English." The SAB notes that  
21 translation options are available and finds that this criterion for omission is unacceptable. Among the 65  
22 papers, others were excluded because they were *in vitro* studies. *In vitro* studies are mentioned in the  
23 assessment (e.g., Janik-Spiechowicz et al. 1998; page 1-46); thus, it was not clear why some *in vitro*  
24 studies were included and others were excluded.

25  
26 The SAB noted that the description of the search strategy did not mention xylenes or ethylbenzene.  
27 Because of the close similarity of xylenes to TMBs and the very similar toxicological effects caused by  
28 xylenes, this may have resulted in important papers being excluded, thus weakening the conclusions of  
29 the assessment. For example, the findings of Chen et al. (1999) and Lee et al. (2005) (cited on p. 1-1)  
30 relating painters' exposure to solvents to neurological problems have a relatively weak association to  
31 TMBs. The SAB notes that the links in these two studies are stronger to xylene and to a mixture of  
32 aromatic solvents including TMBs rather than the TMB isomers. For example, studies such as those of  
33 Ruijten et al. (1994), Qian et al. (2010), Tang et al. (2011), and El Hamid Hassan et al. (2013), are  
34 closely linked to xylene but not cited in the document. The overall association of the effects reported in  
35 these studies in painters with exposures to aromatic solvents like the TMBs is much stronger than the  
36 associations reported by Chen et al. (1999) and Lee et al. (2005).

37  
38 Section B4 in the Supplemental Information provides details on each of the human studies. While not  
39 directly pertinent to the search strategy, the SAB recommends the inclusion of a summary table of the  
40 studies related to each health effect: for example, a table with the 9 studies on neurotoxicity in humans,  
41 reporting study design, inclusion and exclusion criteria, number of subjects, and main results. This is  
42 common practice in epidemiologic reviews and meta-analyses. The current way of presenting the study  
43 has some advantages because it is very analytical, but it is also hard to summarize.

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1 Additional reference that should be considered by the EPA include:  
2

- 3 • Chapter 8 on Trimethylbenzenes (NRC 2013),
- 4 • Health Hazards of Solvents Exposure among Workers in Paint Industry (El Hamid Hassan et al  
5 2013)
- 6 • Xylene-induced auditory dysfunction in humans (Fuente et al. 2013)
- 7 • Hearing loss associated with xylene exposure in a laboratory worker. (Fuente et al. 2012)
- 8 • Visual dysfunction in workers exposed to a mixture of organic solvents. (Gong et al. 2003)
- 9 • Ototoxicity effects of low exposure to solvent mixture among paint manufacturing workers.  
10 (Juárez-Pérez et al. 2014)
- 11 • Short latency visual evoked potentials in occupational exposure to organic solvents (Pratt et al.  
12 2000)
- 13 • Auditory brainstem response in gas station attendants (Quevedo et al. 2012)

14 **3.2.3. Hazard Identification**

15 *Synthesis of Evidence*

16 *Charge Question: A synthesis of the evidence for trimethylbenzene toxicity is provided in Chapter 1,*  
17 *Hazard Identification. Please comment on whether the available data have been clearly and*  
18 *appropriately synthesized for each toxicological effect. Please comment on whether the weight of*  
19 *evidence for hazard identification has been clearly described and scientifically supported.*  
20

21 The synthesis of evidence for the three TMB isomers is nicely divided up into the various target organs  
22 or forms of toxicity, as well as by exposure route and by human versus animal studies. The studies  
23 chosen for review are clearly described and the summary tables and figures well supplement the text.  
24 The tables are clear and useful, and the figures nicely summarize the available data for each effect by  
25 each isomer. An introductory paragraph describing the section layout, including the summary tables for  
26 each endpoint, would improve readability.  
27

28 However, the decision to disregard or limit consideration of the studies on solvent mixtures containing,  
29 but not limited to, the TMBs appears to have affected the synthesis of evidence. Because important  
30 toxicological observations have been made in both animal and human studies involving exposure to  
31 aromatic solvent mixtures, an important toxicological perspective was lost. The IRIS Preamble, in  
32 Section 3.1 on identifying studies (lines 44-47) specifically states, "In assessments of chemical mixtures,  
33 mixture studies are preferred for their ability to reflect interactions among components." The SAB  
34 concluded that because human exposures to the TMBs generally involve complex solvent mixtures, the  
35 available studies on mixtures including the C-9 fraction and white spirit deserve further consideration  
36 and explanation in the TMB assessment.  
37

38 In addition, synthesis of available data appears to have been impaired by the decision not to include  
39 literature on other closely related aromatic solvents. Toluene is briefly mentioned, but the potentially  
40 relevant literature on ethylbenzene, xylenes, and styrene is largely excluded. This eliminated supporting  
41 toxicological information on neurological endpoints which could have helped clarify potential

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1 mechanisms of action. Such information is clearly supported in the IRIS Preamble, section 3.1 (lines 11-  
2 15) "[s]earches for information on mechanisms of toxicity are inherently specialized and may include  
3 studies on other agents that act through related mechanisms." This is further supported in Section 5.4, p.  
4 xxiii (lines 18-21), "Pertinent information may also come from studies of metabolites or of compounds  
5 that are structurally similar or that act through similar mechanisms." It is therefore recommended that  
6 additional animal and human studies on related aromatic solvents be considered in the qualitative and  
7 mechanistic interpretations of TMB toxicity. Examples of such studies are included in comments on the  
8 literature review. (See Section 3.2.2)

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

18  
19 More specifically, in another subchronic evaluation of the C-9 mixture, Douglas et al. (1993) found no  
20 persistent neurotoxicity. In light of the uncertainty involving the neurotoxicity endpoints used in the  
21 draft TMB assessment, this possible discrepancy needs to be addressed in detail. Such a discussion  
22 might involve considerations of the potential for chronic neurotoxicological effects of individual TMB  
23 isomers alone, versus when exposure is to TMBs in mixtures. Data are available from many additional  
24 mixture studies to provide further perspectives on this question, as reviewed, for example, by Richie et  
25 al. (2001).

26  
27 Discussion of the individual endpoints is flawed by questionable statistical statements or inferences. In  
28 several places (pp. 1-3, 1-4, 1-5, 1-7, 1-36), the descriptions of non-statistically significant results infer  
29 that effects have been observed. The SAB recommends that descriptions of results more closely adhere  
30 to the rule that statistical significance provides the criterion of whether an effect has occurred (although  
31 data trends can be cautiously noted).

32  
33 For neurological effects, which are the most consistently observed, the document clearly explains that  
34 although mechanistic data are lacking for the TMBs, there is good rationale for making analogies with  
35 toluene, for which much more information is available. This could have been greatly strengthened, as  
36 mentioned above, by including supportive studies on the three xylene isomers as well as ethylbenzene  
37 and other related solvents and mixtures.

38  
39 The absence of data from related solvents is all the more important because the neurological effect data  
40 that were presented are largely from a single laboratory, with somewhat unconventional endpoints.  
41 Although the quality of the studies seems very high, the functional significance of the observed effects is  
42 not as clear, nor is the extrapolation to humans. A more comprehensive discussion of the evidence for  
43 prolonged effects of aromatic solvents would likely provide more confidence in the use of these  
44 endpoints.

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1 The discussion of respiratory effects should be strengthened by further consideration of the relevance to  
2 humans of the effects observed in the high-dose animal studies. While it's clear that respiratory effects  
3 are observed and are a relevant endpoint in humans, the distinction between the high-dose animal effects  
4 and the human effects could have been made more clearly. The limitations of the human evidence for  
5 hematological and clinical chemistry effect, based on the uncertainties in exposures (mixture  
6 components, doses) should be more clearly described. With regard to carcinogenicity as an endpoint, the  
7 document clearly communicates the inadequacy of the database, including the minimal genotoxicity  
8 database.

9  
10 The SAB recommends that the EPA expand the discussion of the similarities of the TMBs to other  
11 methyl-substituted aromatic compounds. The subsections in the hazard synthesis considering the  
12 similarities between the three TMB isomers are very important but would be improved by further  
13 perspectives on related solvents. This is critical with regard to the decision to base some of the RfDs on  
14 extrapolations among the isomers. The evidence for similar effects and endpoints among methyl-  
15 substituted aromatic compounds seems to be much stronger than what has been presented so far. The  
16 summary table (Page 1-49, Table 1-7) is very helpful in understanding the points made with regard to  
17 toxic effects. A summary table or scheme regarding toxicokinetics and metabolism would also be useful.  
18 Section 1.1.7, which focuses on the toxicokinetic similarities among TMB isomers, would be improved  
19 by summarizing in a table or scheme both the similarities and differences among the isomers in  
20 toxicokinetics and metabolism.

21 ***Noncancer Health Effects***

22 *Charge Question: Does EPA's hazard assessment of noncancer human health effects of*  
23 *trimethylbenzenes clearly integrate the available scientific evidence (i.e., human, experimental animal,*  
24 *and mechanistic evidence) to support the conclusions that trimethylbenzenes pose potential hazards to*  
25 *the nervous system, respiratory system, the developing fetus, and the circulatory system (i.e., blood)?*  
26

27 Hazard assessment results in the identification of the potential adverse health effects attributable to a  
28 specific environmental agent, the mechanisms by which agents exert their toxic effects, and the  
29 associated doses, route, duration, and timing of exposure. Section 1.2.1 (Weight of Evidence for Effects  
30 Other Than Cancer) contains a summary description of the toxicological evidence of effects of the  
31 TMBs on the nervous, respiratory, circulatory and developmental systems. The section, however, does  
32 not adequately describe the limitations and uncertainties within the database or how the results of the  
33 hazard assessment will be utilized in the subsequent dose response evaluation. The SAB recommends  
34 that Section 1.2.1 be revised to include the following:

- 35
- 36 • A short summary of the toxicokinetic similarities and differences among the three isomers early  
37 in the section to provide context to the subsequent effect summaries.
  - 38 • A short summary of the neurological effects database limitations and accompanying  
39 uncertainties such as lack of subchronic data for some isomers, lack of chronic data for all  
40 isomers, questions of reversibility and lack of mechanistic data. The SAB notes that summaries  
41 for the respiratory, hematological and development effects already make these distinctions.

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- 1 • Statement(s) regarding the confidence in the hazard identification results given the limitations of  
2 the available database. This statement(s) should address the question: based on the sensitivity of  
3 endpoints assessed in the limited database, lack of mechanistic information and effects observed  
4 with similar compounds but not assessed for TMBs, what is the confidence that the hazards (i.e.,  
5 sensitive health endpoints) have been adequately identified?
- 6 • Inclusion of a concluding paragraph(s) which states how the results of the hazard identification  
7 (e.g., the effects on the nervous system, respiratory system, the hematological system, and  
8 developing fetus) will be utilized in the subsequent dose-response evaluation as well as  
9 describing the relative importance of the different health effects.

10 ***Carcinogenicity***

11 *Charge Question: Does EPA’s hazard assessment of the carcinogenicity of trimethylbenzenes clearly*  
12 *integrate the available scientific evidence to support the conclusions that under EPA’s Guidelines for*  
13 *Carcinogen Risk Assessment (U.S. EPA, 2005), there is “inadequate information to assess the*  
14 *carcinogenic potential” of trimethylbenzenes?*

15  
16 As noted in the detailed response to the charge question on carcinogenicity (See section 3.2.11), 1,2,4-  
17 TMB has been assessed in only one study. The EPA found that there were a number of deficiencies  
18 concerning this bioassay and the SAB agrees with the agency’s finding. The EPA also noted that no  
19 carcinogenicity bioassays have been conducted with 1,2,3-TMB or 1,3,5-TMB. As such, the SAB  
20 concludes that the EPA’s hazard assessment of the carcinogenicity of the TMBs did integrate all  
21 available scientific evidence and agrees with the EPA that there is “inadequate information to assess the  
22 carcinogenic potential” of trimethylbenzenes.

23 **3.2.4. Toxicokinetics and Pharmacokinetic Modeling**

24 *Charge Question: Data characterizing the toxicokinetics of 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB*  
25 *following inhalation and oral exposures in humans and experimental animals support the use of*  
26 *physiologically-based pharmacokinetic (PBPK) models for 1,2,4-TMB. For the purposes of this*  
27 *assessment, the Hissink et al. (2007) model, originally describing 1,2,4-TMB toxicokinetics following*  
28 *exposure to white spirit (a complex mixture of volatile organic compounds), was modified by EPA to*  
29 *calculate internal dose metrics following exposure to 1,2,4-TMB alone for the derivation of an*  
30 *inhalation RfC for 1,2,4-TMB. Additionally, the model was further modified by the addition of an oral*  
31 *route of exposure for use in a route-to-route extrapolation for the derivation of an oral RfD for 1,2,4-*  
32 *TMB.*

33 *Please comment on whether the selected PBPK model (Hissink et al. 2007) with EPA’s modifications*  
34 *adequately describe the toxicokinetics of 1,2,4-TMB (Appendix B [of the TMB Assessment]). Was the*  
35 *PBPK modeling appropriately utilized and clearly described? Are the model assumptions and*  
36 *parameters scientifically supported and clearly described? Are the uncertainties in the model structure*  
37 *adequately characterized and discussed?*

38  
39 The SAB finds that the selected model did an adequate job of simulating the time-course of TMB in the  
40 blood of human subjects during and following acute inhalation exposures. There was excellent

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1 agreement between predicted and measured blood TMB levels, both during and following 4-hour  
2 exposures, for the subjects of Hissink et al. (2007) inhaling 100 ppm white spirit. All three of these  
3 subjects regularly consumed alcohol, which would induce cytochrome P4502E1 and enhance TMB  
4 metabolism. The model modestly, but consistently underpredicted blood levels in volunteers inhaling 30  
5 ppm TMB for 8 hours (Kostrezewski et al. 1997). The model also consistently underpredicted blood  
6 levels in persons inhaling 2 or 25 ppm TMB for 2 hours (Järnberg et al. 1996, 1997, 1998), but to a  
7 larger degree. Agreement was better at the lower exposure level. These subjects exercised during  
8 exposure, which would increase their systemic uptake of TMB. Post-exposure blood levels were well  
9 predicted for all human data sets.

10  
11 In most instances, the model over-predicted blood TMB levels in rats subjected to single exposures to  
12 white spirit (Hissink et al. 2007) and TMB (Swiercz et al. 2003). The differences between predicted and  
13 empirical levels typically increased from 1½- to 2-fold at lower inhaled concentrations to 4- to 6-fold at  
14  $\geq 100$  ppm. The accuracy of predictions of brain levels was similar to those for blood. The model  
15 reasonably simulated blood and brain levels in rats after repeated TMB exposures. Again, the disparity  
16 between simulated and empirical data increased with increasing vapor concentration. With the repeated  
17 exposure data of Swiercz et al. (2003), there were ~2- and 3-fold differences for the 25 and 50 ppm  
18 exposures, respectively. Differences in brain levels after 606 hours were somewhat greater. There was  
19 more disparity (4- to 5-fold) for blood and brain levels in the rats of Zahlsten et al. (1992) inhaling 100  
20 ppm TMB for 3 days.

21  
22 The poor model prediction for inhaled concentrations  $\geq 100$  ppm in rats is acknowledged by the EPA  
23 authors. Nevertheless, they use the model to provide simulations for exposures outside its application  
24 domain. This is necessitated by the fact that the 100-ppm dose is in the middle of the rat dose-response  
25 range used for benchmark dose modeling. Over-predicting rat dosimetry in this range thus has the  
26 potential to influence the results of dose-response modeling and extrapolation of potency to humans.  
27 Marked over-prediction of high-dose data necessitated omission of the highest dose for benchmark dose  
28 (BMD) modeling.

29  
30 The EPA has two options for alleviating this issue; refine the rat PBPK model to improve fits or conduct  
31 BMD modeling using inhaled concentration first to identify the point of departure (POD), then use the  
32 rat and human PBPK models to determine the human equivalent concentration. Refining the PBPK  
33 model may require recalibration of some type, such as the addition of a first-order metabolic pathway  
34 consistent with the PBPK model of Järnberg and Johanson (1999), or changing hepatic blood flow to 25  
35 percent instead of 17 percent of cardiac output, which is a more common physiologic parameter value.

36  
37 Alternatively, the EPA could conduct BMD modeling of the Korsak and Rydzynski (1996) data using  
38 air TMB concentration as the dose metric to derive the POD. Subsequently, the PBPK model would be  
39 used to convert the POD to the weekly average blood concentration. This alternative approach yields a  
40 BMD of 84 mg/m<sup>3</sup> (17ppm), which would be predicted by the PBPK model to yield a blood  
41 concentration of 0.087mg/L in rats. The result is identical to the values derived by the EPA, suggesting  
42 that the approach of dropping the high-dose group used by the EPA is valid. EPA can use this alternative  
43 approach to support their BMD modeling approach.

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1 The SAB conducted a quality control quality assurance review and confirmed the model simulations  
2 presented in Appendix B of the IRIS document draft. Although a couple minor technical issues were  
3 identified, no fundamental flaws or issues were found. This review is provided in Appendix B of this  
4 report.

5  
6 The EPA's assumptions, in modifying the Hissink et al. (2007) model to predict the kinetics of inhaled  
7 TMB for repeated exposure scenarios, were reasonable and appropriate. The major caveats, however,  
8 were not identified up-front on page B-20 (e.g. that the original model and its parameters were for TMB  
9 and white spirit, lack of parameters for the oral route, lack of parameters for pregnancy). The SAB  
10 recommends that the EPA expand the explanation and justification for the modifications of model  
11 parameters. Specifically, the discussion of the input parameters (e.g., human tissue:blood partition  
12 coefficients (PCs), cardiac output, liver blood flow) should be justified. Additionally the use of scaled-  
13 up rat  $V_{max}$  values, when human values were available, requires further explanation. Metabolic constants  
14 could be questioned, as they summarily reflect the rate of TMB metabolism during mixed exposures to  
15 white spirits, rather than exposure to TMB alone. The use of a liver blood flow of 17.5 percent of  
16 cardiac output should be justified, as it differs substantially from the traditional value of 25 percent. The  
17 EPA did not attempt any re-estimation or adjustment of parameters for chronic exposure (e.g., enzyme  
18 induction, dose-dependency, growth dilution). Results of sensitivity analyses can be used to respond to  
19 related concerns. It was noted that human tissue:blood PCs used in modeling were twice those for rats.  
20 Meuhlenberg and Vijverberg (2000) estimated human brain:blood, fat:blood and kidney:blood PCs that  
21 were higher for rats than for humans. It was suggested that first order and saturable metabolism be  
22 incorporated into the model, and the model run to explore the impact of the change.

23  
24 The SAB did not find a specific discussion of the uncertainties in the model's structure. While these  
25 uncertainties may be implicitly included in the uncertainties discussion, they should be specifically  
26 discussed in reference to the PBPK model.

27  
28 The SAB notes that the PBPK model of Järnberg and Johanson (1999) is a human PBPK model. The  
29 model is for TMB alone, and thus avoids the complications and uncertainties of: (1) concurrent exposure  
30 to other components in white spirit; and (2) species-to-species extrapolations. Empirical human kinetic  
31 data are available from the same laboratory for model parameterization and validation. Human  
32 neurobehavioral data are also available in the literature from other research groups. The results of these  
33 studies identify human NOAELs/LOAELs for acute irritation and central nervous system (CNS) effects  
34 by TMB and white spirit. It is EPA policy to consider utilization of human data and validated human  
35 models when they are available. The SAB recommends that the EPA evaluate the Järnberg and Johanson  
36 model and at a minimum discuss the model selection in future drafts of the assessment.

37  
38 The SAB recommend the EPA conduct BMD modeling of the Korsak and Rydzynski (1999) data using  
39 air TMB concentration as the dose metric to derive the POD and subsequently use the PBPK model to  
40 convert the POD to the weekly average blood TMB concentration. This can be done to either replace the  
41 EPA's current approach or offered as support of the EPA's approach (i.e., to demonstrate the same  
42 answer results from either approach).

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1 *Charge Question: The internal dose metric selected for use in the derivation of the RfC and RfD for*  
2 *1,2,4-TMB was the steady-state weekly average venous blood concentration (mg/L) of 1,2,4-TMB for*  
3 *rats exposed for 6 h/day, 5 days/week. Please comment on whether the selection of this dose metric is*  
4 *scientifically supported and clearly described. If a different dose metric is recommended for deriving the*  
5 *RfC, please identify this metric and provide scientific support for this choice. Are the uncertainties in the*  
6 *selected dose metric adequately characterized and discussed?*

7 The use of any dose metric should be guided by the mode of action (MOA) of the chemical being  
8 examined. For the TMBs, there is a paucity of information on their MOA, and the agency has inferred  
9 the mode of action to be similar to that for chemicals such as toluene. Given the uncertainties in the  
10 MOA, the SAB finds that the selection of the internal dose metric of the venous blood concentration  
11 averaged over a week of exposure is reasonable.

12  
13 In the absence of knowing the MOA, the area under the dose-response curve can be used to estimate the  
14 average venous blood concentration as a viable dose metric. Given that the critical effects upon which  
15 the RfC is being determined are neurological and, therefore, are extrapulmonary effects due to inhalation  
16 of the TMBs, the selection of the internal dose metric comes down to either the maximum venous  
17 concentration or the steady-state weekly average venous blood concentration. While there are acute  
18 effects of 1,2,4-TMB that might bring into play the maximum blood concentration, there were also  
19 effects with 90 days of exposure.

20  
21 Clarification is needed on how the average weekly venous concentration was determined. This is  
22 because the longer phase half-life of the TMB isomers indicates that an exposure period longer than a  
23 week is required for blood levels to achieve a steady state. In addition, the experimental data for both  
24 rats and humans show that steady state is not achieved with only a single week of exposure. Executing  
25 the PBPK model over a 4-week period shows that the average blood levels are still continuing to rise  
26 slightly. The model should be run long enough to come to a weekly steady state and then the associated  
27 venous blood concentration used as the internal dose metric.

28  
29 The multiple tissues of interest for derivation of an RfC are primarily extrapulmonary tissues. However,  
30 the agency has a goal to establish RfCs for multiple endpoints beyond the critical effect endpoint  
31 currently being addressed. If an effect in the respiratory tract is established such as a change in bronchial  
32 alveolar lavage fluid composition and an RfC is to be determined, then the appropriate dose metric  
33 would be based on the mass deposited per unit surface area of the lung rather than on the average venous  
34 blood concentration. A mass per unit lung surface area dose metric enables species with significantly  
35 different lung sizes than humans to be used in the derivation of the RfC.

36  
37 Using the PBPK model-estimated internal dose metrics as the dose inputs for BMD modeling required  
38 the agency to drop the high dose exposures from all modeling efforts because the venous blood dose  
39 metrics consistently over-predicted experimental results for high exposures. This overestimation may be  
40 due in part to the agency using minute ventilation as the driver function for internal dose rather than  
41 decomposing minute ventilation into its two components, namely tidal volume and breathing frequency.  
42 While the exposure level is high and that may lead to a 50 percent reduction in respiratory rate,  
43 respiratory irritants such as the TMBs cause subtle shifts in the breathing pattern while maintaining the

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1 same overall minute ventilation. Shallower breathing leads to a shift upward in the respiratory tract for  
2 the site of deposition. In addition, the PBPK modeling for humans did not take into account the periods  
3 of exercise the subjects underwent, which may explain the model's greater deviations from experimental  
4 results at high exposure levels. While the high doses would not need to be dropped if the agency added  
5 an exponential rising model to their suite of models to be fit, the SAB notes that external air can be used  
6 as the dose metric and then the PBPK model used to back-calculate the appropriate venous blood levels,  
7 arriving at the same result that the agency obtained. If the SAB's suggestions for improvements in the  
8 PBPK model do not lead to a better agreement with the high dose exposures, the agency would be well  
9 advised to include the external air dose metric and corresponding venous blood back-calculations.

10  
11 While uncertainties concerning model parameters, potential for kinetic changes with repeated exposures,  
12 and model estimates of internal dose are discussed, the uncertainties in the selected dose metric (weekly  
13 average venous blood concentration) are not adequately characterized or discussed.

14 **3.2.5. Inhalation Reference Concentration (RfC) for 1,2,4-TMB**

15 *Charge Question: A 90-day inhalation toxicity study of 1,2,4-TMB in male rats (Korsak and Rydzynski,*  
16 *1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection*  
17 *of this study is scientifically supported and clearly described. If a different study is recommended as the*  
18 *basis for the RfC, please identify this study and provide scientific support for this choice.*

19  
20 The SAB generally agrees with the choice of the Korsak and Rydzynski (1996) study as the basis for  
21 derivation of the RfC for 1,2,4-TMB. The study utilized a 90-day exposure period and, thus, the longest  
22 duration exposure study available in the literature; in addition, it included multiple exposure levels. It  
23 was well-conducted and utilized adequate sample sizes of rats such that it was not underpowered. In  
24 addition, it was based on widely-used behavioral assays. An examination of the study indicates these  
25 behavioral studies were carefully carried out and data from control animals were consistent with  
26 previously published observations.

27  
28 Clarification of this choice, however, could be significantly improved in the document in several ways:

- 29
- 30 • The rationale for the choice of Korsak and Rydzynski (1996) is not specifically described and the  
31 reasons for its choice over other studies, e.g., the 4-week exposure studies, need to be more clearly  
32 stated.
  - 33 • As currently written, there is confusion over chronicity of exposure vs. effects. It would be helpful  
34 to modify the terminology particularly related to outcome measures, perhaps as acute effects vs.  
35 long-term effects/irreversible effects and retain the use of the word chronic/subchronic etc. to  
36 descriptions of statements related specifically to exposure.
  - 37 • Separate the write-up into sections that specifically elaborate on the acute effects and provide a  
38 separate section related to effects observed post-exposure. Given the commonality of even the  
39 trends in data across these studies, some mention of the biological significance in the absence of  
40 statistical significance ( $\alpha = 0.05$  as an arbitrarily chosen value) should be mentioned.
  - 41 • The text, where applicable, could include additional qualifications as to "reversibility of effects" at  
42 the 2-week post-exposure time point. This assessment of reversible effects of failures on the

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1 rotarod is based on the finding of lack of statistical difference between treated and control groups  
2 at one week post-exposure following a 13-week exposure period for one of two isomers. However,  
3 this represented a reduction from 40 percent rotarod failure during the final week of exposure  
4 compared to 35 percent one week post-exposure, as compared to 0 percent rates for controls. There  
5 was no such statistical reversal for the other isomer, and for both isomers, the magnitude of the  
6 reduction post exposure was minimal. Further, it is not clear that the statistical analyses of these  
7 data incorporated a repeated measures component that would be required by the experimental  
8 design. Thus, while a case was stated for a statistically significant reversal, it was not consistent  
9 nor did it appear to be biologically meaningful.

- 10 • It was recommended that the EPA re-calculate the RfC as if the study were subchronic (i.e., UF  
11 converts to 1 from 3) and report this value as well.
- 12 • Include more specific mention of the potential cumulative neurotoxicity that is suggested by the  
13 repeated measurement finding of rotarod performance failures across the course of exposure.
- 14 • Include more specific descriptions of the similarity of the animal behavioral endpoints to what has  
15 been observed in humans.

16  
17 *Charge Question: Decreased pain sensitivity (measured as an increased latency to pawlick response*  
18 *after a hotplate test) in male Wistar rats was concluded by EPA to be an adverse effect on the nervous*  
19 *system and was selected as the critical effect for the derivation of the RfC. Please comment on whether*  
20 *the selection and characterization of this critical effect is scientifically supported and clearly described.*  
21 *If a different endpoint(s) is recommended as the critical effect(s) for deriving the RfC, please identify*  
22 *this effect and provide scientific support for this choice.*  
23

24 The SAB agrees that the reduction in pain sensitivity as indicated by an increased latency to pawlick  
25 response in a hotplate test is a valid adverse nervous system effect and appropriately selected as a  
26 critical effect for the derivation of the RfC. This effect was variously seen in response to short-term, 4-  
27 week, and 90-day studies. The associated U-shaped dose-effect curves seen with these isomers,  
28 moreover, are highly consistent with the effects of various other pharmacological agents (e.g., opioids)  
29 on this response and likely reflective of the mechanisms by which these isomers act. This assay is  
30 widely used in the behavioral pharmacology literature and particularly in the study of pain nociception  
31 and opioid pharmacology.  
32

33 The SAB agrees that the observation of prolonged latency in the hot plate test 24 hour post-footshock  
34 delivery that was observed in studies by Gralewicz and colleagues (1997, 2001) also constitutes an  
35 adverse effect. The administration of footshock immediately after the hotplate test trial essentially  
36 maximizes the capabilities of the nervous system and, thus, provides a type of nervous system probe that  
37 then unmask a prolonged latency to a hotplate stimulus 24 hours later. It shows that when the nervous  
38 system is maximally stressed, it cannot respond/recover in a normal timeframe.  
39

40 In addition to the recommendations above for the document related to the nervous system effects, this  
41 section could also benefit from some additional description of the hotplate procedures, including the  
42 rationale/approach for using the footshock intervention in the post-exposure behavioral assessments  
43 carried out after the 4-week exposures.

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1  
2 *Charge Question: In order to characterize the observed dose-response relationship comprehensively,*  
3 *benchmark dose (BMD) modeling was used in conjunction with dosimetric adjustments for calculating*  
4 *the human equivalent concentration (HEC) from a rat and human PBPK model (Hissink et al., 2007) to*  
5 *identify the point of departure (POD) for derivation of the RfC. Please comment on whether this*  
6 *approach is scientifically supported for the available data, and clearly described.*

7 *a. Has the modeling been appropriately conducted and clearly described, based on EPA's*  
8 *Benchmark Dose Technical Guidance (U.S. EPA, 2012)?*

9 *b. Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR*  
10 *equal to 1 standard deviation change in the control mean for the latency to pawlick response)*  
11 *been supported and clearly described?*

12  
13 EPA's decision to omit the high dose group from the Korsak and Rydzynski (1999) study before BMD  
14 modeling is an initial concern. However, an analysis conducted on BMD modeling on the same dataset  
15 using air concentration as the dose metric results in the same POD air concentration as BMD modeling  
16 based on internal dose and using the low and mid-dose groups. As a result, the SAB agrees that the  
17 overall results for the POD generated by the EPA are adequate but strongly suggests that the agency  
18 provide a more robust explanation of any analyses. The SAB also considered Appendix C-2 in the TMB  
19 Assessment as inappropriate and recommends deleting it. If the EPA is so inclined, they could replace it  
20 with the BMD analysis using air concentration as the dose metric.

21  
22 The SAB recommends that the EPA provide better justification for applying the "one standard  
23 deviation" from the mean of the control group for the neurotoxicological endpoint. The EPA should also  
24 provide better explanation of the issues associated with the homogeneity of variance across dose groups  
25 in the Korsak and Rydzynski (1999) study, its implications for BMD modeling, and how the EPA  
26 addressed this in their BMD modeling.

27  
28 *Charge Question: Please comment on the rationale for the selection of the uncertainty factors (UFs)*  
29 *applied to the POD for the derivation of the RfC for 1,2,4-TMB. Are the UFs appropriate based on the*  
30 *recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference*  
31 *Concentration Process (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are*  
32 *proposed, please identify and provide scientific support for the proposed changes.*

33  
34 Consistent with guidance provided in *A Review of the Reference Dose and Reference Concentration*  
35 *Process* (U.S. EPA, 2002), five possible areas of variability and uncertainty were considered by the EPA  
36 in deriving the proposed RfC for 1,2,4-TMB. This consideration is reflected in choices regarding five  
37 specific uncertainty factors, namely:

- 38 1. UF<sub>A</sub> – an interspecies uncertainty factor;
- 39 2. UF<sub>H</sub> – an intraspecies uncertainty factor;
- 40 3. UF<sub>L</sub> – a LOAEL (lowest observed adverse effect level) to NOAEL (no observed adverse effect  
41 level) uncertainty factor;
- 42 4. UF<sub>S</sub> – a subchronic to chronic uncertainty factor; and
- 43 5. UF<sub>D</sub> – a database uncertainty factor.

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1 In responding to this charge question, the SAB evaluated the choice and rationale for each of these UFs,  
2 reaching the following conclusions.

3 **UF<sub>A</sub>.** The SAB agrees with the UF<sub>A</sub> of 3 and its rationale. The default UF<sub>A</sub> of 10 can be divided into two  
4 half-log UF components of 3 each to account for species differences in toxicokinetics and  
5 toxicodynamics, respectively. In developing the RfC for 1,2,4-TMB, the EPA used PBPK modeling to  
6 convert estimated internal doses in rats in toxicity studies of 1,2,4-TMB to corresponding applied doses  
7 in humans. PBPK modeling substantially reduces uncertainty associated with extrapolating animal  
8 exposures to humans based upon toxicokinetic differences, justifying elimination of one of the half-log  
9 components of the default UF<sub>A</sub> of 10 (U.S. EPA, 2002). Uncertainty regarding possible toxicodynamic  
10 differences among species, i.e., different sensitivity to toxicity at equivalent internal doses, remains,  
11 justifying keeping the other half-log component of 3.

12  
13 **UF<sub>H</sub>.** The SAB agrees with the UF<sub>H</sub> of 10 and its rationale, although one TMB Review Panel member  
14 thought that a UF<sub>H</sub> of 3 would be adequate. This UF is intended to account for potential differences  
15 among individuals in susceptibility to toxicity. The EPA concluded that no information on potential  
16 variability in human susceptibility to 1,2,4-TMB toxicity exists with which to justify using a value other  
17 than the default of 10. It was noted during discussion that numerous clinical studies have demonstrated  
18 that humans, including pediatric and geriatric patients, differ by only about 2-fold in their  
19 susceptibility/sensitivity to inhaled lipophilic anesthetics (e.g., chloroform, halothane), indicating to one  
20 Panel member that a UF<sub>H</sub> of 3 would be scientifically defensible given the neurotoxicity endpoint used  
21 to establish the POD. Other TMB Panel members disagreed, stating that the mode of action of  
22 neurotoxicity of 1,2,4-TMB is unknown and that the actions of general anesthetics may have little or no  
23 bearing on variability in TMB susceptibility. In their opinion, the full UF<sub>H</sub> of 10 is warranted.

24  
25 **UF<sub>L</sub>.** The SAB agreed with the EPA's choices for UF<sub>L</sub> values, i.e., a UF<sub>L</sub> of 1 for all endpoints except  
26 increased BAL cells, for which a UF<sub>L</sub> of 10 was selected. However, the SAB suggests that the  
27 justification for the UFL be strengthened. This UF is intended to be used when the POD is a LOAEL  
28 rather than a NOAEL. In conducting BMD modeling, a BMD equal to one standard deviation change in  
29 the control mean for modeled endpoints was selected. The document would be improved by adding an  
30 explanation of the reasoning for selection of one standard deviation (versus one-half standard deviation)  
31 along with a clearer discussion of why this is expected to lead to a POD for which a UF<sub>L</sub> of 1 is  
32 appropriate.

33  
34 **UF<sub>S</sub>.** The SAB agrees with the UF<sub>S</sub> of 3, although one TMB Panel member thought that a UF<sub>S</sub> of 10  
35 would be more appropriate. When the data used to generate a chronic RfC are from subchronic studies, a  
36 UF<sub>S</sub> is used to address uncertainty around whether longer exposures might lead to effects at lower doses.  
37 The EPA justified using less than a full default factor of 10 for this UF stating,

38 "A full subchronic to chronic uncertainty factor of 10 was not applied in this case  
39 as there was evidence of reversibility of not only neurotoxic effects, but also  
40 hematological effects in rats exposed to 1,2,4-TMB for subchronic durations.  
41 Also, the respiratory effects appeared to be inflammatory in nature. Although  
42 reversibility was not investigated for these endpoints, it is possible that adaptive  
43 mechanisms may alleviate these effects following termination of exposure."

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1 Most of the TMB Panel were satisfied with this justification, but some members of the TMB Panel  
2 disputed the evidence for reversibility of effects. In addition several TMB Panel members noted that  
3 reversibility following cessation of exposure was irrelevant since the chronic RfC is applicable to  
4 lifetime of exposure - i.e., there is no post exposure period. The discussion regarding reversibility of  
5 neurotoxic effects is presented in response to the RfC for 1,2,4-TMB (see Section 3.2.5). The TMB  
6 Review Panel discussed that some hematologic effects considered by the EPA appeared to resolve when  
7 exposure ceased, but other effects did not resolve, and that inflammatory pulmonary effects can lead to  
8 persistent injury. The SAB notes that factors other than reversibility could contribute to selection of a  
9 UFs less than 10, such as evidence from PBPK modeling that 1,2,4-TMB does not accumulate in the  
10 body over time and empirical evidence that the POD does not appear to decrease when results from  
11 subchronic studies are compared with studies of shorter duration. One TMB Review Panel member  
12 thought that none of these considerations had sufficient merit to justify using less than the full default  
13 UFs of 10.

14  
15 **UF<sub>D</sub>.** The TMB Panel was divided on whether the UF<sub>D</sub> should be 3, as selected by the agency, or 10.  
16 The purpose of this UF is to account for overall deficiencies in the database of studies available to assess  
17 potential toxicity. The EPA cited strengths in the database in terms of availability of information on  
18 multiple organ systems from three well-designed subchronic toxicity studies in justifying not using the  
19 full default factor of 10. In retaining a half-log factor of 3, the EPA noted the absence of a multi-  
20 generation reproductive/developmental toxicity as a weakness in the database, and specifically concern  
21 for the absence of a developmental neurotoxicity study for 1,2,4-TMB given the importance of  
22 neurotoxicity in establishing the RfC. Among those who agreed with a UF<sub>D</sub> of 3, some found the  
23 justification provided by the EPA to be satisfactory, while others thought that toxicity data available for  
24 C-9 mixtures should contribute to the rationale to lower the value from the default of 10. Others  
25 disagreed with including C-9 mixture data as relevant to the database UF. (See Section 3.2.3). Panel  
26 members who thought that the UF<sub>D</sub> should be 10 cited various reasons, including the absence of data in  
27 other species and the absence of a multi-generational reproductive study, as well as the opinion that the  
28 absence of a developmental neurotoxicity study alone warranted a full factor 10. One TMB Panel  
29 member pointed out that analogy with toluene suggests that the perinatal exposure could lead to  
30 neurodevelopmental effects at doses 10-fold lower than the NOAEL for effects in adults. An additional  
31 point made by another Panel member was that because the RfCs for all of the isomers are being set at  
32 the same value, whereas the database is severely limited for the 1,2,3- and 1,3,5-TMB isomers and the  
33 latter two compounds deserve a UF<sub>D</sub> of 10. Therefore, for consistency, a factor of 10 should be used for  
34 all the isomers.

35  
36 **3.2.6. Inhalation Reference Concentration (RfC) for 1,2,3-TMB**

37  
38 *Charge Question: A 90-day inhalation toxicity study of 1,2,3-TMB in male rats (Korsak and Rydzynski,*  
39 *1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection*  
40 *of this study is scientifically supported and clearly described. If a different study is recommended as the*  
41 *basis for the RfC, please identify this study and provide scientific support for this choice.*  
42

43 The SAB agrees that, as discussed for 1,2,4-TMB in Section 3.2.5, the choice of the Korsak and

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1 Rydzynski (1996) study as the basis for deriving an RfC of 1,2,3-TMB was scientifically supported. As  
2 with 1,2,4-TMB, the SAB finds that the clarification of this choice, however, could be greatly improved  
3 by the same points discussed for 1,2,4-TMB (see section 3.2.5)

4  
5 *Charge Question: Decreased pain sensitivity (measured as an increased latency to pawlick response*  
6 *after a hotplate test) in male Wistar rats was concluded by EPA to be an adverse effect on the nervous*  
7 *system and was selected as the critical effect for the derivation of the RfC. Please comment on whether*  
8 *the selection and characterization of this critical effect is scientifically supported and clearly described.*  
9 *If a different endpoint(s) is recommended as the critical effect(s) for deriving the RfC, please identify*  
10 *this effect and provide scientific support for this choice.*

11  
12 The SAB agrees that, as with 1,2,4-TMB, reduction in pain sensitivity as indicated by an increased  
13 latency to pawlick response in a hotplate test was a valid adverse nervous system effect and  
14 appropriately selected as a critical effect for RfC derivation. The SAB notes that the agency  
15 appropriately uses the same rationale to derive the RfC for 1,2,4-TMB. A detailed response is in Section  
16 3.2.5 and the SAB refers the reader to that section rather than reiterate the response to the Charge  
17 question.

18  
19 *Charge Question: In order to characterize the observed dose-response relationship comprehensively,*  
20 *benchmark dose (BMD) modeling was used in conjunction with default dosimetric adjustments (U.S.*  
21 *EPA, 1994b) for calculating the human equivalent concentration (HEC) to identify the point of*  
22 *departure (POD) for derivation of the RfC. Please comment on whether this approach is scientifically*  
23 *supported for the available data, and clearly described.*

24 *a. Has the modeling been appropriately conducted and clearly described, based on EPA's*  
25 *Benchmark Dose Technical Guidance (U.S. EPA, 2012)?*

26 *b. Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR*  
27 *equal to a 1 standard deviation change in the control mean for the latency to pawlick response)*  
28 *been supported and clearly described?*

29  
30 The SAB response to this charge question deals with the same issues as charge question for 1,2,4-TMB  
31 and did not identify any issues specific to 1,2,3-TMB and refers the reader the section 3.2.5.

32  
33 *Charge Question: Please comment on the rationale for the selection of the uncertainty factors (UFs)*  
34 *applied to the POD for the derivation of the RfC for 1,2,3-TMB. Are the UFs appropriate based on the*  
35 *recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference*  
36 *Concentration Process (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are*  
37 *proposed, please identify and provide scientific support for the proposed changes.*

38  
39 The SAB notes that the uncertainty factor values selected by the EPA for 1,2,3-TMB are identical to  
40 those selected for 1,2,4-TMB, and that the justifications are essentially the same. Thus, the SAB  
41 response to this charge question and recommendation are the same as the response to Charge Question  
42 for 1,2,4-TMB and refers the reader to Section 3.2.5.

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**3.2.7. Inhalation Reference Concentration (RfC) for 1,3,5-TMB**

*Charge Question: One developmental toxicity study (Saillenfait et al. 2005) following inhalation exposure to 1,3,5-TMB was identified in the literature and was considered as a potential principal study for the derivation of the RfC for 1,3,5-TMB. However, the candidate RfC derived for 1,3,5-TMB based on this study (and the critical effect of decreased maternal weight gain) was 20-fold higher than the RfC derived for 1,2,4-TMB (based on decreased pain sensitivity). Given the available toxicological database for 1,2,4-TMB and 1,3,5-TMB, there are several important similarities in the two isomers' neurotoxicity that support an RfC for 1,3,5-TMB that is not substantially different than the RfC derived for 1,2,4-TMB. Additionally, the available toxicokinetic database for the two chemicals indicates that internal dose metrics would be comparable. Thus, EPA concluded that deriving such disparate RfCs for these two isomers was not scientifically supported. Rather, EPA concluded that given the similarities in toxicokinetics and toxicity between the two isomers, there was sufficient evidence to support adopting the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB.*

*Please comment on EPA's conclusion to not base the RfC derivation for 1,3,5-TMB on isomer-specific data. Is the scientific justification for not deriving an RfC based on the available data for 1,3,5-TMB supported and has been clearly described?*

The SAB is not aware of chronic or subchronic studies to support an RfC derivation for 1,3,5-TMB similar to the Korsak and Rydinski (1996) study used for 1,2,4-TMB. The SAB agrees with the EPA's conclusion to adopt the RfC for 1,2,4-TMB (based on decreased pain sensitivity) as the RfC for 1,3,5-TMB. The SAB, however, does not agree with the manner in which the EPA derived the fetal and maternal candidate RfC for 1,3,5-TMB based on Saillenfait et al. (2005).

The Saillenfait study was well-conducted and followed the appropriate European Union guidelines and experimental methods for an inhalation developmental toxicity study (i.e., animal model and strain; exposure chamber generation; five concentration groups; atmosphere sampling and analysis; group sizes; maternal and fetal evaluations; and, statistical data analyses). The SAB acknowledges that the Saillenfait study has two major limitations: (1) no neurotoxic endpoints were collected (decreased pain sensitivity had been determined by the EPA as the critical effect for the other two TMB isomers because it was observed following inhalation exposures in multiple rat studies); and, (2) the exposure period was short (GD 6-15; only 10 days). Nevertheless, 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.

Saillenfait et al. (2005) selected 100 ppm (492 mg/m<sup>3</sup>) for the maternal NOAEL for 1,3,5-TMB with 300 ppm (1476 mg/m<sup>3</sup>) as the maternal LOAEL based on decreased maternal weight gain and food intake. The developmental NOAEL in the study was 300 ppm (1476 mg/m<sup>3</sup>) and the developmental LOAEL was 600 ppm (2952 mg/m<sup>3</sup>) based on decreased mean male fetal body weights.

In the draft TMB Assessment, the EPA set the maternal NOAEL at 300 ppm (1476 mg/m<sup>3</sup>) and the maternal LOAEL at 600 ppm (2952 mg/m<sup>3</sup>) based on decreased corrected body weight gain, higher

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1 exposure levels than Saillenfait et al. The SAB finds that this is not a correct interpretation of a maternal  
2 NOAEL for the Saillenfait et al. paper. Decreased corrected body weight gain was measured only at one  
3 time point (C-section) one day after cessation of exposure. Statistically significant decreased maternal  
4 weights were observed at gestational days (GDs) 13-21 when the fetuses would be contributing far less  
5 to the mother's weight and at GDs 6-21 (entire treatment period). Reduced maternal body weights  
6 correspond exactly with the statistically significant decreased food consumption values recorded at GDs  
7 6-13, 13-21 and 6-21 (entire treatment period). An evaluation of statistical methods used in the  
8 Saillenfait et al. study may also be appropriate.

9  
10 In Section 2.3.2 of the TMB Assessment [Methods of Analysis for 1,3,5-TMB (p. 2-36)] a NOAEL of  
11 300 ppm (1476 mg/m<sup>3</sup>) was used as the POD for the developmental endpoint (decreased male fetal body  
12 weight) that was not the NOAEL (POD = 2974 mg/m<sup>3</sup>) as listed in Table 2-13 (p. 2-38). The NOAEL of  
13 1476 mg/m<sup>3</sup> should have been used for the male fetal POD.

14  
15 Using the maternal NOAEL (100 ppm, 492 mg/m<sup>3</sup>) and male fetal NOAEL (300 ppm, 1476 mg/m<sup>3</sup>) as  
16 recommended above for the PODs and increasing the UF<sub>D</sub> from 3 to 10, to address the lack of  
17 neurological endpoint lowers the fetal and maternal candidate RfCs. The SAB notes that this approach  
18 may not adequately address neurotoxicity endpoint used for the other isomers, but the TMB report  
19 should at a minimum conduct a comparative analysis and improve the justification for using the  
20 extrapolation from the other isomers.

21  
22 In addition to the above analysis and considerations, the SAB noted that there are minor errors in the  
23 description of the 1,3,5-TMB inhalation data. In Section 2.3.1. (Identification of Studies and Effects  
24 Other Than Cancer for 1,3,5-TMB), there were errors in Table 2-12 that need to be addressed:

- 25 • The female fetal body weight average for the 100 ppm (492 mg/m<sup>3</sup>) group should be 5.47  
26 ± 0.21 and not 5.74 ± 0.21 (it is correct in other tables of the document).
- 27 • The level of significance for decreased maternal body weight gain for the 600 ppm  
28 (2,952 mg/m<sup>3</sup>) group should have two (\*\*) and not one (\*) asterisk to indicate p < 0.01.
- 29 • The table also states with a footnote (b) that numbers of live fetuses was not explicitly  
30 reported. However, Saillenfait et al. (2005) did report them in Table 3 of their  
31 manuscript. The total numbers of fetuses were 297, 314, 282, 217, and 236, for the  
32 control and exposure groups, respectively, and should be included in Tables 2-2 and 2-12  
33 of the draft TMB Review document.

34  
35 *Charge Question: Please comment on whether EPA's approach to developing the RfC for 1,3,5-TMB is*  
36 *scientifically supported for the available data and clearly described.*  
37

38 The SAB acknowledges that the agency's approach to developing the RfC for 1,3,5-TMB based on a  
39 structurally and toxicologically related isomer is scientifically appropriate. However, the SAB  
40 recommends that the agency strengthen the justification for using this approach for 1,3,5-TMB by: 1)  
41 following the recommendations provided above regarding generating maternal and fetal-based candidate  
42 RfCs from Saillenfait et al. (2005); and 2) discussing the differences as well as similarities in physical  
43 and toxicological parameters (i.e., Henry's Law constant and toxicokinetics) for 1,3,5-TMB as

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1 compared with the other isomers thereby providing sufficient certainty to set an RfC for this isomer  
2 based on 1,2,4-TMB and/or 1,2,3-TMB.

3 **3.2.8. Oral Reference Dose for 1,2,4-TMB**

4 *Charge Question: The oral database for 1,2,4-TMB was considered inadequate for derivation of an*  
5 *RfD. However, available evidence demonstrates similar qualitative profiles of metabolism and patterns*  
6 *of parent compound distribution across exposure routes (i.e., oral and inhalation). Furthermore, there is*  
7 *no evidence that would suggest the toxicity profiles would differ to a substantial degree between oral*  
8 *and inhalation exposures. Therefore, route-to-route extrapolation, from inhalation to oral, using the*  
9 *modified Hissink et al. (2007) PBPK model was used to derive a chronic oral RfD for 1,2,4-TMB. In*  
10 *order to perform the route-to-route extrapolation, an oral component was added to the model, assuming*  
11 *a constant infusion rate into the liver. Specifically, in the absence of isomer-specific information, an*  
12 *assumption was made that 100% of the ingested 1,2,4-TMB would be absorbed by constant infusion of*  
13 *the oral dose into the liver compartment. The contribution of first-pass metabolism was also evaluated.*

14  
15 *Please comment on whether EPA's conclusion that the oral database for 1,2,4-TMB is inadequate for*  
16 *derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral*  
17 *data are available to support the derivation of an RfD for 1,2,4-TMB. If so, please identify these data.*

18  
19 The SAB agrees that the primary toxicological endpoints for 1,2,4-TMB (neurotoxicity, hematotoxicity)  
20 can be extrapolated across dose routes from the inhalation data with the assistance of PBPK modeling.  
21 There is ample precedent with IRIS assessments to use this approach to derive a reference value for a  
22 chemical with missing data by a particular dose route.

23  
24 The SAB is not aware of adequate repeat dose studies for 1,2,4-TMB via the oral dose route. The  
25 available acute exposure studies offer limited support in developing an RfD. The SAB recognizes that  
26 this represents a data gap. One potential way to fill this data gap is to use oral data for a closely related  
27 TMB. There are subchronic gavage toxicology data available for 1,3,5-TMB (Koch Industries 1995;  
28 Adenuga et al. 2014). The EPA chose not to use the Koch et al. (1995) study for derivation of a RfD,  
29 because it did not assess the potential for neurological effects. The EPA should consider deriving RfD(s)  
30 for endpoints developed in the Koch et al. (1995) and Adenuga et al. (2014), such as liver and kidney  
31 weight changes, which were not seen in inhalation studies. This would be consistent with the EPA's goal  
32 to derive RfDs for multiple endpoints. Such oral RfDs for 1,3,5-TMB could then be considered for  
33 extrapolation to the other TMB isomers. Upon doing so, the EPA can consider the appropriateness of  
34 applying a database uncertainty factor to the oral point of departure to compensate for the data gap of  
35 not having an oral neurotoxicity endpoint in the current approach. This option is commonly utilized for  
36 derivation of RfDs in these situations. By comparing the RfDs generated from the oral studies and from  
37 the extrapolation from the RfC through using route-to-route extrapolation, the EPA can provide a clear  
38 explanation for why the use of the PBPK route-to-route based RfD for 1,2,4-TMB may be preferable to  
39 application of a database uncertainty factor to an oral POD.

40  
41 The SAB notes there were limitations in the Koch Industries study (primarily that it didn't involve  
42 neurotoxicity endpoints) and the study does involve an extrapolation across congeners. Presented with  
43 those limitations, the Koch Industries study does not provide a superior alternative to the PBPK

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1 approach for dose route extrapolation that the EPA implemented. As discussed in Section 3.2.10, the  
2 Koch Industries study may provide a means to derive RfDs for several additional endpoints (e.g., liver,  
3 kidney) for 1,3,5-TMB. The EPA can consider such additional RfDs as potentially useful for 1,2,4-TMB  
4 based upon extrapolation across congeners.

5  
6 *Charge Question: A route-to-route extrapolation from inhalation to oral exposure using the modified*  
7 *Hissink et al. (2007) PBPK model has been used to derive an oral RfD for 1,2,4-TMB. Please comment*  
8 *on whether the PBPK modeling been appropriately utilized and clearly described. Are the model*  
9 *assumptions and parameters scientifically supported and clearly described? Are the uncertainties in the*  
10 *model structure adequately characterized and discussed? Please comment on whether this approach is*  
11 *scientifically supported and clearly described in the document.*

12  
13 The EPA adapted the modified Hissink et al. (2007) model for dose route extrapolation of internal dose  
14 by adding an oral delivery component (continuous gastric infusion, instantaneous and complete  
15 absorption). The Hissink et al. (2007) inhalation human model is a reasonable starting point as it  
16 simulated the available human toxicokinetic data fairly well. While the incorporation of the oral dose  
17 route is simplistic, it is acceptable for the current purposes in that the dose metric used for dose response  
18 modeling (parent compound average weekly venous concentration) is not sensitive to peaks and valleys  
19 of a more normal oral intake pattern. A constant infusion averages out the exposure over the course of  
20 the day, thus creating an average venous concentration that is compatible with the dose metric without  
21 further calculation. Overall, the modified Hissink et al. (2007) model adapted for the oral route is likely  
22 to adequately predict human oral exposures and be useful for dose-response modeling and the derivation  
23 of the RfD.

24  
25 *Charge Question: Please comment on the rationale for the selection of the uncertainty factors (UFs)*  
26 *applied to the POD for the derivation of the RfD for 1,2,4-TMB. Are the UFs appropriate based on the*  
27 *recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference*  
28 *Concentration Processes, and clearly described? If changes to the selected UFs are proposed, please*  
29 *identify and provide scientific support for the proposed changes.*

30  
31 The SAB agrees with the uncertainty factors selected in the development of the oral RfD for 1,2,4-TMB,  
32 but recommends that the discussion of uncertainty be strengthened with respect to bioavailability  
33 assumptions. As discussed in the previous response, the oral RfD for 1,2,4-TMB was derived by  
34 incorporating an oral intake component into the PBPK model for 1,2,4-TMB to obtain a human  
35 equivalent oral dose POD. The EPA used the same uncertainty factors for the oral RfD as were used in  
36 the development of the inhalation RfC. Given that the oral RfD was based upon the same endpoint and  
37 derived from the same study as the RfC, the SAB agrees that it is logical to use the same uncertainty  
38 factors. Thus, the comments and recommendations regarding uncertainty factors are applicable to this  
39 charge question as well (see Section 3.2.5). There was discussion regarding whether there is additional  
40 uncertainty associated with incorporation of the oral intake component in the PBPK model, and  
41 specifically regarding assumptions made with that component regarding oral absorption of 1,2,4-TMB  
42 and first-pass metabolism. Unlike modeling of internal concentrations from inhalation exposure that can  
43 be verified with existing experimental data, there are no data with which to assess model predictions of  
44 internal doses following oral 1,2,4-TMB exposures. The SAB does not consider this additional

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1 uncertainty sufficient to increase the composite UF for the oral RfD, largely because the nature of the  
2 uncertainty (possible lower absorption by the oral route), would add extra health protection. The SAB  
3 recommends that the potential uncertainties associated with oral bioavailability of 1,2,4-TMB be  
4 discussed more clearly in the document.

**3.2.9. Oral Reference Dose (RfD) for 1,2,3-TMB**

5 *Charge Question: The oral database for 1,2,3-TMB was considered to be inadequate for derivation of*  
6 *an RfD. Based on the similarities in chemical properties, toxicokinetics, and toxicity profiles between*  
7 *the 1,2,4-TMB and 1,2,3-TMB isomers, EPA concluded that there was sufficient evidence to support*  
8 *adopting the 1,2,4-TMB RfD as the RfD for 1,2,3-TMB.*

9  
10  
11 *Please comment on whether EPA's conclusion that the oral database for 1,2,3-TMB is inadequate for*  
12 *derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral*  
13 *data are available to support the derivation of an RfD for 1,2,3-TMB. If so, please identify these data.*  
14 *Please comment on whether EPA's approach to developing the RfD for 1,2,3-TMB is scientifically*  
15 *supported and clearly described.*

16  
17 The SAB is not aware of adequate repeat dose studies for 1,2,3-TMB via the oral dose route. The  
18 available acute exposure studies offer limited support in developing an RfD.

19  
20 The SAB agrees that the primary toxicological endpoints used for 1,2,4-TMB (neurotoxicity,  
21 hematotoxicity) and extrapolated across dose routes from the inhalation data with the assistance of  
22 PBPK modeling are appropriate for 1,2,3-TMB. There is ample precedent within the IRIS system for  
23 this approach to derive a reference value for a chemical with missing data by a particular dose route.  
24 The SAB notes that the agency appropriately uses the same rationale to derive the RfD for 1,2,4-TMB.  
25 A detailed response is in Section 3.2.8 and the SAB refers the reader to that section rather than reiterate  
26 the response to the charge question.

**3.2.10. Oral Reference Dose (RfD) for 1,3,5-TMB.**

27  
28  
29 *Charge Question: The oral database for 1,3,5-TMB was considered to be inadequate for derivation of*  
30 *an RfD. EPA concluded that given the similarities in the chemical properties, toxicokinetics, and toxicity*  
31 *profiles between the two isomers, there was sufficient evidence to support adopting the RfD for 1,2,4-*  
32 *TMB as the RfD for 1,3,5-TMB.*

33  
34 *Please comment on whether EPA's conclusion that the oral database for 1,3,5-TMB is inadequate for*  
35 *derivation of an RfD is scientifically supported and clearly described.*  
36 *Please comment on whether oral data are available to support the derivation of an RfD for 1,3,5-TMB.*  
37 *If so, please identify these data.*

38  
39 The Koch Industries study (1995) was the only isomer-specific and route-specific study available in the  
40 peer-reviewed literature for oral exposure to 1,3,5-TMB when the TMB Assessment was drafted. The  
41 SAB finds that the concerns expressed by the EPA do not rise to a sufficient level to reject this study  
42 from consideration. Therefore, the Koch study should be carried through the process for comparative

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1 purposes, and the results reported (i.e., POD or RfD). The EPA should make use of the Koch Industries  
2 study and the recently published data by Adenuga et al. (2014) for comparative purposes.

3 These subchronic gavage toxicology studies of 1,3,5-TMB (Koch Industries, 1995; Adenuga et al.,  
4 2014) conduct and report of the 90-day dosing were found to be consistent with good laboratory  
5 practices and requirements. The Koch study was submitted for an EPA Office of Water test rule and was  
6 peer reviewed by three senior scientists (Versar 2013). Manifestations of CNS depression following  
7 inhalation exposures have been seen in rats and humans. The EPA chose not to use the Koch Industries  
8 (1995) study for derivation of the RfD, because it did not assess the potential for neurological effects  
9 and “presented limited toxicological information” other than that the EPA considered in the TMB  
10 assessment (see the draft TMB assessment, appendix F).

11  
12 EPA should consider deriving RfD(s) for endpoints in the Koch Industries (1995) and Adenuga et al.  
13 (2014), such as liver and kidney weight changes, which were not seen in inhalation studies. This would  
14 be consistent with EPA’s goal to derive RfDs for multiple endpoints. Such oral RfDs for 1,3,5-TMB  
15 could then be considered for comparison and possibly extrapolation to the other isomers. Upon doing so,  
16 the EPA can consider the appropriateness of applying a database uncertainty factor to the oral POD to  
17 compensate for the lack of data to support an oral neurotoxicity endpoint. This option is commonly  
18 utilized for derivation of RfDs in these situations. By comparing the RfDs generated from the Koch  
19 Industries (1995) and Adenuga et al. (2014) and from the RfC using route-to-route extrapolation, the  
20 EPA can then provide a clear explanation for why the use of the PBPK route-to-route based RfD for 1,  
21 2, 4-TMB may be preferable to application of a database uncertainty factor to an orally-based point of  
22 departure.

23  
24 **3.2.11. Carcinogenicity of 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB**

25  
26 *Charge Question: The draft Toxicological Review of Trimethylbenzenes did not conduct a quantitative*  
27 *cancer assessment for any isomer due to the lack of available studies. Please comment on whether data*  
28 *are available to support the derivation of a quantitative cancer risk estimate.*  
29

30 The SAB finds that the evidence for carcinogenicity of trimethylbenzenes is limited and that this fact  
31 was well presented by the EPA in the draft toxicological review.

32 The carcinogenicity of 1,2,4-TMB has been assessed in a single study (Maltoni et al. 1997), in which a  
33 single dose level was administered to rats for two years. The SAB determined that this study had a  
34 number of shortcomings. For example, it is unclear how the dose was selected, and only one dose was  
35 used so nothing can be said about dose-response. The dosing schedule was quite unusual and the authors  
36 stated that a more frequent schedule (i.e., 5 or 6 days per week) would have resulted in unacceptable  
37 toxicity. Survival was affected by treatment, but quantitative data and statistical analyses were not  
38 presented. Body weights were collected, but the data were not reported. The only remarkable finding  
39 from the study was neuroesthesioepitheliomas, a tumor arising from the olfactory neuroepithelium,  
40 which occurred in treated but not control animals. No statistical analyses were presented in the paper but  
41 a Fishers Exact test conducted by the EPA indicated that the result was not significant. Nonetheless,

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1 these tumors are very rare in rats and it is noteworthy that in the same study ethylbenzene also induced  
2 neuroesthesioepitheliomas. Carcinogenicity bioassays do not appear to have been conducted with 1,2,3-  
3 TMBor 1,3,5-TMB.

4 Trimethylbenzenes do not appear to be genotoxic when assessed in a standard battery of genotoxicity  
5 assays. The one exception was 1,2,3-TMB in the Ames assay in the absence of S9. The SAB concluded  
6 that the significance of the finding was uncertain because it was not clear what mechanism could lead to  
7 such a response.

8 The SAB is not aware of any human studies on carcinogenicity of TMBs, and notes that a number of  
9 biomarker studies and their association with cancer of various sites have been published. These  
10 biomarker studies should be reviewed and included. Some examples are:

- 11
- 12 • Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of  
13 potential urinary cancer biomarkers--a powerful strategy for breast cancer diagnosis. (Silva et al.  
14 2012)
- 15 • Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-  
16 phase microextraction in combination with gas chromatography-mass spectrometry. (Silva et al.  
17 2011)
- 18 • Cellular responses after exposure of lung cell cultures to secondary organic aerosol particles.  
19 (Gaschen et al. 2010)
- 20

21 Based upon the deficiencies of the Maltoni et al. (1997) study, the lack of bioassays with 1,2,3-TMB and  
22 1,3,5-TMB, and the lack of human studies, the SAB agrees that the EPA could not conduct a  
23 quantitative cancer assessment for any isomer due to the lack of appropriate studies.

### 24 **3.3. Additional Recommendations**

25 The SAB identified two additional topics not addressed directly in the Charge that warrant additional  
26 consideration by the agency: (1) an expanded discussion of sensitive life stages and vulnerable  
27 populations, and (2) deriving the subchronic RfC and RfD for the TMB isomers.

#### 28 **3.3.1. Susceptible Populations and Lifestages**

29

30 The draft TMB Assessment provides one paragraph on this subject, spanning pages 1-54 to 1-55. It  
31 correctly identifies various types of immaturity (metabolism, renal clearance) as potentially leading to  
32 greater vulnerability in early life. However, this section could provide a better outline of the kinds of  
33 information needed to understand the potential vulnerabilities in early life, including key aspects of  
34 TMB mode of action and key developmental features.

35

36 Regarding mode of action, it is important to know:

- 37 • whether it is the parent compound or metabolites (or both) that contribute to toxic effect;
- 38 • which metabolic systems are responsible for removing the parent compound and creating  
39 important metabolites; and

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- what is the role of distributional phenomena (e.g., uptake into brain; partitioning into fat) and other clearance mechanisms in determining chemical fate and access to target sites.

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.

Regarding toxicodynamic vulnerability, perhaps the most relevant data would be developmental neurotoxicity information for the TMBs themselves. This has been acknowledged as a data gap by the EPA but a Hungarian study (Lehotsky et al. 1985) did test a C-9 mixture containing trimethylbenzenes (Aromatol) for developmental neurotoxicity in rats. That study had minimal reporting of results, simply stating that there were no effects of Aromatol on dams or offspring at any time point (Lehotsky et al. 1985). This is in spite of the fact that the high dose of Aromatol was 2000 mg/m<sup>3</sup>, a dose that one would expect to have a neurotoxic effect in dams during and after exposure, based upon results of other testing. The lack of any toxicity in dams or offspring, combined with the lack of reporting of any data (including Aromatol treatment group neurological testing or Aromatol composition) and the fact that it was a mixture and not a specific TMB, makes this study of minimal utility. Therefore, the developmental neurotoxicity data gap remains.

Some discussion is warranted concerning what is known about early life vulnerability to aromatic solvent neurotoxicity. Several studies are available suggesting a vulnerable window of brain development in mice to the neurotoxic effects of toluene (Win-Shwe et al. 2010, 2012). The USEPA should evaluate this evidence relative to other developmental neurotoxicity studies that may be available for toluene and other related alkylbenzenes to determine whether this data gap represents a large uncertainty.

This section should conclude with a statement as to whether any specific data exist for TMBs that would show the extent of early life vulnerability based upon toxicokinetic and toxicodynamic considerations and the degree to which such data for related alkylbenzenes helps to fill these data gaps.

### **3.3.2. Developing Subchronic RfCs and RfDs**

In addition to responding to the charge questions related to development of chronic toxicity values for 1,2,4-, 1,2,3-, and 1,3,5-TMB, the TMB Review Panel discussed using the analysis presented in the

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1 TMB Assessment to support development of subchronic toxicity values (i.e., subchronic RfCs and oral  
2 RfDs) for these chemicals.

3  
4 The EPA and other environmental regulatory agencies are frequently required to address the risks  
5 associated with exposures lasting less than a lifetime. Because the toxic endpoint(s) of concern for a  
6 given chemical, as well as threshold doses or concentrations for toxicity, can change with exposure  
7 duration, the toxicity value used in risk assessment should be matched to the extent possible to the  
8 length of exposure associated with the scenario of interest. Recognizing the need for toxicity values for  
9 less-than-lifetime exposures, the EPA Risk Assessment Forum recommended that the agency develop  
10 such values and incorporate them into the IRIS database (U.S. EPA, 2002).

11  
12 In the case of the TMBs, the principal studies used to create the proposed RfCs and RfDs are all  
13 subchronic in duration, and the analysis needed to support a robust set of subchronic toxicity values has  
14 in effect already been done for these chemicals. The SAB acknowledges that the derivation of  
15 subchronic RfCs and RfDs may not always be appropriate. However, the toxic endpoints and dose-  
16 response relationships for the TMBs in the draft report are clearly relevant for subchronic exposure, and  
17 the same PODs and the same uncertainty factors — except UFs, which is used to generate a chronic  
18 toxicity value from subchronic study data — would apply to the development of a set of subchronic  
19 RfCs and RfDs.

20  
21 Given the potential usefulness of these toxicity values for risk assessment, the importance of having the  
22 values available on IRIS, and the very small amount of additional work required to add them to the  
23 TMB Assessment, the SAB suggests that IRIS consider including subchronic RfCs and RfDs for 1,2,4-  
24 TMB, 1,2,3-TMB, and 1,3,5-TMB. These values would be calculated using the same inputs as for the  
25 chronic toxicity values, but omitting the UFs. The SAB anticipates that incorporation of these values will  
26 require minimal edits to existing tables and text.

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**APPENDIX A**

NCEA Charge to the Science Advisory Board for the  
IRIS Toxicological Review of Trimethylbenzenes  
August 2013 (Updated May 2014)<sup>2</sup>

**Introduction**

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the draft Toxicological Review of Trimethylbenzenes (1,2,3-trimethylbenzene [1,2,3-TMB], 1,2,4-trimethylbenzene [1,2,4-TMB], and 1,3,5-trimethylbenzene [1,3,5-TMB]) that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). This is a new assessment; there is currently no entry on the IRIS database for any isomer of trimethylbenzene.

IRIS is a human health assessment program that evaluates scientific information on effects that may result from exposure to specific chemical substances in the environment. Through IRIS, EPA provides high quality science-based human health assessments to support the Agency's regulatory activities and decisions to protect public health. IRIS assessments contain information for chemical substances that can be used to support the first two steps (hazard identification and dose-response assessment) of the human health risk assessment process. When supported by available data, IRIS provides health effects information and toxicity values for chronic health effects (including cancer and effects other than cancer). Government and others combine IRIS toxicity values with exposure information to characterize public health risks of chemical substances; this information is then used to support risk management decisions designed to protect public health.

The external review draft Toxicological Review of Trimethylbenzenes is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. This draft IRIS assessment includes:

- a *Preamble* to describe the methods used to develop IRIS assessments;
- an *Executive Summary* to concisely summarize the major conclusions of the assessment;
- a *Literature Search Strategy and Study Selection* section to describe the process for identifying and evaluating the evidence for consideration in developing the assessment;
- a *Hazard Identification* section to systematically synthesize and integrate the available evidence of organ/system-specific hazards; and
- a *Dose-Response Analysis* section to describe the selection of studies for consideration in calculating toxicity values and to provide details of the analysis and methodology in deriving and selecting toxicity values.

Additionally, appendices for chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental Information* (See Appendix A to C) to the draft Toxicological Review. The draft assessment was developed according to guidelines and technical reports published by EPA (see Preamble) and contains a qualitative characterization of the

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<sup>2</sup> The charge for TMBs was updated to include general charge question #4 requesting comment from the external peer review panel on the adequacy of EPA's assessment revisions and response to the public comments. The CAAC Augmented for the TMB Panel discussed and revised this charge question on the May 22, 2014 teleconference.

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1 hazards for TMBs, including a cancer descriptor of a chemical's human carcinogenic potential, and  
2 noncancer toxicity values, including a chronic oral reference dose (RfD) and a chronic inhalation reference  
3 concentration (RfC) for all three trimethylbenzene isomers. A quantitative cancer assessment for  
4 trimethylbenzenes was not conducted due to inadequate data.

5 **Charge Questions**

6 In April 2011, the National Research Council (NRC) released its *Review of the Environmental Protection*  
7 *Agency's Draft IRIS Assessment of Formaldehyde* (NRC 2011). In addition to offering comments specifically  
8 about EPA's draft formaldehyde assessment, the NRC included comments and recommendations for  
9 improving the development of IRIS assessments. The IRIS Program's implementation of the NRC  
10 recommendations is following a phased approach. Phase 1 of implementation has focused on a subset of  
11 the short-term recommendations, such as editing and streamlining documents, increasing transparency  
12 and clarity, and using more tables, figures, and appendices to present information and data in assessments.  
13 Phase 1 also focused on assessments that had been near the end of the development process and close to  
14 final posting. The IRIS Program is now in Phase 2 of implementation which addresses all of the short-term  
15 NRC recommendations. The Program is implementing all of these recommendations but recognizes that  
16 achieving full and robust implementation of certain recommendations will be an evolving process with  
17 input and feedback from the public, stakeholders, and external peer review committees. This phased  
18 approach is consistent with the NRC's *Roadmap for Revision* as described in Chapter 7 of the formaldehyde  
19 review report. The NRC stated that "the committee recognizes that the changes suggested would involve a  
20 multi-year process and extensive effort by the staff at the National Center for Environmental Assessment  
21 and input and review by the EPA Science Advisory Board and others."

22 Below is a set of charge questions that address scientific issues in the draft IRIS Toxicological Review of  
23 Trimethylbenzenes. The charge questions also seek feedback on whether the document is clear and  
24 concise, a central concern expressed in the NRC report. Please provide detailed explanations for responses  
25 to the charge questions. EPA will also consider the Science Advisory Board review panel's comments on  
26 other major scientific issues specific to the hazard identification and dose-response assessment of  
27 trimethylbenzenes. Please consider the accuracy, objectivity, and transparency of EPA's analyses and  
28 conclusions in your review.

29  
30 **General Charge Questions:**

- 31 1. NRC (2011) indicated that the introductory section of IRIS assessments needed to be expanded to  
32 describe more fully the methods of the assessment. NRC stated that they were "not recommending  
33 the addition of long descriptions of EPA guidelines to the introduction, but rather clear, concise  
34 statements of criteria used to exclude, include, and advance studies for derivation of [toxicity  
35 values]." Please comment on whether the new Preamble provides a clear and concise description of  
36 the guidance and methods that EPA uses in developing IRIS assessments.
- 37 2. NRC (2011) provided comments on ways to improve the presentation of steps used to generate  
38 IRIS assessments and indicated key outcomes at each step, including systematic review of evidence,  
39 hazard identification, and dose-response assessment. Please comment on the new IRIS document  
40 structure and whether it will increase the ability for assessment to be more clear, concise and easy  
41 to follow.
- 42 3. NRC (2011) state that "all critical studies need to be thoroughly evaluated with standardized  
43 approaches that are clearly formulated" and that "strengthened, more integrative, and more

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1 transparent discussions of weight of evidence are needed.” NRC also indicated that the changes  
2 suggested would involve a multiyear process. Please comment on EPA’s success thus far in  
3 implementing these recommendations.

- 4 4. EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes and has  
5 revised the assessment to respond to the scientific issues raised in the comments. A summary of  
6 the public comments and EPA’s responses are provided in Appendix F of the Supplemental  
7 Information to the Toxicological Review of Trimethylbenzenes. Are there scientific issues that  
8 were raised by the public as described in Appendix F that may not have been adequately  
9 addressed by EPA?

10  
11 **Chemical-Specific Charge Questions**

12 **A. Executive Summary**

- 13 1. The major conclusions of the assessment pertaining to the hazard identification and dose-response  
14 analysis have been summarized in the Executive Summary. Please comment on the whether the  
15 conclusions have been clearly and sufficiently described for purposes of condensing the  
16 Toxicological Review information into a concise summary.

17 **B. Literature Search Strategy/Study Selection**

- 18 1. The process for identifying and selecting pertinent studies for consideration in developing the  
19 assessment is detailed in the Literature Search Strategy/Study Selection section. Please comment on  
20 the whether the literature search approach, screening, evaluation, and selection of studies for inclusion  
21 in the assessment are clearly described and supported. Please identify any additional peer-reviewed  
22 studies from the primary literature that should be considered in the assessment of noncancer and  
23 cancer health effects of 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB.

24 **C. Hazard Identification**

25 ***Synthesis of Evidence***

- 26 1. A synthesis of the evidence for trimethylbenzene toxicity is provided in Chapter 1, *Hazard*  
27 *Identification*. Please comment on whether the available data have been clearly and appropriately  
28 synthesized for each toxicological effect. Please comment on whether the weight of evidence for hazard  
29 identification has been clearly described and scientifically supported.

30 ***Summary and Evaluation***

- 31 1. Does EPA’s hazard assessment of noncancer human health effects of trimethylbenzenes clearly  
32 integrate the available scientific evidence (i.e., human, experimental animal, and mechanistic evidence)  
33 to support the conclusions that trimethylbenzenes pose potential hazards to the nervous system,  
34 respiratory system, the developing fetus, and the circulatory system (i.e., blood)?
- 35 2. Does EPA’s hazard assessment of the carcinogenicity of trimethylbenzenes clearly integrate the  
36 available scientific evidence to support the conclusions that under EPA’s *Guidelines for Carcinogen Risk*  
37 *Assessment* ([U.S. EPA, 2005](#)), there is “inadequate information to assess the carcinogenic potential” of  
38 trimethylbenzenes?

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**D. Toxicokinetics and Pharmacokinetic Modeling**

Data characterizing the toxicokinetics of 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB following inhalation and oral exposures in humans and experimental animals supports the use of physiologically-based pharmacokinetic (PBPK) models for 1,2,4-TMB. For the purposes of this assessment, the Hissink et al. (2007) model, originally describing 1,2,4-TMB toxicokinetics following exposure to white spirit (a complex mixture of volatile organic compounds), was modified by EPA to calculate internal dose metrics following exposure to 1,2,4-TMB alone for the derivation of an inhalation RfC for 1,2,4-TMB. Additionally, the model was further modified by the addition of an oral route of exposure for use in a route-to-route extrapolation for the derivation of an oral RfD for 1,2,4-TMB.

1. Please comment on whether the selected PBPK model (Hissink et al., 2007) with EPA's modifications adequately describe the toxicokinetics of 1,2,4-TMB (Appendix B). Was the PBPK modeling appropriately utilized and clearly described? Are the model assumptions and parameters scientifically supported and clearly described? Are the uncertainties in the model structure adequately characterized and discussed?
2. The internal dose metric selected for use in the derivation of the RfC and RfD for 1,2,4-TMB was the steady-state weekly average venous blood concentration (mg/L) of 1,2,4-TMB for rats exposed for 6 h/day, 5 days/week. Please comment on whether the selection of this dose metric is scientifically supported and clearly described. If a different dose metric is recommended for deriving the RfC, please identify this metric and provide scientific support for this choice. Are the uncertainties in the selected dose metric adequately characterized and discussed?

**E. Inhalation Reference Concentration (RfC) for 1,2,4-TMB**

1. A 90-day inhalation toxicity study of 1,2,4-TMB in male rats (Korsak and Rydzyński, 1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.
2. Decreased pain sensitivity (measured as an increased latency to pawlick response after a hotplate test) in male Wistar rats was concluded by EPA to be an adverse effect on the nervous system and was selected as the critical effect for the derivation of the RfC. Please comment on whether the selection and characterization of this critical effect is scientifically supported and clearly described. If a different endpoint(s) is recommended as the critical effect(s) for deriving the RfC, please identify this effect and provide scientific support for this choice.
3. In order to characterize the observed dose-response relationship comprehensively, benchmark dose (BMD) modeling was used in conjunction with dosimetric adjustments for calculating the human equivalent concentration (HEC) from a rat and human PBPK model (Hissink et al., 2007) to identify the point of departure (POD) for derivation of the RfC. Please comment on whether this approach is scientifically supported for the available data, and clearly described.
  - a. Has the modeling been appropriately conducted and clearly described, based on EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012)?
  - b. Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR equal to 1 standard deviation change in the control mean for the latency to pawlick response) been supported and clearly described?

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- 1 4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD  
2 for the derivation of the RfC for 1,2,4-TMB. Are the UF's appropriate based on the recommendations  
3 described in Section 4.4.5 of *A Review of the Reference Dose and Reference Concentration Processes* ([U.S.  
4 EPA, 2002](#)), and clearly described? If changes to the selected UF's are proposed, please identify and  
5 provide scientific support for the proposed changes.

6 **F. Inhalation Reference Concentration (RfC) for 1,2,3-TMB**

- 7 1. A 90-day inhalation toxicity study of 1,2,3-TMB in male rats ([Korsak and Rydzyński, 1996](#)) was selected  
8 as the basis for the derivation of the RfC. Please comment on whether the selection of this study is  
9 scientifically supported and clearly described. If a different study is recommended as the basis for the  
10 RfC, please identify this study and provide scientific support for this choice.
- 11 2. Decreased pain sensitivity (measured as an increased latency to pawlick response after a hotplate test)  
12 in male Wistar rats was concluded by EPA to be an adverse effect on the nervous system and was  
13 selected as the critical effect for the derivation of the RfC. Please comment on whether the selection and  
14 characterization of this critical effect is scientifically supported and clearly described. If a different  
15 endpoint(s) is recommended as the critical effect(s) for deriving the RfC, please identify this effect and  
16 provide scientific support for this choice.
- 17 3. In order to characterize the observed dose-response relationship comprehensively, benchmark dose  
18 (BMD) modeling was used in conjunction with default dosimetric adjustments ([U.S. EPA, 1994b](#)) for  
19 calculating the human equivalent concentration (HEC) to identify the point of departure (POD) for  
20 derivation of the RfC. Please comment on whether this approach is scientifically supported for the  
21 available data, and clearly described.
- 22 a. Has the modeling been appropriately conducted and clearly described, based on EPA's  
23 *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#))?
- 24 b. Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR  
25 equal to a 1 standard deviation change in the control mean for the latency to pawlick  
26 response) been supported and clearly described?
- 27 4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD  
28 for the derivation of the RfC for 1,2,3-TMB. Are the UF's appropriate based on the recommendations  
29 described in Section 4.4.5 of *A Review of the Reference Dose and Reference Concentration Processes* ([U.S.  
30 EPA, 2002](#)), and clearly described? If changes to the selected UF's are proposed, please identify and  
31 provide scientific support for the proposed changes.

32 **G. Inhalation Reference Concentration (RfC) for 1,3,5-TMB**

33 One developmental toxicity study ([Saillenfait et al., 2005](#)) following inhalation exposure to 1,3,5-TMB was  
34 identified in the literature and was considered as a potential principal study for the derivation of the RfC  
35 for 1,3,5-TMB. However, the candidate RfC derived for 1,3,5-TMB based on this study (and the critical effect  
36 of decreased maternal weight gain) was 20-fold higher than the RfC derived for 1,2,4-TMB (based on  
37 decreased pain sensitivity). Given the available toxicological database for 1,2,4-TMB and 1,3,5-TMB, there  
38 are several important similarities in the two isomers' neurotoxicity that support an RfC for 1,3,5-TMB that  
39 is not substantially different than the RfC derived for 1,2,4-TMB. Additionally, the available toxicokinetic  
40 database for the two chemicals indicates that internal dose metrics would be comparable. Thus, EPA  
41 concluded that deriving such disparate RfCs for these two isomers was not scientifically supported. Rather,

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1 EPA concluded that given the similarities in toxicokinetics and toxicity between the two isomers, there was  
2 sufficient evidence to support adopting the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB.

- 3 1. Please comment on EPA's conclusion to not base the RfC derivation for 1,3,5-TMB on isomer-specific  
4 data. Is the scientific justification for not deriving an RfC based on the available data for 1,3,5-TMB  
5 supported and has it been clearly described?
- 6 2. Please comment on whether EPA's approach to developing the RfC for 1,3,5-TMB is scientifically  
7 supported for the available data and clearly described.

8 **H. Oral Reference Dose (RfD) for 1,2,4-TMB**

9 The oral database for 1,2,4-TMB was considered inadequate for derivation of an RfD. However, available  
10 evidence demonstrates similar qualitative profiles of metabolism and patterns of parent compound  
11 distribution across exposure routes (i.e., oral and inhalation). Furthermore, there is no evidence that would  
12 suggest the toxicity profiles would differ to a substantial degree between oral and inhalation exposures.  
13 Therefore, route-to-route extrapolation, from inhalation to oral, using the modified Hissink et al. (2007)  
14 PBPK model was used to derive a chronic oral RfD for 1,2,4-TMB. In order to perform the route-to-route  
15 extrapolation, an oral component was added to the model, assuming a constant infusion rate into the liver.  
16 Specifically, in the absence of isomer-specific information, an assumption was made that 100% of the  
17 ingested 1,2,4-TMB would be absorbed by constant infusion of the oral dose into the liver compartment.  
18 The contribution of first-pass metabolism was also evaluated.

- 19 1. Please comment on whether EPA's conclusion that the oral database for 1,2,4-TMB is inadequate for  
20 derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral  
21 data are available to support the derivation of an RfD for 1,2,4-TMB. If so, please identify these data.
- 22 2. A route-to-route extrapolation from inhalation to oral exposure using the modified Hissink et al. (2007)  
23 PBPK model has been used to derive an oral RfD for 1,2,4-TMB. Please comment on whether the PBPK  
24 modeling been appropriately utilized and clearly described. Are the model assumptions and  
25 parameters scientifically supported and clearly described? Are the uncertainties in the model structure  
26 adequately characterized and discussed? Please comment on whether this approach is scientifically  
27 supported and clearly described in the document.
- 28 3. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD  
29 for the derivation of the RfD for 1,2,4-TMB. Are the UFs appropriate based on the recommendations  
30 described in Section 4.4.5 of *A Review of the Reference Dose and Reference Concentration Processes* ([U.S.](#)  
31 [EPA, 2002](#)), and clearly described? If changes to the selected UFs are proposed, please identify and  
32 provide scientific support for the proposed changes.

33 **I. Oral Reference Dose (RfD) for 1,2,3-TMB**

34 The oral database for 1,2,3-TMB was considered to be inadequate for derivation of an RfD. Based on the  
35 similarities in chemical properties, toxicokinetics, and toxicity profiles between the 1,2,4-TMB and 1,2,3-  
36 TMB isomers, EPA concluded that there was sufficient evidence to support adopting the 1,2,4-TMB RfD as  
37 the RfD for 1,2,3-TMB.

- 38 1. Please comment on whether EPA's conclusion that the oral database for 1,2,3-TMB is inadequate for  
39 derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral  
40 data are available to support the derivation of an RfD for 1,2,3-TMB. If so, please identify these data.

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- 1 2. Please comment on whether EPA's approach to developing the RfD for 1,2,3-TMB is scientifically  
2 supported and clearly described.

3 **J. Oral Reference Dose (RfD) for 1,3,5-TMB**

4 The oral database for 1,3,5-TMB was considered to be inadequate for derivation of an RfD. EPA concluded  
5 that given the similarities in the chemical properties, toxicokinetics, and toxicity profiles between the two  
6 isomers, there was sufficient evidence to support adopting the RfD for 1,2,4-TMB as the RfD for 1,3,5-TMB.

- 7 1. Please comment on whether EPA's conclusion that the oral database for 1,3,5-TMB is inadequate for  
8 derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral  
9 data are available to support the derivation of an RfD for 1,3,5-TMB. If so, please identify these data.
- 10 2. Please comment on whether EPA's approach to developing the RfD for 1,3,5-TMB is scientifically  
11 supported and clearly described.

12 **K. Carcinogenicity of 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB**

- 13 1. The draft Toxicological Review of Trimethylbenzenes did not conduct a quantitative cancer assessment  
14 for any isomer due to the lack of available studies. Please comment on whether data are available to  
15 support the derivation of a quantitative cancer risk estimate.

16  
17  
18

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## **APPENDIX B**

### **Results of Review of Timethylbenzene**

#### **PBPK Model Internal Metrics**

##### **Model Overview**

A PBPK model for white spirit constituents was developed and published by TNO Quality of Life, The Netherlands (Hissink et al., 2007). This model was reviewed along with other trimethyl benzene (1,2,4-TMB) models by the U.S. EPA (the Agency) and chosen to use for internal dose metric estimation (U.S. EPA, 2013). In this process, a detailed computer code analysis was conducted, and generally found to be acceptable, but some corrections were necessary.

The changes to the model code (.*csf* file) consisted of addressing a coding error in the supplied file (not discussed in the manuscript) that resulted in metabolic rate changing over the course of exposure ( $V_{MAX} = K_{VMAX} * (ABS(T-TLEG) + (T-TLEG)) / 2 + V_{MAX0}$ ).  $K_{VMAX}$  was set equal to 0, so metabolic rates are consistent throughout time. Second, flow mass-balance was corrected by adding a simple equation to calculate total as 1-summed flows ( $Q_{STOTC} = 1 - Q_{RTOTC}$ ). Finally, the description of inhaled/exhaled concentrations from inhaled exposures were altered to fit conventions of alveolar volume (70% of total). The Agency version of the model achieves this 70% by adding a second ventilation rate ( $Q_{PC}$ ) that represents alveolar and  $Q_{P2C}$  that represents entire lung volume. Changes in input parameters (.*m* files) were also incorporated including anatomical parameters which were updated to base them on the conventionally used parameters listed in (Brown et al., 1997) (Tables 1 and 2)

##### **Rat Internal Dose Metrics**

After implementing the modest model corrections, the Agency numerically optimized metabolic parameters ( $V_{max}$  and  $K_m$ ) to fit the rodent data. The Agency chose the

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repeat dosing data of Swiercz et al. (2003) to calibrate the model and optimized parameters are shown in Table 1. The model fits to the data sets from Hissink et al., 2007 and Swiercz et al., 2003 are shown in Figures 1 and 2 and a comparison of predicted blood concentrations to study-specific end of exposure measures concentrations for these two studies are shown in Table 3.

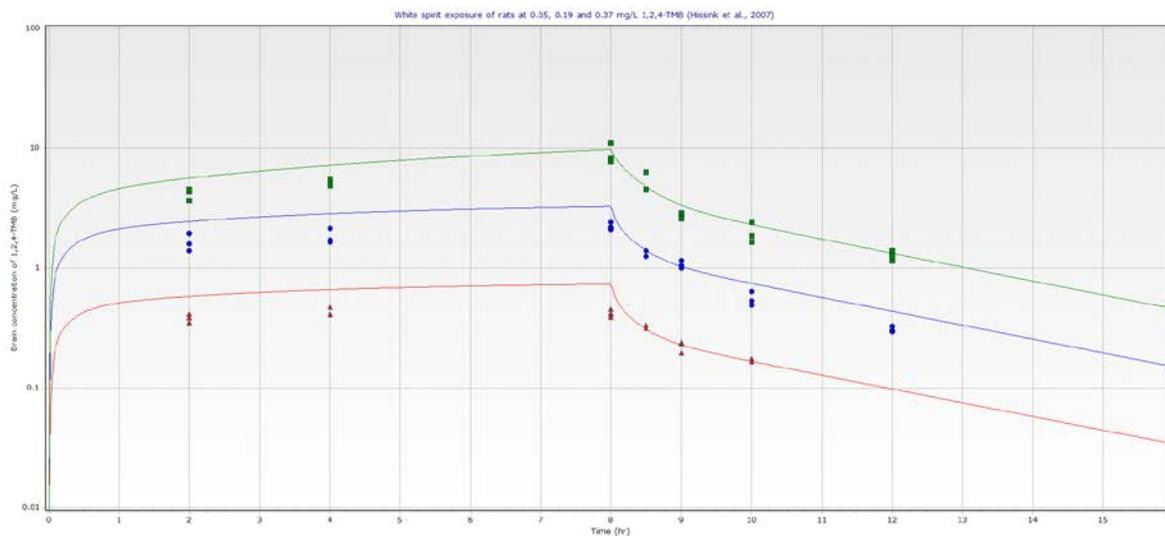
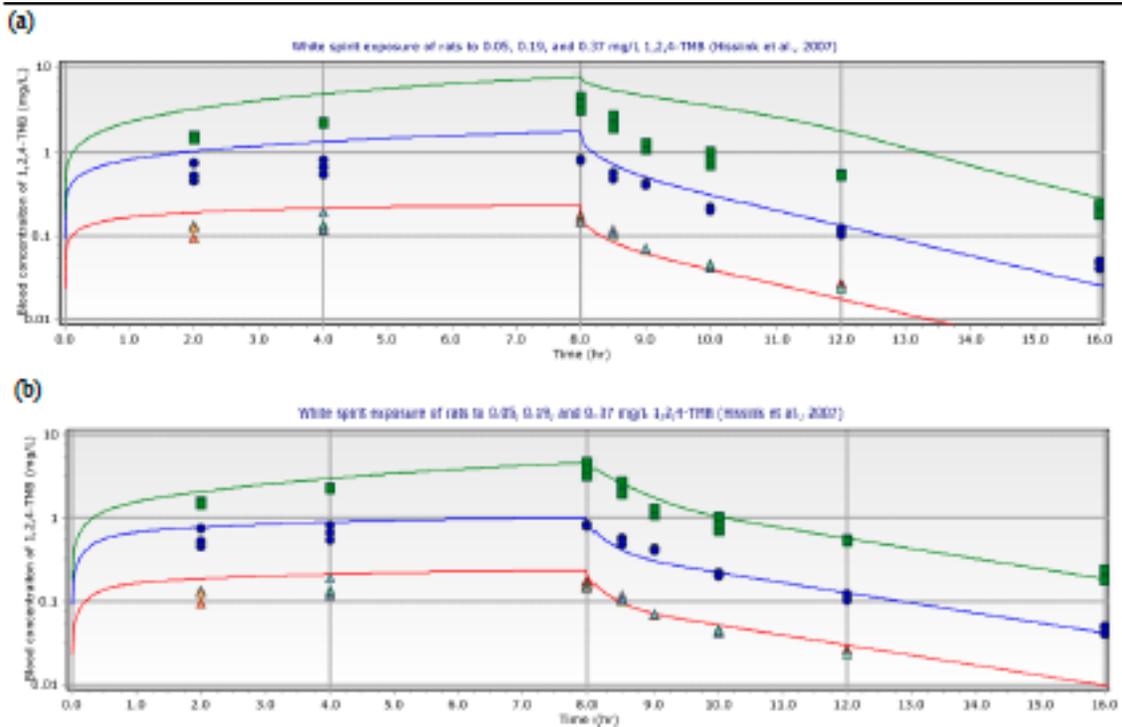


Figure 1. Model predicted blood concentrations for the study described in Hissink et al., 2007. Compare this figure to B-10(b) of U.S. EPA (2013). This figure represents the fit to the final model parameters and thus replicates Figure B-10(b).

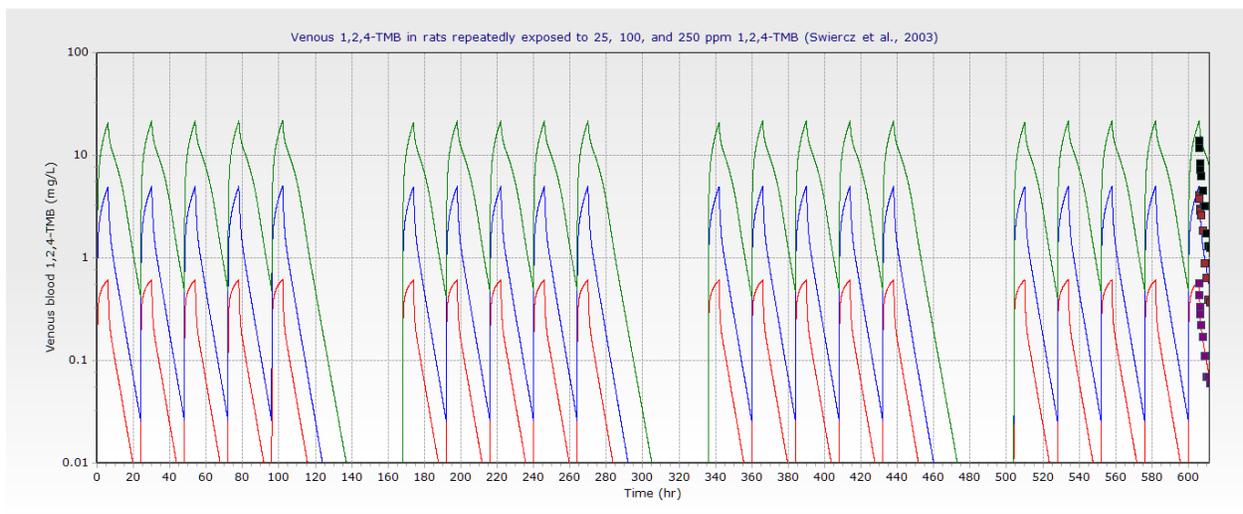
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Note: Rats exposed to 1,2,4-TMB in white spirit (WS) (Hissink et al., 2007) (a) before and (b) after numerical optimization. See Legend, Figures B-7 and B-8.

**Figure B-10. Comparisons of model predictions to measured blood concentrations in rats exposed to 1,2,4-TMB in WS.**



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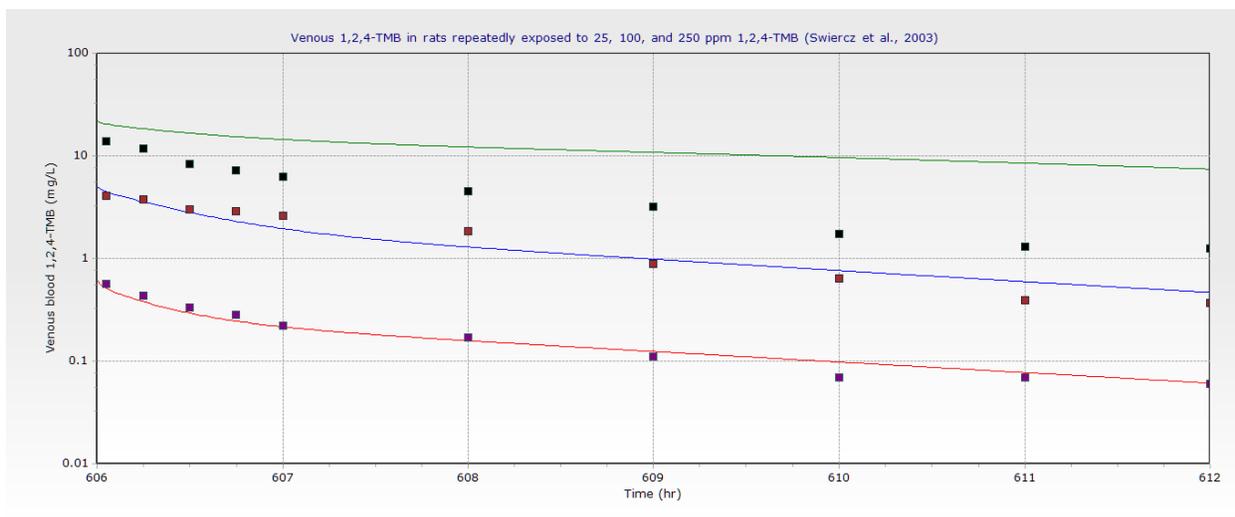
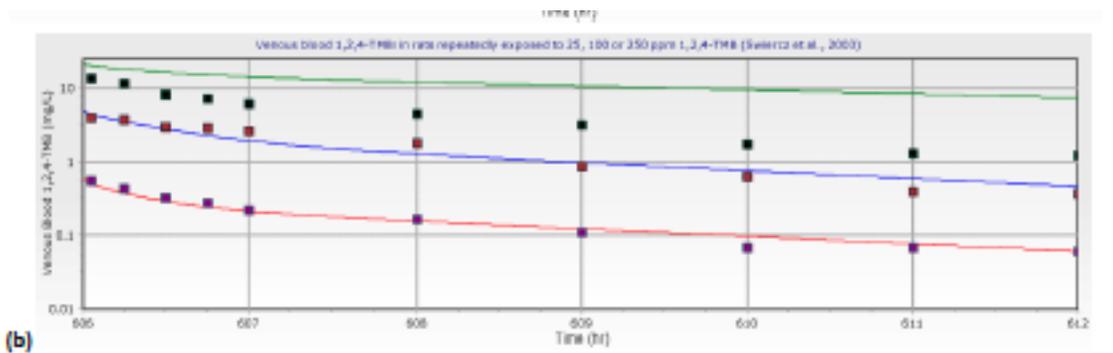


Figure 2. Model predicted blood concentrations for the study described in Swiercz et al., 2003. Rats were exposed to TMB 6 hr/day, 5 days/wk for 4 weeks. Blood was collected from the tail vein after the last exposure. Top) whole timecourse, Bottom) last 6 hr. Compare this figure to B-12 of U.S. EPA (2013).



Swiercz et al. (2003) in rats repeatedly exposed to 1,2,4-TMB: (a) before and (b) after numerical optimization. See Legend in Figures B-7 and B-8.

**Figure B-12. Comparisons of model predictions to measured venous blood concentrations by Swiercz et al. (2003) in rats repeatedly exposed to 1,2,4-TMB.**

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Internal blood 1,2,4-TMB metrics predicted by the model were compared to a few other studies and consistently over-predicted the data, as reported in U.S. EPA 2013 (Tables 4 and 5).

### **Human Internal Dose Metrics**

In the report (U.S. EPA 2013), the human exposure data of Hissink et al., 2007 was shown with the  $V_{max}$  and  $K_m$  optimized to fit the rat data from the same study, and was not shown using the  $V_{max}$  and  $K_m$  optimized from the Swiercz et al. (2003) rat data which was used in the final model (Table 2). Figure 3 shows the fit of that data using the  $V_{max}C/K_m$  used for internal dose metric determinations.

In agreement with figures B-14 and B-15, the model also under-predicts the data from Järnberg et al. (1998, 1997a; 1996) (Figure 4) and Kostrzewki et al, 1997 (Figure 5).

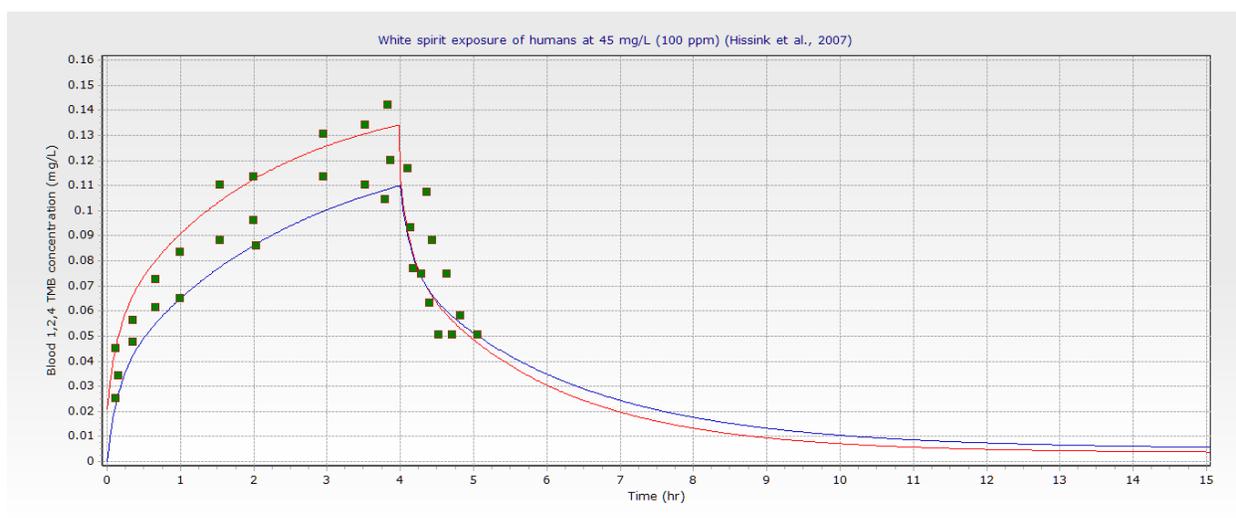


Figure 3. Comparisons of model predictions to measured human venous blood in human volunteers exposed to 100 ppm WS with 7.8% 1,2,4-TMB (39 mg/m<sup>3</sup> 1,2,4-TMB). The red line shows the fit when the metabolic parameters optimized to fit rat data

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from the same study (Hissink et al., 2007) are used, the blue line shows the fit when the  $V_{maxC}$  and  $K_m$  optimized from the study of Swiercz et al., 2003 is used.

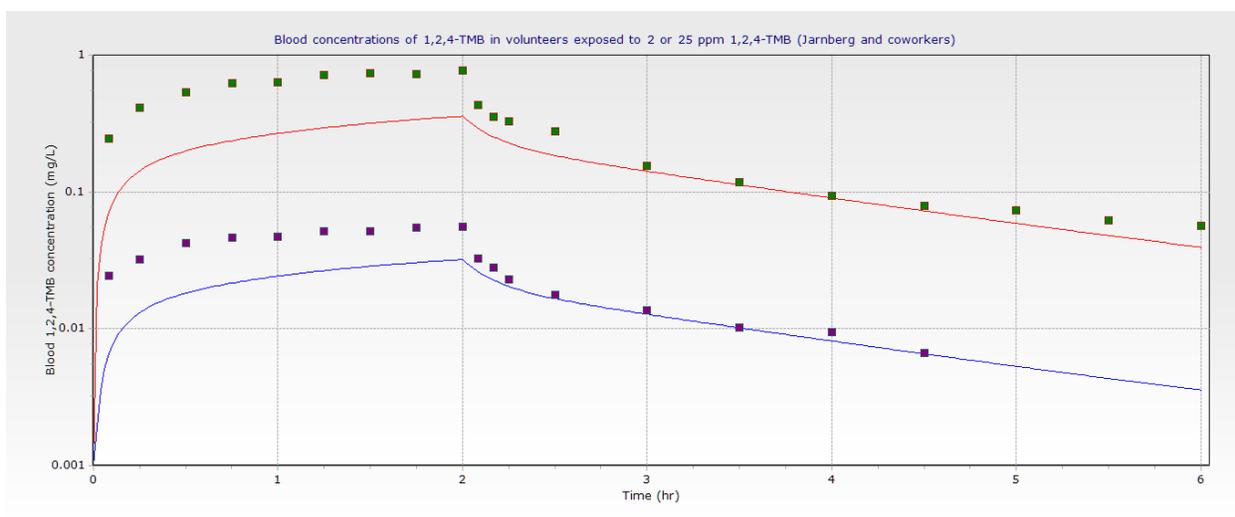
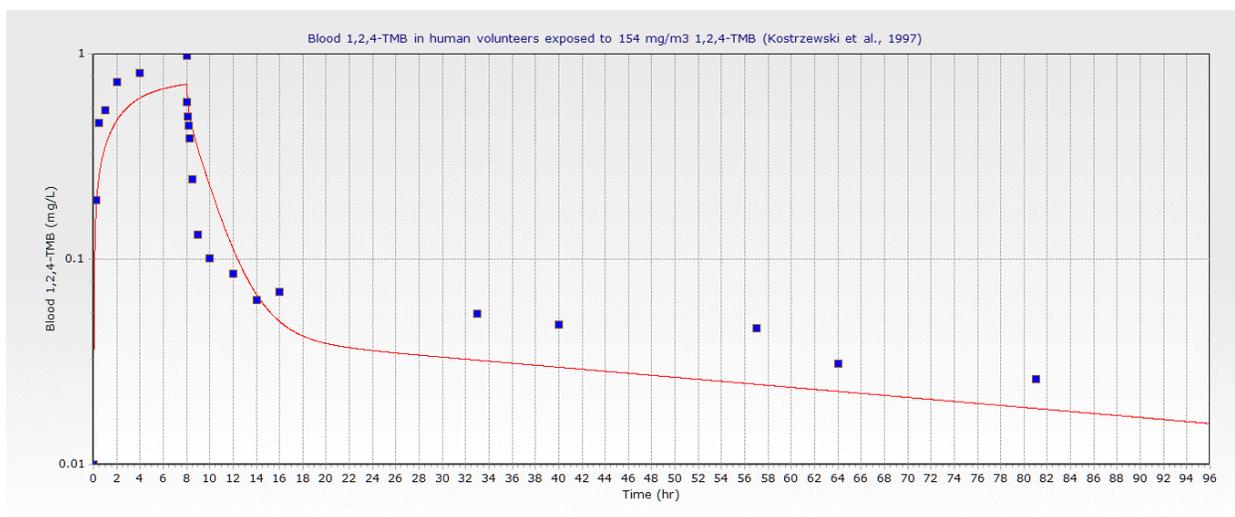


Figure 4. Comparisons of model predictions to measured human venous blood concentrations of Järnberg et al. (1998, 1997a; 1996) in volunteers exposed to 2 or 25 ppm (~10 or 123 mg/m<sup>3</sup>) 1,2,4-TMB for 2 hours while riding a bicycle (50 W).



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**Figure 5. Comparisons of model predictions to measured human venous blood concentrations in Kostrzewki et al. (1997) in human volunteers exposed to 154 mg 1,2,4-TMB/m<sup>3</sup> for 8 hours.**

#### Conclusions

Agency changes are consistent with state of the art PBPK modeling and well-implemented. The Agency version of the model consistently underpredicts compared to the Hissink parameterization (Data not shown). The model still consistently overpredicts rat data. According to U.S. EPA 2013:

*The measured Wistar rat arterial blood and tissue concentrations were consistently overpredicted by the model, suggesting collection delays in the studies. The model also consistently overpredicted the measured Sprague-Dawley rat tissue and blood concentrations, including the “recovery” (12 hr post-exposure) samples, which should not be subject to collection delays. Many of the “validation” comparisons were made at exposure concentrations (250 ppm [1,230 mg/m<sup>3</sup>] or greater) for which the optimized model did not provide accurate venous blood concentrations. It cannot be determined with the available data whether the 2–3-fold differences between the model and Sprague-Dawley rat blood concentrations at lower concentrations (75 and 150 ppm [369 and 738 mg/m<sup>3</sup>]) are due to methodological differences (e.g., in sample collections and analysis) or true strain differences. Overall, we conclude that the optimized model produces acceptable simulations of venous blood 1,2,4-TMB for chronic exposure to  $\leq 100$  ppm (492 mg/m<sup>3</sup>) for rats or  $\leq 30$  ppm (147.6 mg/m<sup>3</sup>) for humans 1,2,4-TMB by inhalation*

Because the overprediction is consistent between rodent strains and across studies, the model optimization choices should maybe be reconsidered. An attempt was made to evaluate the model optimizations, but the data files used to conduct those optimizations (e.g. swiercz-2003-ven-low.csv) were not found and thus the optimizations would not run.

Conversely, the human model may be underpredicting blood concentrations. A comparison of Figure B-16 (U.S. EPA, 2013) to the output produced in this assessment indicates that the fit to the human data of Hissink et al 2007 matches for the elimination phase, but ~25% lower peak blood concentrations are predicted (Figure 3). Because fat

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content in these volunteers was measured, the study-specific fat percentage was used, resulting in a slight additional decrease in the peak. Although holding the Km constant and optimizing the Vmax did not result in a significant improvement to the fit to the data (U.S. EPA, 2013), since human data is available, it might be advisable to determine human-specific metabolic rates. Three different human exposure studies were identified and blood TMB concentrations are under-predicted post-exposure in all of them (Figures 3-5 and U.S. EPA, 2013 figures B14 and B15).

Apart from the consistent over-prediction of rat data and under-prediction of human data, this model simulates the data overall and parameterization and implementation seem correct, although a complete model review was not conducted.

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**Suggested Conventions to Facilitate PBPK Model Review**

The US EPA needs to implement a rigorous and consistent approach to having their PBPK models and approach is peer-reviewed. This peer-review should be implemented in a consistent and thorough manner and should be conducted by an external panel, either the CAAC or some other assembled peer-review panel. This peer-review should yield a report detailing the findings of the peer-review. The review can follow EPA's own method for reviewing PBPK models (McLanahan et al., 2012). As the CAAC reviews assessments that utilize PBPK models, the Agency can facilitate the panels ability to review and confirm the uses of the PBPK model. These include:

- The inclusion of an “about these files” script is excellent and highly recommended. This file is very important and should be checked carefully. The file should include information to:
  - Describe generated figures (publication and figure #1)
  - Dosing and parameters.
  - other pertinent information.
- Over-arching setup files should be included. Parameters set in individual .m files should be discouraged to assure a unified parameterization is in place.
  - Because files may not be run in order, each file must setup all parameters through the use of standardized setup files and must either contain the data needed to produce figures or must call a central data file.
- Files should be put organized in a logical progression. Suggested order might be:
  - Setup files for difference species/conditions
  - Rodent studies via a route
  - Rodent studies via alternate routes...
  - Human studies
  - Simulations
- All files should be annotated

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- Especially note changes or different from standardize approaches
  - Should indicate which, if any figures they reproduce from EPA reports and/or manuscripts.
  - Data source should be identified (Digitized from figure, supplied by author...)
- Files should show the model mass-balance

**References**

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* 13, 407–84.

Hissink, A.M., Krüse, J., Kulig, B.M., Verwei, M., Muijser, H., Salmon, F., Leenheers, L.H., Owen, D.E., Lammers, J.H.C.M., Freidig, A.P., McKee, R.H., 2007. Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents III. PBPK modeling of white spirit constituents as a tool for integrating animal and human test data. *Neurotoxicology* 28, 751–60.

U.S. EPA (2013). Toxicological Review of Trimethylbenzenes (CASRN 25551-13-7, 95-63-6, 526-73-8, and 108-67-8). In Support of Summary Information on the Integrated Risk Information System (IRIS). Supplemental Information. EPA/635/R-13/171b Revised External Review Draft [www.epa.gov/iris](http://www.epa.gov/iris)

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**TABLE 1. COMPARISON OF RAT MODEL INPUT PARAMETERS**

| Parameter   | (Hissink et al., 2007) | Transmitted to EPA | Transmitted to Summit | Comments                                |
|---|------------------------|--------------------|-----------------------|---|
| <b>Partitioning</b>                                 |                        |                    |                       |   |
| Saline:Air  | 3                      |                    |                       | QC by EPA, as reported in Hissink et al |
| Olive oil:Air                                       | 13200                  |                    |                       | QC by EPA, as reported in Hissink et al |
| Blood:Air - rat                                     | 148                    |                    |                       | QC by EPA, as reported in Hissink et al |
| Rapidly perfused:Blood                              | 2.53                   |                    |                       | QC by EPA, as reported in Hissink et al |
| Slowly perfused:Blood                               | 1.21                   |                    |                       | QC by EPA, as reported in Hissink et al |
| Fat:Blood   | 62.7                   |                    |                       | QC by EPA, as reported in Hissink et al |
| Brain:Blood   | 2.53                   |                    |                       | QC by EPA, as reported in Hissink et al |
| Liver:Blood   | 2.53                   |                    |                       | QC by EPA, as reported in Hissink et al |
| <b>Anatomical and Physiological</b>                 |                        |                    |                       |   |
| Alveolar ventilation rate (L/hr/kg <sup>0.7</sup> ) | 20                     |                    | 14*                   | (Brown et al., 1997)                    |
| Total cardiac output (L/hr/kg <sup>0.7</sup> )      | 20                     |                    | 14*                   | (Brown et al., 1997)                    |
| <b>Blood flow (% cardiac output)</b>                |                        |                    |                       |   |
| Liver (total)                                       | 25                     |                    | 17.6                  | (Brown et al., 1997)                    |
| Fat   | 9                      |                    |                       |   |
| Brain   | 1.2                    |                    | 2                     | (Brown et al., 1997)                    |

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| Parameter                            | (Hissink et al., 2007) | Transmitted to EPA | Transmitted to Summit | Comments  |
|--------------------------------------|------------------------|--------------------|-----------------------|---|
| Rapidly perfused (total)             | 49.8                   | 76 <sup>†</sup>    | 57.4 <sup>§</sup>     | (Brown et al., 1997)  |
| Slowly perfused (total)              | 15                     | NA                 | Calculated            |   |
| <b>Tissue volume (% body weight)</b> |                        |                    |                       |   |
| Liver                                | 4                      |                    |                       |   |
| Fat                                  | 7                      |                    |                       | (Brown et al., 1997)  |
| Brain                                | 0.72                   |                    | 0.57                  | (Brown et al., 1997)  |
| Rapidly perfused                     | 4.28                   | NA                 | 9 <sup>§</sup>        | (Brown et al., 1997)  |
| Slowly perfused                      | 75                     | NA                 | 82 <sup>§</sup>       | (Brown et al., 1997)  |
| <b>Metabolism</b>                    |                        |                    |                       |   |
| VmaxC (mg/hr/kg <sup>0.7</sup> )     | 3.5                    |                    | 4.17                  | Hissink et al visibly optimized: US EPA used ACSL.x to numerically optimize. Also used Swiercz et al. (2003) inhalation data to optimize. |
| Km (mg/L)                            | 0.25                   |                    | 0.322                 |   |

\* Within EPA version of model code, this is raised to the 0.74 power, not 0.7.  $QP = QPC \cdot BW^{0.74}$ ,  $QC = QCC \cdot BW^{0.74}$ . Since this is generally thought of as a “body surface area” correction, either is acceptable, the use of a different power is noted in footnote of table B-13. In addition, the EPA version of the model uses two different QPC values to correct for alveolar volume ( $QC/QC2=0.7$ ).

|| parameter is the same as reported in Hissink et al., 2007.

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§In the final EPA version of the model, values for total rapid flow and volume (QRTOTC,VRTOTC) and for total slow volume (VSTOTC), are used to calculate blood flow to rapidly perfused tissues (designated Rich within the .csi) and slow compartment volumes and flows. For example,  $QR = QRTOTC * QC - QL - QBR$ . Where QC is total cardiac output, QL and QBR are liver and brain flows, respectively. The EPA did this to correct mass-balance issues. Therefore, a direct comparison cannot be made to the values from Hissink et al.

¥ According to USEPA 2013, this should have been 9%

‡ The way in which total rapid compartment is presented in the updated version of the model, it is unclear what this value represents here. It may be a calculation performed by the EPA to approximate the initial value.

NA – Because the way in which total rapid and slow compartments are presented in the updated version of the model, these values would not be used in the model and were not provided to Summit for review.

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**TABLE 2. COMPARISON OF HUMAN MODEL INPUT PARAMETERS**

| Parameter   | (Hissink et al., 2007) | Transmitted<br>Summit   | to | Comments   |
|---|------------------------|-------------------------|----|--|
| <b>Partitioning</b>                                 |                        |                         |    |  |
| Saline:Air  | 3                      |                         |    | QC by EPA, as reported in Hissink et al  |
| Olive oil:Air                                       | 13200                  |                         |    | QC by EPA, as reported in Hissink et al  |
| Blood:Air - human                                   | 85                     |                         |    | QC by EPA, as reported in Hissink et al  |
| Rapidly perfused:Blood                              | 2.53                   |                         |    | QC by EPA, as reported in Hissink et al  |
| Slowly perfused:Blood                               | 2.11                   |                         |    | QC by EPA, as reported in Hissink et al  |
| Fat:Blood   | 62.7                   |                         |    | QC by EPA, as reported in Hissink et al  |
| Brain:Blood   | 2.53                   |                         |    | QC by EPA, as reported in Hissink et al  |
| Liver:Blood   | 2.53                   |                         |    | QC by EPA, as reported in Hissink et al  |
| <b>Anatomical and Physiological</b>                 |                        |                         |    |  |
| Alveolar ventilation rate (L/hr/kg <sup>0.7</sup> ) | 20                     | 15*                     |    | (Brown et al., 1997)   |
| Total cardiac output (L/hr/kg <sup>0.7</sup> )      | 20                     | 16*                     |    | (Brown et al., 1997)   |
| <b>Blood flow (% cardiac output)</b>                |                        |                         |    |  |
| Liver (total)                                       | 26                     | 17.5                    |    | (Brown et al., 1997)   |
| Fat   | 5                      | 8.5                     |    | (Brown et al., 1997)   |
| Brain   | 14                     | 11.4                    |    | (Brown et al., 1997)   |
| Rapidly perfused (total)                            | 30                     | 66.6                    |    |  |
| Slowly perfused (total)                             | 25                     | Calculated <sup>§</sup> |    |  |
| <b>Tissue volume (% body weight)</b>                |                        |                         |    |  |
| Liver   | 2.6                    |                         |    |  |
| Fat   | 14.6                   | 21.4                    |    | Hissink et al., 2007, were describing the specific population from their study – average body fat (measured using calipers) was 14.6%. |
| Brain   | 2                      |                         |    |  |

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| Parameter                        | (Hissink et al., 2007) | Transmitted<br>Summit | to | Comments                     |
|----------------------------------|------------------------|-----------------------|----|------------------------------|
| Rapidly perfused                 | 3                      | 7.6                   |    | (Brown et al., 1997)         |
| Slowly perfused                  | 66.4                   | 81 <sup>§</sup>       |    | (Brown et al., 1997)         |
| VmaxC (mg/hr/kg <sup>0.7</sup> ) | 3.5                    | 4.17                  |    | Scaled from rat Optimization |
| Km (mg/L)                        | 0.25                   | 0.322                 |    | Scaled from rat Optimization |

\* Within EPA version of model code, this is raised to the 0.74 power, not 0.7.  $QP = QPC * BW^{**0.74}$ ,  $QC = QCC * BW^{**0.74}$ . Since this is generally thought of as a “body surface area” correction, either is acceptable, the use of a different power is noted in footnote of table B-13. In addition, the EPA version of the model uses two different QPC values to correct for alveolar volume ( $QP/QP2=0.7$ ).

|| Parameter is the same as reported in Hissink et al., 2007

§In all versions of the model, values for total rapid flow and volume (QRTOTC,VRTOTC) and for total slow volume (VSTOTC), are used to calculate blood flow to rapidly perfused tissues (designated Rich within the .csl) and slow compartment volumes and flows. For example,  $QR = QRTOTC * QC - QL - QBR$ . Where QC is total cardiac output, QL and QBR are liver and brain flows, respectively. The EPA added a mass-balance equation ( $QSTOTC=1-QRTOTC$ ) to correct mass-balance issues. Therefore, a direct comparison cannot be made to the values from Hissink et al. for Flows to the slow compartment.

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1

2 **TABLE 3. STUDY-REPORTED CMAX COMPARED TO PREDICTED CMAX**

| Exposure Concentration (mg/l) | Data AVG*    | Model Prediction | Model Prediction/Data |
|-------------------------------|--------------|------------------|-----------------------|
| Hissink et al, 2007 (8 hr)    |              |                  |                       |
| 0.047                         | 0.16 ± 0.010 | 0.27             | 1.7                   |
| 0.19                          | 0.81 ± NA    | 1.2              | 1.5                   |
| 0.37                          | 4.0 ± 0.70   | 3.7              | 0.93                  |
| Swiercz et al. 2003,          |              |                  |                       |
| 0.12                          | 0.56         | 0.55             | 0.98                  |
| 0.49                          | 4.1          | 4.7              | 1.1                   |
| 1.23                          | 14           | 21.0             | 1.5                   |

3 Comparison of model-predicted Blood 1,2,4-TMB to study-specific data. For Hissink et al.  
4 2007, data is at the end of the 8 hr exposure, for Swiercz et al., 2003 data is first collected on  
5 the last day of repeated exposures. For .\* ± SD when available.

6 **TABLE 4. MODEL SIMULATED AND EXPERIMENTAL MEASURED CONCENTRATIONS OF 1,2,4-TMB IN**  
7 **MALE SPRAGUE-DAWLEY RATS EXPOSED TO 1,2,4-TMB AT THE END OF 12 HOUR EXPOSURE**  
8 **(ZAHLSSEN, 1996).: TABLE B-11 FROM U.S. EPA 2013**

| Exposure Concentration (mg/l) | Experiment (mg/L) | Model Prediction | Model Prediction/Data |
|-------------------------------|-------------------|------------------|-----------------------|
| 0.37                          | 1.7               | 4.2              | 2.5                   |

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|      |     |    |     |
|------|-----|----|-----|
| 0.74 | 6.9 | 18 | 2.6 |
| 1.5  | 14  | 48 | 3.5 |

1

2 **TABLE 4. MODEL SIMULATED AND EXPERIMENTAL MEASURED CONCENTRATIONS OF 1,2,4-TMB IN**  
 3 **MALE SPRAGUE-DAWLEY RATS EXPOSED TO 1,000 PPM (4,920 MG/M3) 1,2,4-TMB (12 HR/DAY,**  
 4 **FOR 14 DAYS) AT THE END OF EXPOSURE: TABLE B-12 FROM U.S. EPA 2013**

| Day | Experiment (mg/L) | Model Prediction | Model Prediction/Data |
|-----|-------------------|------------------|-----------------------|
| 1   | 63.5              | 181              | 2.8                   |
| 3   | 43.1              | 293              | 6.8                   |
| 7   | 33.4              | 372              | 11.1                  |
| 10  | 34.0              | 395              | 11.6                  |
| 14  | 35.2              | 399              | 11.3                  |

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8 **NON-CANCER ENDPOINT DOSE-RESPONSE MODELING FOR 1,2,4-TMB:KORSAK ET AL., 2000**

|      | 1<br>US EPA 2013<br>Average mg/l | 2<br>Model Average<br>mg/l | 3<br>Hissink Model<br>Average mg/l | 4<br>Hissink/Model<br>Average mg/l |
|------|----------------------------------|----------------------------|------------------------------------|------------------------------------|
| Low  | 0.1339                           | 0.13                       | 0.16                               | 1.2                                |
| Mid  | 0.8671                           | 0.87                       | 1.9                                | 2.2                                |
| High | 5.248                            | 5.4                        | 12.2                               | 2.3                                |

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1 Column 1 is the data taken from U.S. EPA, 2013 Table C-1 (Korsak et al., 2000a). Column 2  
2 are the weekly average blood concentrations produced using average exposures and body  
3 weights from that study in this assessment. Column 3 shows the same assessment using the  
4 rat parameters from Hissink et al, 2017 (Table 1). Column 4 shows the difference between the  
5 Hissink and U.S. EPA, 2013 parameterization.

6

7

8 *HUMAN INTERNAL METRIC COMPARISON AFTER CONTINUOUS INHALATION EXPOSURE: VENOUS TMB*  
9 *CONCENTRATION (SS)*

| Exposure Concentration (mg/m <sup>3</sup> ) | Model mg/l | Hissink Model mg/l | Hissink/Model mg/l |
|---|------------|--------------------|--------------------|
| 16  | 0.09       | 0.10               | 1.1                |
| 24.5  | 0.13       | 0.15               | 1.1                |
| 84  | 0.50       | 0.62               | 1.2                |
| 134   | 0.89       | 1.4                | 1.6                |

10

11