UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

September 29, 2015

EPA-SAB-15-013

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

Subject: 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 (IRIS). 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 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 that were nearly completed. For these assessments, the agency is focusing on streamlining the documents, increasing the transparency and clarity of the assessment, and presenting the data and information using 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 prior to the NRC recommendations and only implements the first phase of the agency's efforts to enhance the IRIS process. The SAB 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 improving IRIS toxicological assessments. Specific comments on developing the preamble and executive summary for future assessments, as well as the TMB assessment, are provided in the enclosed report. The SAB also found that the tables and presentation of data and information considered are an improvement. The SAB provides 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 findings and recommendations from the reviews and will provide the agency, through the Chartered SAB, advice and comments on the agency's progress to enhance IRIS assessments.

The SAB agrees with the agency that physiologically based pharmacokinetic (PBPK) modeling is an appropriate approach to developing reference concentrations and reference doses for trimethylbenzenes. When implementing a PBPK modeling approach the SAB strongly recommends that the EPA provide a transparent and detailed discussion of the rationale for selecting this approach. The discussion should include the available studies, data, and information considered by the agency, how these data were compared and considered, and why these analyses led the agency to use a PBPK approach rather than chemical-specific studies. 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. The SAB encourages the EPA to conduct independent peer review of PBPK model and modeling results used in assessments when the model is a new version, was previously unpublished, or is a modification of a published model. The SAB finds that the PBPK modeling approach and extrapolating inhalation data to an oral exposure is appropriate for the reference concentration and reference dose for 1,2,3-TMB, 1,2,4-TMB and 1,3,5-TMB. However, 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.

There are inhalation and oral toxicology studies for 1,3,5-TMB and the analyses of these studies should be expanded to develop candidate toxicity values for other endpoints (i.e., developmental and liver toxicity) in addition to the critical effect the EPA selected. The SAB notes that the endpoints for these studies are not the same neurotoxicological effects used in the PBPK and extrapolation from 1,2,4-TMB. The SAB recommends that the agency derive candidate toxicity values for 1,3,5-TMB using available toxicology studies for 1,3,5-TMB to provide a clearer explanation of its selection and the rationale for using PBPK modeling and extrapolating inhalation data to an oral exposure. The SAB recognizes there may be uses of candidate toxicity values in addition to selecting an overall toxicity value and strongly supports the agency developing candidate reference concentrations and reference doses for multiple endpoints. The Board finds that the agency needs to further clarify how candidate toxicity values should be developed and whether they have other potential uses.

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

There is a limited discussion of sensitive life stages and vulnerable populations for the TMB assessment due to lack of data on the toxicological responses in these populations. The SAB encourages the agency to expand the description and importance of these analyses in future assessments.

Regulatory agencies are frequently required to address risks associated with short-term exposures. The principal studies used to derive the proposed reference concentrations and reference doses for the TMBs are subchronic in duration and the analysis needed to generate subchronic reference concentrations and

reference doses has already been done. Given the usefulness of subchronic toxicity values and the small amount of additional work needed to add them to the TMB Assessment, the SAB recommends that the review be expanded to include the presentation of subchronic reference concentrations and reference doses.

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

Sincerely,

/Signed/

Peter S. Thorne
Chair
Science Advisory Board

/Signed/

Cynthia M. Harris
Chair
SAB Chemical Assessment Advisory Committee
Augmented for the Review of the Draft IRIS Trimethylbenzenes Assessment

Enclosure

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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TABLE OF CONTENTS

Acronyms and Abbreviations

ADME	absorption, distribution, metabolism, and excretion
BMD	benchmark dose
C-9	aromatic hydrocarbon fraction including ethyltoluenes and trimethylbenzenes
CNS	central nervous system
CYP450	cytochrome P450
EPA	U.S. Environmental Protection Agency
GD	gestational day
HEC	human equivalent concentration
IRIS	Integrated Risk Information System
K_m	Michaelis-Menten constant
LOAEL	lowest-observed-adverse-effect level
MOA	mode of action
NCEA	National Center for Environmental Assessment
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PC	partition coefficients
POD	point of departure
ppm	parts per million
RfC	reference concentration
RfD	reference dose
SAB	Science Advisory Board
SD	standard deviation
TMB	trimethylbenzene
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_D	database deficiency uncertainty factor
V_{max}	maximum rate of uptake/conversion

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), hereafter referred to as the TMB assessment, developed by the Integrated Risk Information System (IRIS) program. 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 TMBs at Superfund sites. Of the 38 sites on the EPA's National Priorities List that report TMB isomer contamination, 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,* 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 that were nearly developed. For these assessments, the agency focused on streamlining the documents, increasing the transparency and clarity of the assessments, and better presenting the data and information 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 implemented 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 improving IRIS toxicological assessments by addressing the NRC recommendations. Specific comments on developing the preamble and executive summary for future assessments, as well as the TMB Assessment, are provided in the enclosed report. 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, through the Chartered SAB, with advice and comments on the agency's progress to enhance IRIS assessments.

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.

The SAB conducted a review of the modified Hissink PBPK model and provides specific recommendations to improve the use of modeling for trimethylbenzenes. A review of the PBPK modeling used to develop the TMB assessment is provided in Appendix B of this report. The SAB report provides specific recommendations to improve the use of PBPK modeling for 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 modified PBPK models application in the risk assessment.

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.

There are inhalation and oral toxicology studies for 1,3,5-TMB and the analyses of these studies should be expanded to develop candidate reference toxicity values for other endpoints (i.e., developmental and liver toxicity). The SAB notes that the endpoints for these studies are not the same neurotoxicological effects used in the PBPK modeling and extrapolation from 1,2,4-TMB. The SAB recommends that the agency derive candidate reference concentrations and reference doses for 1,3,5-TMB using available toxicology studies for 1,3,5-TMB to provide a clearer explanation of the EPA's rationale for using the PBPK modeling approach and extrapolating inhalation data to an oral exposure and compare those results to the reference concentrations and reference doses developed for 1,3,5-TMB using the PBPK approach extrapolating from 1,2,4-TMB.

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 to fill gaps in the TMB database. The SAB finds that the available studies on closely related aromatic solvents and mixtures may provide qualitative and mechanistic insights into the toxicity of TMBs. Therefore these studies, including the C-9 fraction and white spirit studies, deserve further discussion to transparently describe the EPA's consideration 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.

Additional Recommendations

The SAB identified three additional topics not addressed directly in the Charge that warrant additional consideration by the agency: (1) clarification of how the EPA considers candidate toxicity values and their intended use, (2) an expanded discussion of sensitive life stages and vulnerable populations, and (3) deriving the subchronic RfC and RfD for the TMB isomers.

The SAB recognizes there are uses of candidate toxicity values in addition to selecting an overall toxicity value and strongly supports the agency developing candidate reference concentrations and reference doses for multiple endpoints. The overall toxicity value is one that is intended to be protective of toxicity of all types, and this is taken into consideration when selecting the uncertainty factor. Another use of candidate RfCs/RfDs is to better understand the effects of combined or cumulative chemical exposures and this context may change the considerations for an uncertainty factor for databases from those used to develop the overall toxicity value. The SAB is unaware of any discussion of this issue by EPA or clear description of how candidate RfC/RfD values are to be developed and used. As the IRIS process moves forward, it will be important to provide much greater clarity on this subject. The Board encourages the agency to further clarify how the candidate toxicity values should be developed and considerations for other potential uses.

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

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

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 the following sections:

- Preamble to describe the methods used to develop IRIS assessments;
- Executive Summary to concisely summarize the major conclusions of the assessment;
- Literature Search Strategy and Study Selection section to describe the process for identifying and evaluating the evidence for consideration in developing the assessment;
- Hazard Identification section to systematically synthesize and integrate the available evidence of organ/system-specific hazards; and
- 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 for technical reports published by the EPA and contains a qualitative characterization of the hazards for the TMBs, including a cancer descriptor of the isomers' human carcinogenic potential, and noncancer toxicity values, including a chronic oral RfD and a chronic inhalation RfC for all three TMB isomers. A quantitative cancer assessment for TMBs was not conducted due to inadequate data.

2.2. Charge to the Science Advisory Board

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

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

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

The SAB provides recommendations for three issues that were not addressed in the charge. The SAB noted that (1) additional potential uses of the candidate toxicity values developed to select the overall toxicity value were not well described; (2) the toxicological review provided a limited discussion of health effects from exposure to vulnerable life stages; and (3) the approach used to develop the RfC and RfD in this assessment built upon developing a subchronic RfC and RfD for each of the isomers and applying uncertainty factors to arrive at a chronic value for inhalation and ingestion yet the assessment does not provide a subchronic RfC or RfD. The SAB provides discussion and recommendations relating to these issues after the responses to the EPA charge. Charge questions are included in italics in relevant sections of this report and the full charge is included as Appendix A.

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

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

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

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

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

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

- p. xxii, line 67 that negative genetic toxicity studies carry less weight than positive ones;
- p. xxiii, line 78 that funding source can downgrade the credibility of studies;
- the assessment includes 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 assessment should include some discussion 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.
- 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 its 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 (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 lot of the details to well-structured appendices is helpful. The set of focused appendices helps a reader to find the place where particular study aspects or analyses of issues are to be found. The organization of the appendices — and the consistency of presentation across IRIS documents — are important in making the place to find details clear. Although the general structure of the appendix entries can be discerned, the plans for the structure and consistency have not been provided, so it will take some time and examination of other documents following the same plan for readers to find things easily. The use of appendices simultaneously allows presentation of more detail than may have been captured in earlier generations of IRIS documents and also avoids cluttering the main body of the IRIS document -- where interpretation and evaluation are considered. The appendix approach also frees the main document from seeming to need to present all the details before drawing any interpretation.

Consistent Presentation of the Studies Considered

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

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

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

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

Describing the Literature Search

The Literature Search Strategy section is brief and focuses only on identification of pertinent studies from the literature. The SAB is concerned that the general description of the process and the specific implementation for TMBs may be too exclusive, missing potentially informative ancillary studies that could help in interpretation or evaluation of those studies strictly observing toxicity outcomes of the TMBs alone in controlled settings. The SAB recommends that, in the literature search process, a clear distinction be drawn between, on the one hand, the primary search for sources of evidence that would be used directly in the identification and characterization of potential hazards for TMBs as well as in quantitative analysis of dose-response properties, and on the other hand, the secondary identification of studies that describe effects and properties of similar chemicals or that illuminate underlying biological processes that might be targets of toxic action. The primary literature search should be comprehensive and subjected to an orderly process of systematic review. The secondary search is for literature that is useful to provide context, in terms of what might be expected given the knowledge of other chemicals and of the potential pathways of toxic action. As such, the secondary search need not be comprehensive and could include reviews as well as original experimental studies; the aim should be to provide enough context so that the assessment of the first set of literature can be informed by what might be expected, given existing knowledge of similar chemicals, which can be further evaluated with respect to how these similar cases may help to fill data gaps which exist in the TMBs primary literature.

It should be clear that literature search is only the first step of systematic review, which needs to be followed by evaluation of each study in terms of design, quality, shortcomings, main findings (including both positive and negative findings), evaluation of the reliability of individual study results, and identification of other studies, particularly on mechanisms, that could address uncertainties in the primary database. This supports a further process of comparing results across studies to assess both the consistency of specific effects, and also the manifestation of related effects that would be expected from hypothesized underlying causative processes, both of which bear on the use of specific study results as evidence regarding the existence and nature of hazards in human target populations.

Describing the Hazard Identification Steps

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

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

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

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

The tabulation of studies is useful, and the dose levels and dose-specific responses are important details to include. The hyperlinks to the detailed description of studies in the appendices helps to make those appendices directly supportive and makes finding of relevant information more efficient. The exposure-response arrays are useful summary devices to aid communication, though they should not be read as meta-analysis forest plots or otherwise be used as the primary basis of conclusions. Nonetheless, they provide a valuable overview of the data.

Describing the Dose-Response Steps

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

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

Presenting the Outcomes

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

In summary the key recommendations are:

- Each study should be in a consistently formatted table and present the study-specific considerations of study quality and pertinence, including shortcomings and assumptions that are needed to interpret the study's outcomes. Consistency of format is important within each document, but it would also be a useful goal to achieve from one IRIS assessment to another.

- The literature search process should be described to draw a clear distinction between the primary search for sources of evidence and a secondary identification of studies used to describe effects similar chemicals or studies that illuminate the underlying biological processes that might be targets of toxic action used to fill data gaps. The primary literature search should be used directly

in the identification and characterization of potential hazards for TMBs as well as in quantitative analysis of dose-response properties.

- Add a brief summary of the main features of assessments before the section on Hazard Identification to provide context for the interpretation of studies and the more the detailed treatment should remain in an appendix.

- Both the Hazard Identification and the Dose-Response sections should have an initial paragraph setting out the main issues that will be considered and indicating which considerations (to be developed in the subsequent text) are the most notable for the larger assessment process. A parallel paragraph at the end of each of these chapters could summarize what its contents have provided to the larger assessment process.

3.1.3. Standardized Evaluation of Critical Studies

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

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

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

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

The SAB recognizes that an important challenge facing the agency is that assessments must go ahead even as this further development proceeds and before all aspects are complete. The Board notes that a strategy of working on the structure of the assessment, focusing on text to document the process and the agency's choices and analytical options, is a good way to begin.

The recommendations for revision of the IRIS process come from the NRC "Roadmap" (Chapter 7 of the Formaldehyde review) and other sources. The SAB recommends that a good principle to follow in conducting assessments during the process of revision is to consider the reasons behind the recommendations for change, and to make efforts to address the issues and to explain how the chosen approaches seek to reflect the NRC recommendations, although the methods may not yet be fully developed and agreed upon. That is, trying to address as well as one can the issues behind the recommended methodological and procedural changes is a good way to make assessments as reformed as they can be, and improve acceptance as the overall IRIS process continues to advance.

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

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

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

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

The TMB Review Panel was divided, however, on the adequacy of the responses and the advisability of the dispositions that were made as presented in the summary. In particular, there were a variety of views on the role that testing of the C-9 fraction should have in the assessment, with some panelists accepting the reasons for omission of this from the main evaluation and others feeling that these results had a role that had not been adequately explored. The use of the C-9 fraction in the TMB assessment is further discussed in section 3.2.3 of this report. The SAB concludes that there is value to considering the C-9 mixtures studies along with data for related alkylbenzenes (e.g., toluene, ethylbenzene, xylene, styrene) in helping to inform gaps in the TMBs database. There was also disagreement among the TMB Panelists related to the interpretation of the pain sensitivity data, with some members questioning whether the document adequately examined the question of reversibility following termination of exposure, which further bears on whether ongoing or repeated exposures to TMBs should be deemed to have accumulating toxicity beyond effects evident in shorter-term exposure; other panel members believed

that the data were consistent with cumulative toxicity and lack of reversibility. The full discussion of these issues and their treatment in the TMBs assessment is covered in the responses to the charge questions in Section 3.2 of this report.

3.2. Toxicological Review of Trimethylbenzenes

3.2.1. Executive Summary

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

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

Recommendations to improve the Executive Summary include:

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

3.2.2. Literature Search Strategy/Study Selection

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

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

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

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

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

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

In summary key recommendations are:

- Provide citations for the 4,300 papers and group them according to the rationales for their exclusion. This could be accomplished in several ways, ranging from an appendix at the end of the document, to a link between the document and the Medline search that was used, to an on-line searchable data base.

- Include a summary table of the studies related to each health effect: for example, a table with the 9 studies on neurotoxicity in humans, reporting study design, inclusion and exclusion criteria, number of subjects, and main results.

Additional references that should be considered by the EPA include:

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

3.2.3. Hazard Identification

Synthesis of Evidence for TMBs

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

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

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

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

The summary table (Page 1-49, Table 1-7) is very helpful in understanding the points made with regard to toxic effects. A summary table or scheme regarding toxicokinetics and metabolism would also be useful. Section 1.1.7, which focuses on the toxicokinetic similarities among TMB isomers, would be improved by summarizing in a table or scheme both the similarities and differences among the isomers in toxicokinetics and metabolism.

The synthesis has sections that summarize weight of evidence determinations for cancer and non-cancer endpoints as well as a summary of the uncertainties raised by potentially susceptible populations. The SAB recommends that this would also be a good place (in its own separate subsection) to describe the major uncertainties and gaps present in the TMBs toxicological database. A synthesis of the literature should naturally lead the reader to a summary of what is known and what is unknown for the chemical(s) under review. This then leads to a discussion of data gaps, which in the current draft does not appear until Page 2-11. The current discussion is brief and does not weigh the value of evidence from related chemicals or from studies done on the C-9 mixture. Structurally related alkylbenzenes such as toluene, xylene, ethylbenzene and styrene have similarities in neurotoxic effect and metabolic disposition. Such information is clearly supported in the IRIS Preamble, section 3.1 (lines 11-15) "[s]earches for information on mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms." This is further supported in Section 5.4, p. xxiii (lines 18-21), "Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms." It is therefore recommended that additional animal and human studies on related aromatic solvents be considered in the qualitative and mechanistic interpretations of TMB toxicity. Examples of such studies are included in comments on the literature review. (See Section 3.2.2)

Information on mixtures containing TMBs and on compounds that are structurally related to TMBs can be pertinent in several ways. First, the degree to which the effects seen in TMB-only studies are consistent across related chemicals may help to evaluate the evidence for the existence and nature of hazards, while also potentially informing MOA. Clearly, perfect consistency is not needed nor expected; however, major discrepancies in comparably conducted studies should be noted as part of the determination of the robustness of findings from the TMB-only studies. Where consistency of effect is seen across related structures, studies with these related chemicals that go beyond the testing done on TMBs may help fill data gaps, identify additional endpoints of potential concern, or better characterize uncertainties that arise in the interpretation of TMB-only studies.

The main data gaps for the TMBs appear to be the lack of a developmental neurotoxicity study, the lack of a multi-generational reproduction study and the lack of a chronic noncancer (neurotoxicity) study. The EPA could potentially utilize data from these analogous alkylbenzenes to inform these data gaps and determine whether the database uncertainty factor (now 3 fold) and the subchronic to chronic uncertainty factor (now 10 fold) should be modified on this basis. Further, discussion of the existing C-9 mixtures studies should be brought into the main document at this juncture by describing their strengths and weaknesses and relevance to the setting of RfDs/RfCs for individual TMB isomers, with particular emphasis on whether they provide evidence to inform the aforementioned data gaps. For example, regarding the developmental neurotoxicity data gap, 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/m3, 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 limited utility for filling the developmental neurotoxicity data gap. Thus, chemical relevance and study quality need to be considered when bringing in other chemicals or mixtures to help fill data gaps.

If data for individual alkylbenzenes (toluene, ethylbenzene, xylene, styrene) or from the C-9 mixtures studies are used by the EPA to modify the assignment of uncertainty factors, a comparative discussion would be needed to describe similarities and differences between the surrogate chemical (or mixture) and the TMBs and whether the extrapolation across chemicals reduces overall uncertainty or merely reduces uncertainty in one area (e.g., subchronic to chronic extrapolation) only to add back a different type of uncertainty (extrapolation across chemicals). The EPA's use of surrogate chemicals (toluene, Page 1-19) to draw inferences regarding TMB mode of action is appropriate and as suggested below, early life toxicokinetic data with toluene may be useful to decrease the uncertainty associated with early life exposures. There may be additional value to review these surrogate chemicals and mixtures, not only from the perspective of filling TMB data gaps, but to identify other effects of potential concern that have not received adequate attention in TMB studies. This can include consideration of the human clinical toxicology and epidemiology studies that may exist for these related chemicals.

The testing of the C-9 fraction has interpretative issues but is relevant to the TMB isomers under review because this mixture, as tested, was about half TMBs; therefore, much of the observed effects could have been due to the TMBs. The various C-9 components of the mixture may have created competition for metabolic clearance that could have increased duration of exposure to the TMBs. However, certain components may have induced metabolic clearance enzymes or competed for distributional pathways (e.g., uptake into the CNS) which might have decreased the response. Thus, the minimal observed toxicity in several C-9 studies (e.g., Douglas et al. 1993) provides relevant information for the evaluation of individual TMB isomers and the agency should carefully explain its reasoning of the role of C-9 studies in its final evaluation of TMBs. Relevance increases to the extent that application of the IRIS assessment for TMBs may be used in certain settings for the evaluation of exposure to mixtures containing TMBs and related alkylbenzenes. Thus as stated above the C-9 mixtures studies have relevance and need further discussion. Within this context, data from additional mixture studies may provide further perspectives on this question, as reviewed, for example, by Richie et al. (2001).

Noncancer Health Effects

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

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

- A short summary of the toxicokinetic similarities and differences among the three isomers early in the section to provide context to the subsequent effect summaries.
- A short summary of the neurological effects database limitations and accompanying uncertainties such as lack of subchronic data for some isomers, lack of chronic data for all isomers, questions of reversibility and lack of mechanistic data. The SAB notes that summaries for the respiratory, hematological and development effects already make these distinctions.
- Statement(s) regarding the confidence in the hazard identification results given the limitations of the available database. This statement(s) should address the question: based on the sensitivity of endpoints assessed in the limited database, lack of mechanistic information and effects observed with similar compounds but not assessed for TMBs, what is the confidence that the hazards (i.e., sensitive health endpoints) have been adequately identified?
- Inclusion of a concluding paragraph(s) which states how the results of the hazard identification (e.g., the effects on the nervous system, respiratory system, the hematological system, and developing fetus) will be utilized in the subsequent dose-response evaluation as well as describing the relative importance of the different health effects.

Carcinogenicity

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

As noted in the detailed response to the charge question on carcinogenicity (See section 3.2.11), 1,2,4-TMB has been assessed in only one study. The EPA found that there were a number of deficiencies concerning this bioassay and the SAB agrees with the agency's finding. The EPA also noted that no carcinogenicity bioassays have been conducted with 1,2,3-TMB or 1,3,5-TMB. As such, the SAB concludes that the EPA's hazard assessment of the carcinogenicity of the TMBs did integrate all available scientific evidence and agrees with the EPA that there is "inadequate information to assess the carcinogenic potential" of trimethylbenzenes. The SAB provided recommendations to use data for analogous compounds qualitatively (see section 3.2.3 Hazard Identification) to fill data gaps and uncertainty factors. The SAB found that the available carcinogenic data for the TMB isomers is inadequate to assess quantitatively as discussed later in this report.

3.2.4. Toxicokinetics and Pharmacokinetic Modeling

Charge Question: 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 support 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.

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 [of the TMB Assessment]). 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?

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

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

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

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

Alternatively, the EPA could conduct BMD modeling of the Korsak and Rydzynski (1996) data using air TMB concentration as the dose metric to derive the POD. Subsequently, the PBPK model would be used to convert the POD to the weekly average blood concentration. This alternative approach yields a

BMD of 84 mg/m^3 (17ppm), which would be predicted by the PBPK model to yield a blood concentration of 0.087mg/L in rats. The result is identical to the values derived by the EPA, suggesting that the approach of dropping the high-dose group used by the EPA does not introduce any bias. EPA can use this alternative approach to support their BMD modeling approach.

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

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

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

One TMB panel member noted that there is a published human PBPK model (Järnberg and Johanson 1999). The EPA requested the model through email and was unable to obtain the model. The model is for TMB alone, and avoids the complications and uncertainties of: (1) concurrent exposure to other components in white spirit; and (2) species-to-species extrapolations. Empirical human kinetic data are available from the same laboratory for model parameterization and validation. Human neurobehavioral data are also available in the literature from other research groups. The results of these studies identify human NOAELs/LOAELs for acute irritation and central nervous system (CNS) effects by TMB and white spirit. The SAB notes that EPA policy is to use and consider human data and validated human models when available. Because the EPA could not obtain Järnberg and Johanson model, the SAB has provided recommendations to improve the use of the Hissink model and encourages the EPA to, at a minimum, be more transparent in its discussion of available models and model selection in this and future assessments.

The SAB recommends the EPA conduct BMD modeling of the Korsak and Rydzynski (1999) data using air TMB concentration as the dose metric to derive the POD and subsequently use the PBPK model to

convert the POD to the weekly average blood TMB concentration. This can be done to either replace the EPA's current approach or offered as support of the EPA's approach (i.e., to demonstrate the same answer results from either approach).

Charge Question: 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?

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

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

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

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

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

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

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

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

Charge Question: A 90-day inhalation toxicity study of 1,2,4-TMB in male rats (Korsak and Rydzynski, 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.

The SAB generally agrees with the choice of the Korsak and Rydzynski (1996) study as the basis for derivation of the RfC for 1,2,4-TMB. The study utilized a 90-day exposure period and, thus, the longest duration exposure study available in the literature; in addition, it included multiple exposure levels. It was well-conducted and utilized adequate sample sizes of rats. In addition, it was based on widely-used behavioral assays. An examination of the study indicates these behavioral studies were carefully carried out and data from control animals were consistent with previously published observations.

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

- The rationale for the choice of Korsak and Rydzynski (1996) is not specifically described and the reasons for its choice over other studies, e.g., the 4-week exposure studies, need to be more clearly stated.
- As currently written, there is confusion over chronicity of exposure vs. effects. It would be helpful to modify the terminology particularly related to outcome measures, perhaps as acute effects vs. long-term effects/irreversible effects and retain the use of the word chronic/subchronic etc. to descriptions of statements related specifically to exposure.
- Separate the write-up into sections that specifically elaborate on the acute effects and provide a separate section related to effects observed post-exposure. Given the commonality of even the trends in data across these studies, some mention of the biological significance in the absence of statistical significance ($\alpha = 0.05$ as an arbitrarily chosen value) should be mentioned.
- The text, where applicable, could include additional qualifications as to "reversibility of effects" at the 2-week post-exposure time point. This assessment of reversible effects of failures on the rotarod is based on the finding of lack of statistical difference between treated and control groups at one week post-exposure following a 13-week exposure period for one of two isomers. Some TMB Panel members felt that this was sufficient evidence for reversibility. For other members, however, this did not provide sufficient evidence. Specifically, this interpretation of a reversal relied on a reduction from 40 percent rotarod failure during the final week of exposure compared to 35 percent one week post-exposure, as compared to 0 percent rates for controls. There was no

such statistical reversal for the other isomer, and for both isomers, the magnitude of the reduction post exposure was minimal. Further, it was not clear that the statistical analyses of these data incorporated a repeated measures component that would be required by the experimental design. Thus, while a case was stated for a statistically significant reversal, several TMB Panel members felt that it was not consistent across nor did it appear to be biologically meaningful.

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

Charge Question: 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.

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

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

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

Charge Question: 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?

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

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

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

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

- UFA – an interspecies uncertainty factor;
- UFH – an intraspecies uncertainty factor;
- UFL – a LOAEL (lowest observed adverse effect level) to NOAEL (no observed adverse effect level) uncertainty factor;
- UFS – a subchronic to chronic uncertainty factor; and
- UFD – a database uncertainty factor.

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

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

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

UF_L. The SAB agrees with the EPA's choices for UF_L values, i.e., a UF_L of 1 for all endpoints except increased bronchoalveolar lung cells, for which a UF_L of 10 was selected. However, the SAB suggests that the justification for the UF_L be strengthened. This UF is intended to be used when the POD is a LOAEL rather than a NOAEL. In conducting BMD modeling, a BMD equal to one standard deviation change in the control mean for modeled endpoints was selected. The document would be improved by adding an explanation of the reasoning for selection of one standard deviation (versus one-half standard deviation) along with a clearer discussion of why this is expected to lead to a POD for which a UF_L of 1 is appropriate.

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

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

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

UF_D. The TMB Panel was divided on whether the UF_D should be 3, as selected by the agency, or 10. The purpose of this UF is to account for overall deficiencies in the database of studies available to assess potential toxicity. The EPA cited strengths in the database in terms of availability of information on

multiple organ systems from three well-designed subchronic toxicity studies in justifying not using the full default factor of 10. In retaining a half-log factor of 3, the EPA noted the absence of a multi-generation reproductive/developmental toxicity as a weakness in the database, and specifically concern for the absence of a developmental neurotoxicity study for 1,2,4-TMB given the importance of neurotoxicity in establishing the RfC. Among those who agreed with a UF_D of 3, some found the justification provided by the EPA to be satisfactory, while others thought that toxicity data available for C-9 mixtures should contribute to the rationale to lower the value from the default of 10. Others disagreed with including C-9 mixture data as relevant to the database UF. (See Section 3.2.3). Panel members who thought that the UF_D should be 10 cited various reasons, including the absence of data in other species and the absence of a multi-generational reproductive study, as well as the opinion that the absence of a developmental neurotoxicity study alone warranted a full factor 10. One TMB Panel member pointed out that analogy with toluene suggests that the perinatal exposure could lead to neurodevelopmental effects at doses 10-fold lower than the NOAEL for effects in adults. An additional point made by another Panel member was that because the RfCs for all of the isomers are being set at the same value, whereas the database is severely limited for the 1,2,3- and 1,3,5-TMB isomers and the latter two compounds deserve a UF_D of 10. Therefore, for consistency, a factor of 10 should be used for all the isomers.

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

Charge Question: A 90-day inhalation toxicity study of 1,2,3-TMB in male rats (Korsak and Rydzynski, 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.

The SAB agrees with the EPA's conclusion not to base the RfC derivation for 1,2,3-TMB on isomer-specific data. The justification for this conclusion is supported and clearly described. The SAB is not aware of chronic or subchronic studies that could be used to support an RfC derivation for 1,2,3-TMB with neurotoxicity as the critical endpoint, similar to the Korsak and Rydynski (1996) study used to develop the 1,2,4-TMB RfC. As with 1,2,4-TMB, the SAB finds that the clarification of this choice, however, could be greatly improved by expanding the assessment on the same points discussed for 1,2,4-TMB (see section 3.2.5)

Charge Question: 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.

The SAB agrees that reduction in pain sensitivity as indicated by an increased latency to pawlick response in a hotplate test was a valid adverse nervous system effect and appropriately selected as a critical effect for RfC derivation of 1,2,3-TMB. The SAB notes that the agency appropriately uses the same rationale to derive the RfC for 1,2,4-TMB and uses this information. A detailed response is in Section 3.2.5 and the SAB refers the reader to that section rather than reiterate the response to the Charge question.

Charge Question: In order to characterize the observed dose-response relationship comprehensively, benchmark dose (BMD) modeling was used in conjunction with default dosimetric adjustments (U.S. EPA, 1994b) for calculating the human equivalent concentration (HEC) 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 a 1 standard deviation change in the control mean for the latency to pawlick response) been supported and clearly described?

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

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

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

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 agrees with the EPA conclusion not to base the RfC derivation for 1,3,5-TMB on isomer-specific data. The justification for this conclusion is supported and clearly described. The SAB is not aware of chronic or subchronic studies that could be used to support an RfC derivation for 1,3,5-TMB with neurotoxicity as the critical endpoint, similar to the Korsak and Rydynski (1996) study used to develop the 1,2,4-TMB RfC. The candidate RfCs for 1,3,5-TMB, based on maternal and fetal toxicity from the study of Saillenfait et al. (2005) are presented by EPA, but were not chosen as the overall RfC. Although the SAB takes issue with the PODs selected by EPA in their analysis of the Saillenfait et al. study, as discussed below, it nevertheless agrees with the decision not to use this study to derive the overall RfC for 1,3,5-TMB. The SAB concurs with EPA that the best approach under the circumstances is to adopt the RfC for 1,2,4-TMB, based on decreased pain sensitivity, as the overall RfC for 1,3,5-TMB.

The SAB provides the following recommendations to develop a candidate RfC based on Saillenfait et al. (2005). This 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 the calculations for the fetal and maternal endpoint-based candidate RfCs.

Saillenfait et al. (2005) selected 100 ppm (492 mg/m3) for the maternal NOAEL for 1,3,5-TMB with 300 ppm (1476 mg/m^3) 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^3) and the developmental LOAEL was 600 ppm (2952 mg/m^3) 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^3) and the maternal LOAEL at 600 ppm (2952 mg/m^3) based on decreased corrected body weight gain and higher

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

The SAB recommends that EPA use decreased maternal body weight gain data from GDs 6-13 and 6-21 as the basis of the maternal endpoint POD and candidate RfC rather than corrected maternal weight gain data. If BMD modeling is unsuccessful, the SAB recommends that EPA use the maternal NOAEL of 492 mg/m^3 as the POD.

Section 2.3.2 of the TMB Assessment [Methods of Analysis for 1,3,5-TMB)] incorrectly identifies 2,974 mg/m^3 as the NOAEL) for the developmental endpoint (decreased male fetal body weight). The SAB recommends using the NOAEL of 1476 mg/m^3 as the POD for derivation of a developmental endpoint RfC. The SAB also suggests that EPA consider increasing the UF_D from 3 to 10, to address the lack of neurodevelopmental testing, in the derivation of the developmental candidate RfC. The SAB notes that this approach may not fully address neurological effects which serve as the basis for the other isomers. However, the revised developmental endpoint RfC calculation will be based on a more appropriate POD and improve the justification for using the extrapolation from the lower neurological-based RfC from 1,2,4-TMB.

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

- The female fetal body weight average for the 100 ppm (492 mg/m3) group should be 5.47 ± 0.21 and not 5.74 ± 0.21 (it is correct in other tables of the document).
- The level of significance for decreased maternal body weight gain for the 600 ppm (2,952 mg/m3) group should have two (**) and not one (*) asterisk to indicate $p < 0.01$.
- The table also states with a footnote (b) that numbers of live fetuses was not explicitly reported. However, Saillenfait at al. (2005) did report them in Table 3 of their manuscript. The total numbers of fetuses were 297, 314, 282, 217, and 236, for the control and exposure groups, respectively, and should be included in Tables 2-2 and 2-12 of the draft TMB Review document.

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

The SAB acknowledges that the agency's approach to developing the overall RfC (based on neurological effects) for 1,3,5-TMB based on a structurally and toxicologically related isomer is scientifically appropriate. However, the SAB recommends that the agency strengthen the justification for using this approach for 1,3,5-TMB by: 1) following the recommendations provided above regarding recalculating the maternal and developmental-based candidate RfCs from Sallenfait et al. (2005); and 2) discussing the differences as well as similarities in physical and toxicological parameters (i.e., Henry's Law constant and toxicokinetics) for 1,3,5-TMB as compared with the other isomers.

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

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

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

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

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

The SAB notes there were limitations in the Koch Industries study (primarily that it didn't involve neurotoxicity endpoints) and the study does involve an extrapolation across congeners. Presented with those limitations, the Koch Industries study does not provide a superior alternative to the PBPK approach for dose route extrapolation that the EPA implemented. As discussed in Section 3.2.10, the Koch Industries study may provide a means to derive RfDs for several additional endpoints (e.g., liver, kidney) for 1,3,5-TMB. The EPA can consider such additional RfDs and whether they are potentially useful for 1,2,4-TMB based upon extrapolation across congeners.

Charge Question: A route-to-route extrapolation from inhalation to oral exposure using the modified Hissink et al. (2007) PBPK model has been used to derive an oral RfD for 1,2,4-TMB. Please comment

on whether the PBPK modeling been 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? Please comment on whether this approach is scientifically supported and clearly described in the document.

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

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

The SAB agrees with the uncertainty factors selected in the development of the oral RfD for 1,2,4-TMB, but recommends that the discussion of uncertainty be strengthened with respect to bioavailability assumptions. As discussed in the previous response, the oral RfD for 1,2,4-TMB was derived by incorporating an oral intake component into the PBPK model for 1,2,4-TMB to obtain a human equivalent oral dose POD. The EPA used the same uncertainty factors for the oral RfD as were used in the development of the inhalation RfC. Given that the oral RfD was based upon the same endpoint and derived from the same study as the RfC, the SAB agrees that it is logical to use the same uncertainty factors. Thus, the comments and recommendations regarding uncertainty factors are applicable to this charge question as well (see Section 3.2.5). There was discussion regarding whether there is additional uncertainty associated with incorporation of the oral intake component in the PBPK model, and specifically regarding assumptions made with that component regarding oral absorption of 1,2,4-TMB and first-pass metabolism. Unlike modeling of internal concentrations from inhalation exposure that can be verified with existing experimental data, there are no data with which to assess model predictions of internal doses following oral 1,2,4-TMB exposures. The SAB does not consider this additional uncertainty sufficient to increase the composite UF for the oral RfD, largely because the nature of the uncertainty (possible lower absorption by the oral route), would add extra health protection. The SAB recommends that the potential uncertainties associated with oral bioavailability of 1,2,4-TMB be discussed more clearly in the document.

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

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

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

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

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

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

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

Please comment on whether EPA's conclusion that the oral database for 1,3,5-TMB is inadequate for derivation of an RfD is scientifically supported and clearly described.

Please comment on whether oral data are available to support the derivation of an RfD for 1,3,5-TMB. If so, please identify these data.

The SAB agrees with the EPA's approach to extrapolating the RfD of 1,2,4-TMB to 1,3,5-TMB. The SAB is aware of an isomer specific study (Koch Industries 1995) and the recently released data on 1,3,5-TMB (Adenuga et al. 2014) provided by public commenters.

The Koch Industries study (1995) was the only isomer-specific and route-specific study available in the peer-reviewed literature for oral exposure to 1,3,5-TMB when the TMB Assessment was drafted in 2013. The EPA chose not to use this study for derivation of an RfD because it did not assess the potential for neurological effects and "presented limited toxicological information." (see Appendix F of the draft TMB Toxicological Review). Although the rationale for this decision is clearly described, the SAB disagrees and considers the Koch Industries study suitable for development of one or more candidate RfDs for 1,3,5-TMB. The Koch Industries study of 1,3,5-TMB toxicity after subchronic (90-day) gavage treatment was consistent with good laboratory practices and requirements and, when submitted for an EPA Office of Water test rule, was peer reviewed by three senior scientists (Versar 2013). Although the study does not include neurological endpoints, it does provide information on toxicity to other organ systems such as liver and kidney. In the opinion of the SAB, this study is suitable for providing candidate RfDs for one or more endpoints in the same way that, for example, candidate RfC values based upon a variety of endpoints were developed and presented for 1,2,4-TMB (see Table 2-4 of the draft TMB Toxicological Review). In view of the principle of toxicological equivalence among TMB isomers for the purposes of development of toxicity values proposed by the EPA and

accepted by the SAB (see response to charge questions in Section 3.2.7), these candidate RfDs could also be considered for 1,2,3- and 1,2,4-TMB as well.

Given the importance of neurotoxicity as a critical endpoint for inhalation exposure to TMB isomers, there should be confidence that any value selected as the RfD for 1,3-5-TMB is adequately protective of this type of effect. In order to produce an RfD protective of neurotoxicity using PODs from the Koch Industries study, a large UF_D (e.g., 10) could be used to account for the absence of isomer- and route-specific neurotoxicity data. However, in the opinion of the SAB, there is stronger scientific support for use of PBPK-extrapolated RfD for 1,2,4-TMB based on a neurotoxic endpoint as the overall RfD for 1,3,5-TMB. Thus, while the SAB recommends use of the Koch Industries data and Adenuga et al. (2014) to develop candidate RfDs for comparison purposes, it agrees with the overall RfD for 1,3,5-TMB as proposed by EPA.

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

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

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

The carcinogenicity of 1,2,4-TMB has been assessed in a single study (Maltoni et al. 1997), in which a single dose level was administered to rats for two years. The SAB determined that this study had a number of shortcomings. For example, it is unclear how the dose was selected, and only one dose was used so nothing can be said about dose-response. The dosing schedule was quite unusual and the authors stated that a more frequent schedule (i.e., 5 or 6 days per week) would have resulted in unacceptable toxicity. Survival was affected by treatment, but quantitative data and statistical analyses were not presented. Body weights were collected, but the data were not reported. The only remarkable finding from the study was neuroesthesioepitheliomas, a tumor arising from the olfactory neuroepithelium, which occurred in treated but not control animals. No statistical analyses were presented in the paper but a Fishers Exact test conducted by the EPA indicated that the result was not significant. Nonetheless, these tumors are very rare in rats and it is noteworthy that in the same study ethylbenzene also induced neuroesthesioepitheliomas. Carcinogenicity bioassays do not appear to have been conducted with 1,2,3-TMB or 1,3,5-TMB.

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

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

- Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of potential urinary cancer biomarkers--a powerful strategy for breast cancer diagnosis. (Silva et al. 2012)

- Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry. (Silva et al. 2011)
- Cellular responses after exposure of lung cell cultures to secondary organic aerosol particles. (Gaschen et al. 2010)

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

3.3. Additional Recommendations

The SAB identified three additional topics not addressed directly in the Charge that warrant additional consideration by the agency: (1) A clarification of how the EPA considers candidate toxicity values and their intended use, (2) an expanded discussion of sensitive life stages and vulnerable populations, and (3) deriving subchronic RfC and RfD for the TMB isomers.

3.3.1. Candidate Toxicity Values

Section 7.6 of the preamble describes how IRIS assessments derive candidate values for each suitable data set and effect that is credibly associated with an agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures using guidance on methods to derive RfCs and RfDs. The assessment process develops an organ- or system-specific reference value for each organ or system affected by the agent and selects an overall reference dose and an overall reference concentration for the agent to represent lifetime human exposure levels where effects are not anticipated to occur. Providing these organ/system-specific candidate reference values, IRIS assessments may facilitate subsequent risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms.

In considering the merits and use of candidate toxicity values, the SAB encountered an issue where further clarification by EPA is strongly encouraged. Interest by the EPA in developing PODs and RfCs/RfDs for multiple endpoints in new IRIS profiles is noted. As shown in this toxicological review, one of the uses of RfCs/RfDs for various endpoints is as candidates for selection as the overall toxicity value. The overall toxicity value is one that is intended to be protective of toxicity of all types, and this is taken into consideration when selecting the UF_D. Another use of candidate RfCs/RfDs is to better understand the effects of combined chemical exposures. Risks from combined or cumulative exposures to chemicals is generally of greatest concern when the chemicals affect the same targets organs. While an overall RfC or RfD is based upon one effect chosen as the critical effect, that chemical may produce other types of toxicity at doses that are only marginally higher than the selected overall toxicity value. To illustrate the problem, consider the situation in which individuals are exposed to three chemicals, each with an RfC based upon a different endpoint, but all have the potential to affect the liver. For the risk assessor, the combined effect of the three chemicals on the liver may be greater concern than the effects of the individual chemicals on other organ systems. In order to evaluate the risk of liver injury from combined exposure, the risk assessor needs a liver RfC for each compound. Conceivably this information could come from candidate RfCs for the chemicals, if available for the liver, but there is a difference in the way that an RfC for this use would be developed versus an RfC suitable for selection as the overall RfC. The difference is in the way that the UF_D is selected – on one hand to insure that the RfC is protective against all forms of toxicity and on the other that it is reliably protective of toxicity to a specific target organ. Conceivably, the UF_D values selected for those two purposes, and the resulting

RfC/RfD values, could be quite different. The SAB is unaware of any discussion of this issue by EPA or clear description of how candidate RfC/RfD values are to be developed and used. As the IRIS process moves forward, it will be important to provide much greater clarity on this subject.

3.3.2. Susceptible Populations and Lifestages

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

Regarding mode of action, it is important to know:

- whether it is the parent compound or metabolites (or both) that contribute to toxic effect;
- which metabolic systems are responsible for removing the parent compound and creating important metabolites; and
- 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 useful for describing the degree of toxicokinetic uncertainty presented by early life stage exposure to TMBs.

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.

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

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

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

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

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APPENDIX A: CHARGE TO THE SAB

NCEA Charge to the Science Advisory Board for the
IRIS Toxicological Review of Trimethylbenzenes
August 2013 (Updated May 2014)[2]

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 hazards for TMBs, including a cancer descriptor of a chemical's human carcinogenic potential, and noncancer toxicity values, including a chronic oral reference dose (RfD) and

[2] 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.

a chronic inhalation reference concentration (RfC) for all three trimethylbenzene isomers. A quantitative cancer assessment for trimethylbenzenes was not conducted due to inadequate data.

Charge Questions

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

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

General Charge Questions:

1. 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.
2. 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.
3. NRC (2011) state that "all critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated" and that "strengthened, more integrative, and more transparent discussions of weight of evidence are needed." NRC also indicated that the changes suggested would involve a multiyear process. Please comment on EPA's success thus far in implementing these recommendations.
4. EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes and has

revised the assessment to respond to the scientific issues raised in the comments. A summary of the public comments and EPA's responses are provided in Appendix F of the Supplemental Information to the Toxicological Review of Trimethylbenzenes. Are there scientific issues that were raised by the public as described in Appendix F that may not have been adequately addressed by EPA?

Chemical-Specific Charge Questions

A. Executive Summary

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

B. Literature Search Strategy/Study Selection

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

C. Hazard Identification

Synthesis of Evidence

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

Summary and Evaluation

1. Does EPA's hazard assessment of noncancer human health effects of trimethylbenzenes clearly integrate the available scientific evidence (i.e., human, experimental animal, and mechanistic evidence) to support the conclusions that trimethylbenzenes pose potential hazards to the nervous system, respiratory system, the developing fetus, and the circulatory system (i.e., blood)?
2. Does EPA's hazard assessment of the carcinogenicity of trimethylbenzenes clearly integrate the available scientific evidence to support the conclusions that under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is "inadequate information to assess the carcinogenic potential" of trimethylbenzenes?

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

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

1. A 90-day inhalation toxicity study of 1,2,3-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 default dosimetric adjustments (U.S. EPA, 1994b) for calculating the human equivalent concentration (HEC) 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 a 1 standard deviation change in the control mean for the latency to pawlick response) been supported and clearly described?
4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC for 1,2,3-TMB. Are the UFs appropriate based on the recommendations described in Section 4.4.5 of *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

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

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.

1. 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 it been clearly described?
2. Please comment on whether EPA's approach to developing the RfC for 1,3,5-TMB is scientifically supported for the available data and clearly described.

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

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

1. Please comment on whether EPA's conclusion that the oral database for 1,2,4-TMB is inadequate for derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral data are available to support the derivation of an RfD for 1,2,4-TMB. If so, please identify these data.
2. A route-to-route extrapolation from inhalation to oral exposure using the modified Hissink et al. (2007) PBPK model has been used to derive an oral RfD for 1,2,4-TMB. Please comment on whether the PBPK modeling been 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? Please comment on whether this approach is scientifically supported and clearly described in the document.
3. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD for 1,2,4-TMB. Are the UFs appropriate based on the recommendations described in Section 4.4.5 of *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

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

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

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

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

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

1. Please comment on whether EPA's conclusion that the oral database for 1,3,5-TMB is inadequate for derivation of an RfD is scientifically supported and clearly described. Please comment on whether

oral data are available to support the derivation of an RfD for 1,3,5-TMB. If so, please identify these data.

2. Please comment on whether EPA's approach to developing the RfD for 1,3,5-TMB is scientifically supported and clearly described.

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

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

APPENDIX B: RESULTS OF REVIEW OF TRIMETHYLBENZENE 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 (*.csl* 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 (*VMAX = KVMAX*(ABS(T-TLEG)+(T-TLEG))/2+VMAX0*). KVMAX 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 (QSTOTC = 1 – QRTOTC). 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 (QPC) that represents alveolar and QP2C 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 (Vmax and Km) to fit the rodent data. The Agency chose the 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.

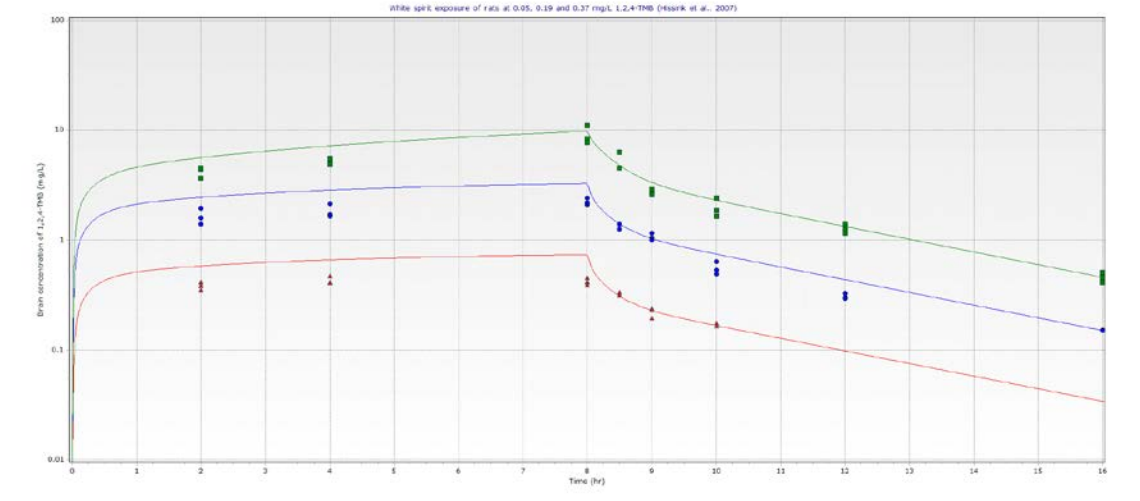
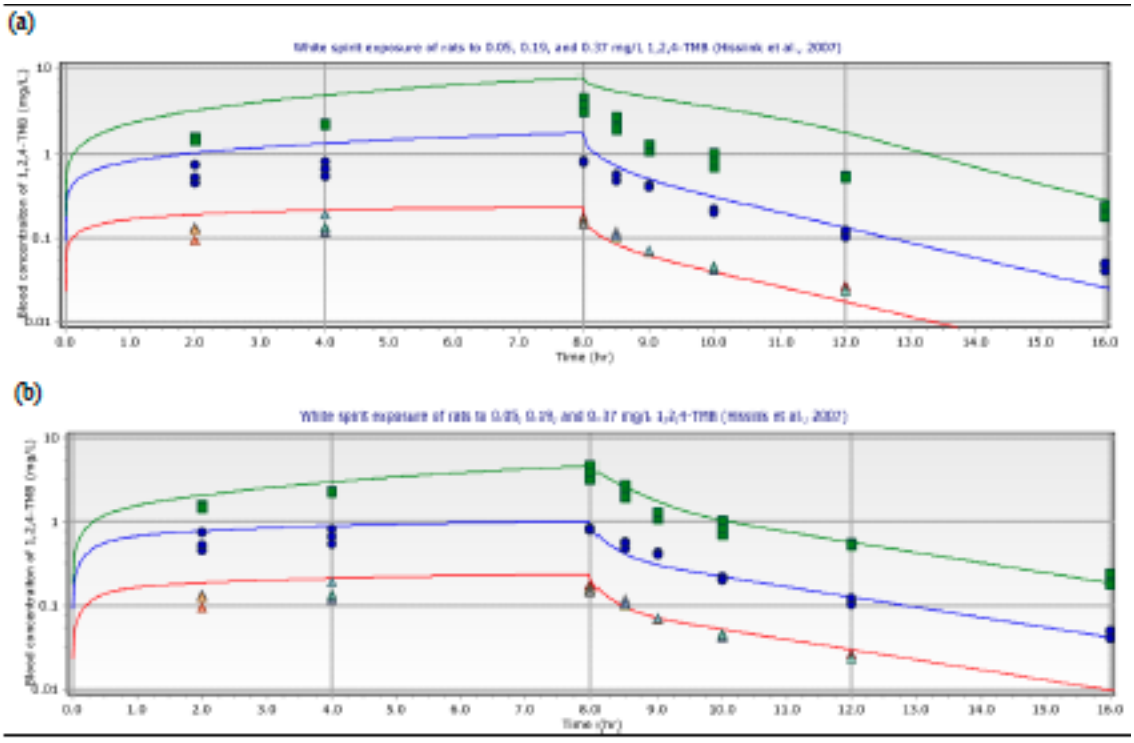
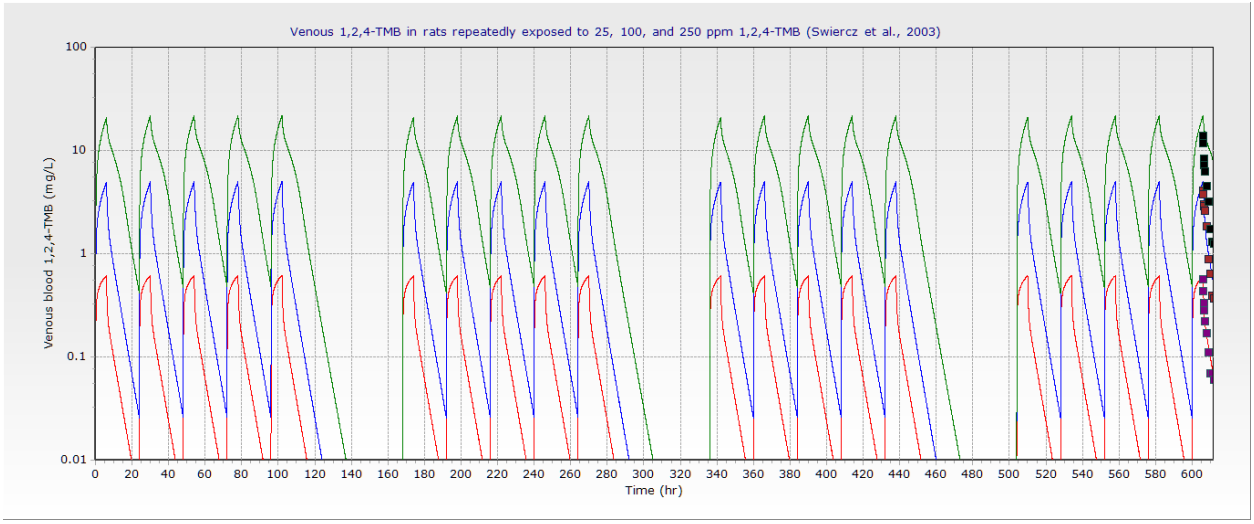


Figure 1. Model predicted blood concentrations for the study described in Hissink et al., 2007. Compare this figure to B-10(b) of U.S. EPA (2013). This figure represents the fit to the final model parameters and thus replicates Figure B-10(b).



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.



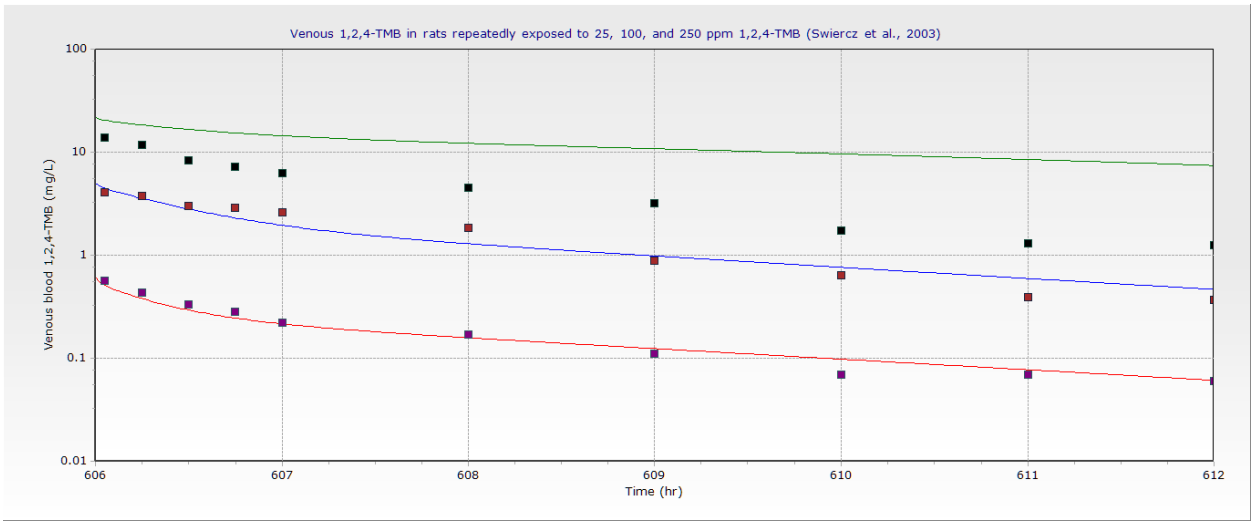
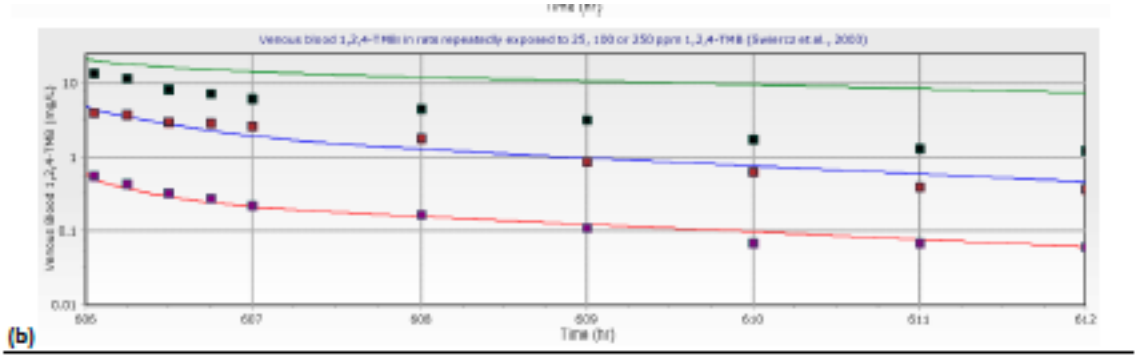


Figure 2. Model predicted blood concentrations for the study described in Swiercz et al., 2003. Rats were exposed to TMB 6 hr/day, 5 days/wk for 4 weeks. Blood was collected from the tail vein after the last exposure. Top) whole timecourse, Bottom) last 6 hr. Compare this figure to B-12 of U.S. EPA (2013).



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.

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 Vmax and Km optimized to fit the rat data from the same study, and was not shown using the Vmax and Km 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 VmaxC/Km 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).

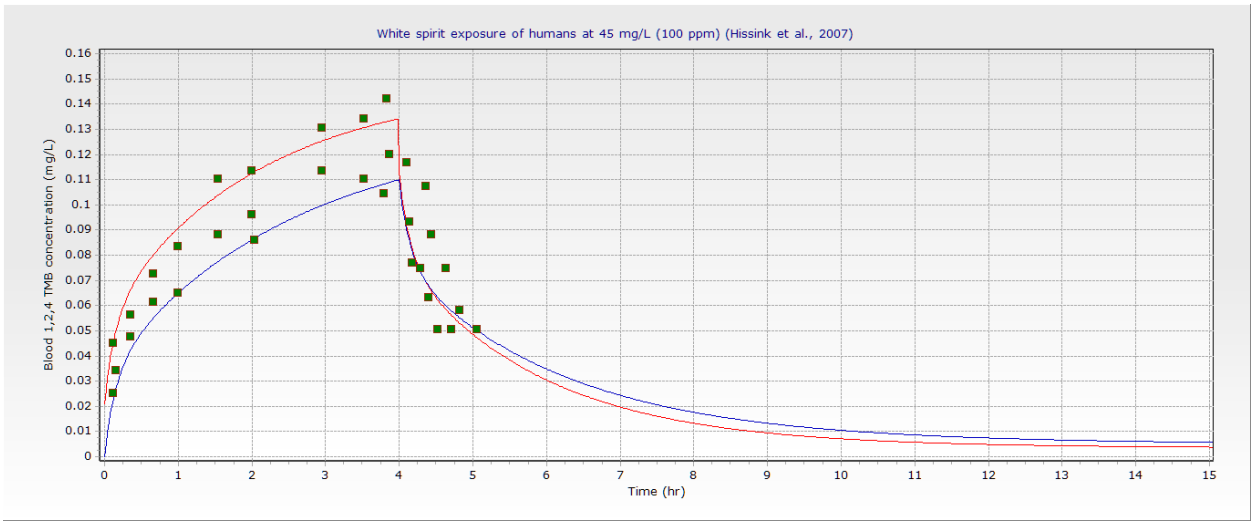


Figure 3. Comparisons of model predictions to measured human venous blood in human volunteers exposed to 100 ppm WS with 7.8% 1,2,4-TMB (39 mg/m3 1,2,4-TMB). The red line shows the fit when the metabolic parameters optimized to fit rat data from the same study (Hissink et al., 2007) are used, the blue line shows the fit when the VmaxC and Km optimized from the study of Swiercz et al., 2003 is used.

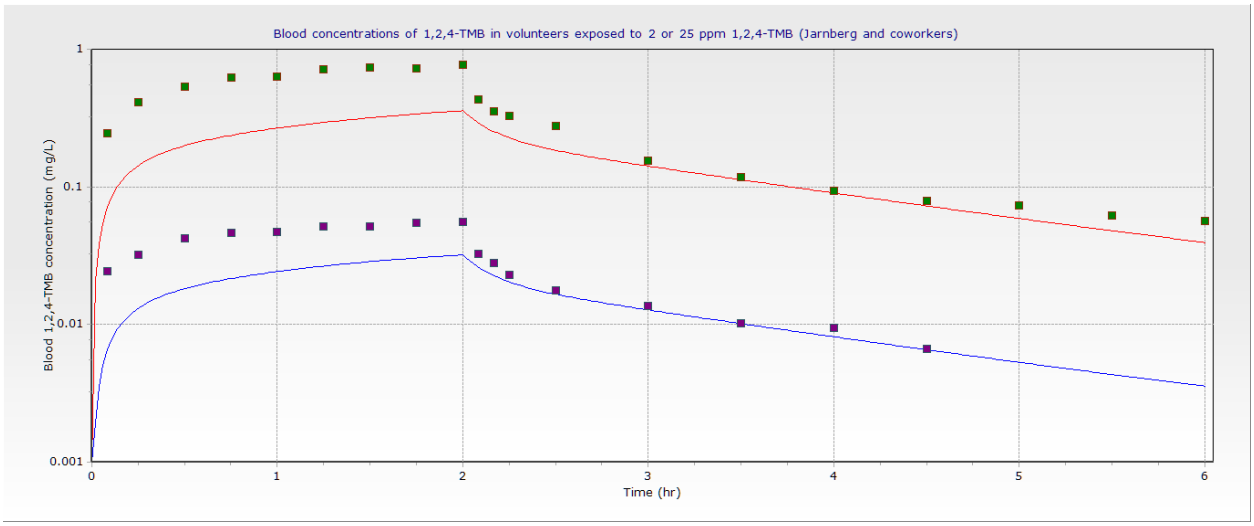


Figure 4. Comparisons of model predictions to measured human venous blood concentrations of Järnberg et al. (1998, 1997a; 1996) in volunteers exposed to 2 or 25 ppm (~10 or 123 mg/m3) 1,2,4-TMB for 2 hours while riding a bicycle (50 W).

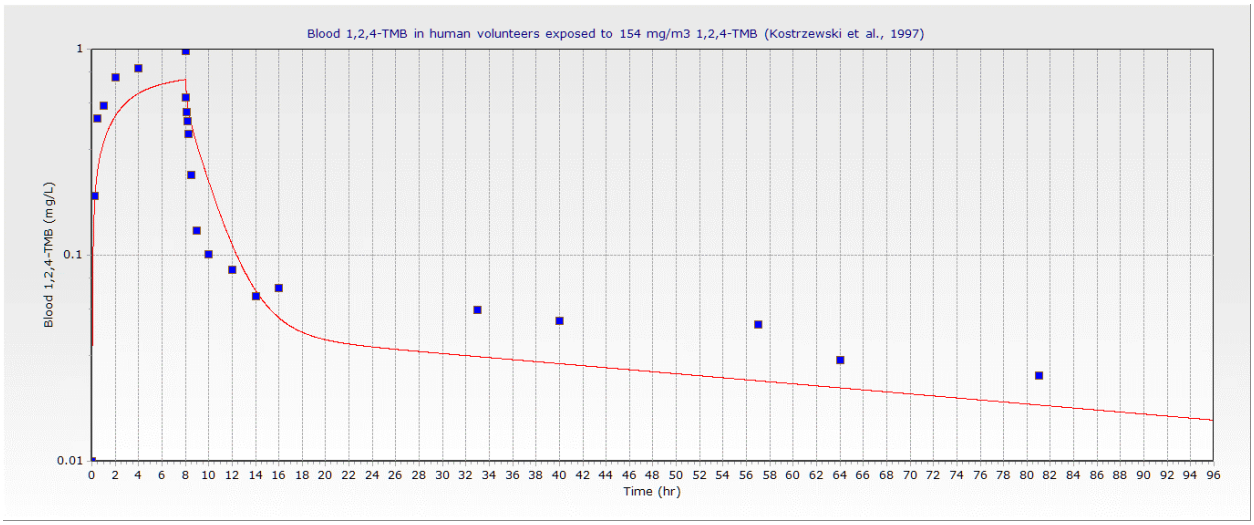


Figure 5. Comparisons of model predictions to measured human venous blood concentrations in Kostrzewki et al. (1997) in human volunteers exposed to 154 mg 1,2,4-TMB/m3 for 8 hours**.**

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 over-predicts 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/m3]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/m3]) 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 ≤ 100 ppm (492 mg/m3) for rats or ≤ 30 ppm (147.6 mg/m3) 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 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.

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
    - 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, and108-67-8). 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

TABLE 1. COMPARISON OF RAT MODEL INPUT PARAMETERS

Parameter	(Hissink et al., 2007)	Transmitted to EPA	Transmitted to Summit	Comments
Partitioning				
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
Anatomical and Physiological				
Alveolar ventilation rate (L/hr/kg$^{0.7}$)	20	||	14*	(Brown et al., 1997)
Total cardiac output (L/hr/kg$^{0.7}$)	20	||	14*	(Brown et al., 1997)
Blood flow (% cardiac output)				
Liver (total)	25	||	17.6	(Brown et al., 1997)
Fat	9	||	||	
Brain	1.2	||	2	(Brown et al., 1997)
Rapidly perfused (total)	49.8	76[‡]	57.4[§]	(Brown et al., 1997)
Slowly perfused (total)	15	NA	Calculated	
Tissue volume (% body weight)				
Liver	4	||	||	
Fat	7	||	||	(Brown et al., 1997)
Brain	0.72	||	0.57	(Brown et al., 1997)
Rapidly perfused	4.28	NA	9[§]	(Brown et al., 1997)
Slowly perfused	75	NA	82[§]	(Brown et al., 1997)
Metabolism				
VmaxC (mg/hr/kg0.7)	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*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 (QC/QC2=0.7).

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

§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 .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 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%

ǂ 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.

TABLE 2. COMPARISON OF HUMAN MODEL INPUT PARAMETERS

Parameter	(Hissink et al., 2007)	Transmitted to Summit	Comments
Partitioning			
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
Anatomical and Physiological			
Alveolar ventilation rate ($L/hr/kg^{0.7}$)	20	15*	(Brown et al., 1997)
Total cardiac output ($L/hr/kg^{0.7}$)	20	16*	(Brown et al., 1997)
Blood flow (% cardiac output)			
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[§]	
Tissue volume (% body weight)			
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	||	
Rapidly perfused	3	7.6	(Brown et al., 1997)
Slowly perfused	66.4	81[§]	(Brown et al., 1997)
VmaxC (mg/hr/kg0.7)	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.

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

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

TABLE 4. MODEL SIMULATED AND EXPERIMENTAL MEASURED CONCENTRATIONS OF 1,2,4-TMB IN MALE SPRAGUE-DAWLEY RATS EXPOSED TO 1,2,4-TMB AT THE END OF 12 HOUR EXPOSURE (ZAHLSEN, 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
0.74	6.9	18	2.6
1.5	14	48	3.5

TABLE 4. MODEL SIMULATED AND EXPERIMENTAL MEASURED CONCENTRATIONS OF 1,2,4-TMB IN MALE SPRAGUE-DAWLEY RATS EXPOSED TO 1,000 PPM (4,920 MG/M3) 1,2,4-TMB (12 HR/DAY, 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

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

	1 US EPA 2013 Average mg/l	2 Model Average mg/l	3 Hissink Model Average mg/l	4 Hissink/Model 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

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

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

Exposure Concentration (mg/m3)	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