

**Science Advisory Board (SAB) Draft Report (October 9, 2014) to Assist
Meeting Deliberations -- Do Not Cite or Quote –**

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

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6 EPA-SAB-15-xxx

7
8 The Honorable Gina McCarthy
9 Administrator
10 U.S. Environmental Protection Agency
11 1200 Pennsylvania Avenue, N.W.
12 Washington, D.C. 20460

13
14 Subject: Science Advisory Board Review of the IRIS Draft Toxicological Review of
15 Trimethylbenzenes

16
17 Dear Administrator McCarthy:

18
19 The U.S. Environmental Protection Agency's National Center for Environmental Assessment requested
20 a peer review of the scientific basis supporting the draft *Toxicological Review of Trimethylbenzenes*
21 developed for the agency's Integrated Risk Information System. The assessment is based on a
22 comprehensive review of the available scientific literature on the noncancer and cancer health effects in
23 humans and experimental animals exposed to three trimethylbenzene isomers: 1,2,3-TMB, 1,2,4-TMB,
24 or 1,3,5-TMB.

25
26 In April 2011, the National Research Council released its *Review of the Environmental Protection*
27 *Agency's Draft IRIS Assessment of Formaldehyde* and included comments and recommendations for
28 improving the development of IRIS assessments in general. The *Toxicological Review of*
29 *Trimethylbenzenes* is one of the first IRIS assessments to address the NRC recommendations for
30 improving the development of IRIS assessments.

31
32 The SAB was asked to review the scientific and technical analyses used to develop reference
33 concentrations and reference doses for the three trimethylbenzene isomers and to comment on the
34 agency's enhancements to the IRIS Program in response to the implemented to address the NRC
35 recommendations. The SAB Chemical Assessment Advisory Committee was augmented with additional
36 toxicological experts to conduct this review.

37
38 The agency implemented a phased approach to address the NRC recommendations for several
39 assessments near the end of their development focusing on streamlining the documents, increasing the
40 transparency and clarity of the assessment, and better presenting the data and information considered
41 through the use of standard tables editing and formatting. The SAB acknowledges the improvement in
42 the new format for IRIS assessments and commends the agency for its progress in addressing the NRC
43 recommendations. The SAB recognizes that the TMB assessment was developed "mid-stream" in the
44 EPA's efforts to enhance the IRIS process and looks forward to reviewing future IRIS assessments with

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

9
10 The SAB agrees with the agency that physiologically based pharmacokinetic modeling is an appropriate
11 approach to developing reference concentrations and reference doses. When implementing a PBPK
12 modeling approach the SAB strongly recommends that the EPA provide a transparent and detailed
13 discussion of the rationale for selecting this approach. The discussion should include the available
14 studies, data, and information considered by the agency, how they were compared and considered, and
15 why these analyses led the agency to use a PBPK approach rather than chemical specific studies. The
16 EPA should conduct independent peer review of the PBPK model and modeling results if it is a new
17 version, previously unpublished or is a modification of a published model. In the enclosed report the
18 SAB conducts a review of the PBPK model and provides specific recommendations to improve the use
19 of modeling for trimethylbenzenes.

20
21 The SAB finds that the physiologically based pharmacokinetic modeling approach and extrapolating
22 inhalation data to an oral exposure is appropriate for the reference concentration and reference dose for
23 1,2,4-TMB and 1,2,3-TMB. However, the SAB notes that the presentation of the analysis should be
24 expanded to better describe the inhalation and oral toxicology studies considered and rationale for using
25 the PBPK model.

26
27 The SAB notes that there are oral toxicology studies for 1,3,5-TMB and the physical toxicological
28 properties are sufficiently different that further analysis and explanation are required to explain using the
29 extrapolation and PBPK approach for this isomer. The SAB notes that the endpoints for these studies are
30 not the same neurotoxicological effects used in the PBPK approach used for 1,2,4- and 1,2,3-TMB. The
31 SAB recommends that the agency derive an reference dose for 1,3,5-TMB using available oral dosing
32 toxicology studies for 1,3,5-TMB and compare those results to the approach EPA used to develop the
33 reference concentrations and reference doses for 1,3,5-TMB using the PBPK approach extrapolating
34 from 1,2,4-TMB.

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

39
40 The SAB also notes that there is a limited discussion of sensitive life stages and vulnerable populations
41 and encourages the agency to expand the description and importance of these analyses in the current
42 TMB assessment and future assessments.

43

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

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

12
13 Sincerely,
14

15
16
17
18 Chair
19 Science Advisory Board
20

Dr. Cynthia Harris
Chair
SAB Chemical Assessment Advisory Committee
Augmented for the TMB Review
21

22 Enclosure
23

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NOTICE

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

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

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3

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Acronyms and Abbreviations

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

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1. EXECUTIVE SUMMARY

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

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

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

Enhancements to the IRIS Program

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

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1 Advisory Committee, through the Chartered SAB, will compare the reviews to provide the agency with
2 advice and comments on the agency's progress to enhance the IRIS program's assessments.

3
4 ***Chronic Hazard Assessment of Trimethylbenzenes***

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

14
15 The SAB finds that the PBPK modeling approach, which extrapolates inhalation data to an oral
16 exposure, is appropriate for the RfC and RfD for 1,2,4-TMB and 1,2,3-TMB. However, the SAB notes
17 that the presentation of the analysis should be expanded to better describe the oral toxicology studies
18 considered and rationale for using the PBPK model. This approach may not be appropriate for 1,3,5-
19 TMB. The SAB notes that there are oral toxicology studies for 1,3,5-TMB and the physical
20 toxicological properties are sufficiently different from the other TMBs that further analysis and
21 explanation are required to clarify using the extrapolation and PBPK approach for 1,3,5-TMB. The SAB
22 notes that the endpoints for these studies are not the same neurotoxicological effects used in the PBPK
23 approach used for 1,2,4- and 1,2,3-TMB. The SAB recommends that the agency derive an RfD for
24 1,3,5-TMB using available oral dosing toxicology studies for 1,3,5-TMB and compare those results to
25 the approach EPA used to develop the RfCs and RfDs for 1,3,5-TMB using the PBPK approach
26 extrapolating from 1,2,4-TMB.

27
28 The SAB finds that while the search strategy and rationale to select studies was clearly articulated the
29 exclusion criteria and implementation of those criteria was not as transparent. The breadth of the
30 literature review and discussion should be expanded to include other closely related aromatic solvents
31 and possibly mixtures. The SAB concludes that because human exposures to the TMBs generally
32 involve complex mixtures, the available studies on mixtures – including the C-9 fraction and white spirit
33 studies – deserve further consideration and discussion.

34
35 The SAB concludes that EPA's hazard assessment of the carcinogenicity of TMBs integrates all
36 available scientific evidence and agrees with the EPA that there is "inadequate information to assess the
37 carcinogenic potential" of TMBs. The carcinogenicity of 1,2,4-TMB has been assessed in only a single
38 study. The EPA found that there were a number of deficiencies concerning this bioassay and the SAB
39 agrees that there are not sufficient data to conduct a quantitative assessment. The SAB also notes that no
40 carcinogenicity bioassays have been conducted with 1,2,3-TMB or 1,3,5-TMB.

41
42 ***Sensitive Life Stages and Subchronic Reference values***

43 In addition to responding to the EPA Charge Questions the SAB identified two additional topics that
44 warrant further consideration by the agency: (1) an expanded discussion of sensitive life stages and

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1 vulnerable populations; and (2) the benefit of publishing the subchronic RfC and RfD that were
2 developed using the analysis for the trimethylbenzene isomers.

3
4 There is a limited discussion of sensitive life stages and vulnerable populations in the preamble and the
5 SAB encourages the agency to expand the description and importance of these analyses in the TMB
6 assessment and address the hazard identification and dose-response for sensitive life stages and
7 vulnerable populations in future toxicological assessments.

8
9 The SAB notes that the agency's derivation of chronic RfCs and RfDs for the TMBs is built upon
10 subchronic values. The principal studies used to derive the proposed RfCs and RfDs are all subchronic
11 in duration, and the analysis needed to support a robust set of subchronic toxicity values has in effect
12 already been done. The toxic endpoints and dose-response relationships are clearly relevant for
13 subchronic exposure, and the same points of departures would apply to the development of a set of
14 subchronic RfCs and RfDs. Given the potential usefulness of these toxicity values for risk assessment,
15 the importance of having the values available on IRIS, and the very small amount of additional work
16 required to add them to the TMB Assessment, the SAB recommends that the review be expanded to
17 include the presentation of subchronic RfCs and RfDs for 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB.
18

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2. INTRODUCTION

2.1. Background

The EPA requested a peer review of the scientific basis supporting the draft *Toxicological Review of Trimethylbenzenes (1,2,3-trimethylbenzene [1,2,3-TMB], 1,2,4-trimethylbenzene [1,2,4-TMB], and 1,3,5-trimethylbenzene [1,3,5-TMB])* that will appear on the agency's online database, the Integrated Risk Information System (IRIS). This is a new assessment; there is currently no entry on 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 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, 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). 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 draft *Toxicological Review of Trimethylbenzenes* (August 2013), hereafter referred to as the TMB Assessment, is based on a review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. This draft IRIS assessment includes:

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

In addition the Draft TMB Assessment includes appendices on chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and a summary of the public comments

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1 received on the May 2012 draft. The draft assessment was developed according to guidelines and
2 technical reports published by EPA and contains a qualitative characterization of the hazards for the
3 trimethylbenzenes, including a cancer descriptor of the isomers' human carcinogenic potential, and
4 noncancer toxicity values, including a chronic oral RfD and a chronic inhalation RfC for all three
5 trimethylbenzene isomers. A quantitative cancer assessment for trimethylbenzenes was not conducted
6 due to inadequate data.

7 **2.2. Charge to the Science Advisory Board**

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

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

29
30 The EPA Charge to the SAB includes one question about the response to public comments on the May
31 2012 TMB Assessment and specific questions about the scientific and technical approaches aspect used
32 to develop the RfC and RfD for each of the individual isomers. Because the agency uses PBPK
33 modeling and extrapolation of data for 1,2,4-TMB to develop the RfC and RfD for 1,2,3-TMB and
34 1,3,5-TMB the responses to questions often raised similar if not identical issues and advice for the
35 agency to consider. Where appropriate this report refers the reader to issues discussed in previous
36 sections rather than repeating the same information and advice for each isomer.

37
38 During deliberations the TMB Review Panel identified two issues that were not addressed in the EPA
39 charge to the SAB. First, the toxicological review provided a limited discussion of health effects from
40 exposure to vulnerable life stages. Second, the approach used to develop the RfC and RfD in this
41 assessment built upon developing a subchronic RfC and RfD for each of the isomers and applying
42 uncertainty factors to arrive at a chronic value for inhalation and ingestion. The SAB provides

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- 1 recommendations for these issues after the response to the EPA charge. Charge questions are included in
- 2 italics at the beginning of each response of this report and the full charge is included as Appendix A.

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3. RESPONSE TO CHARGE QUESTIONS

This review provides responses to the each of the charge questions in the order presented by the EPA. Section 3.1 provides advice on how the agency has addressed the NRC recommendation to improve the IRIS process. There are three general Charge questions that focus on the IRIS program enhancements and the response are based on the TMB assessment to identify recommendations for further improvement. There is also a question on the response to public comments the agency received on a draft May 2012 Draft of the TMB Assessment. Section 3.2 focuses on the description of the analyses for each of the three isomers for inhalation, ingestion, and carcinogenic effects and the charge questions, respectively. The SAB notes that RfC and RfD for the isomers are based on the available data and studies for 1,2,4-TMB. The charge questions for the 1,2,4-TMB and 1,2,3 TMB isomers are almost identical and raise similar toxicological issues. Rather than repeating the advice and comments for the 1,2,3-TMB and its limited dataset the responses to the charge questions for 1,2,3-TMB, where appropriate, refer to the discussion of 1,2,4-TMB and identify issues unique to 1,2,3-TMB. Section 3.3 discusses the two additional issues identified by the TMB Panel. Section 3.3.1 provides advice on how the agency should consider and address adverse health effects for vulnerable life stages and Section 3.3.2 provides a recommendation to publish subchronic RfCs and RfDs.

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 it lacks any overarching statement about what IRIS seeks to accomplish, its ultimate purposes, and its intentions for what its assessments are meant to represent to their users.

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

8
9 Section 2 on the IRIS Process is clear and concise in the description of the seven steps to develop
10 assessments and the multiple levels of review and what happens at each one. It is rather vague, however,
11 on the nature of the problem formulation step. It should include some discussion (without seeking to
12 constrain the agency's further actions) about the issues needing to be addressed, the prospects for
13 addressing them with available data, and the uncertainties and plausible alternative interpretations that
14 would need to be worked through. Although the section clearly indicates that the agency will prepare a
15 summary record of response to peer review comments, it is not clear who will be the ultimate EPA
16 decision-maker. The discussion of Step 5 notes that newly published studies that are critical to
17 conclusions can be brought into consideration, but a more explicit reference to the stopping rule policy
18 (and where its details can be found) would be appropriate.

19
20 The SAB recommends that the agency take measures to ensure that the Preamble in this and future
21 assessments be structured so that it refers the reader to the appropriate guidance and cannot be construed
22 to contradict policy by over summarizing existing guidance.

23
24 Sections 3 to 7 lay out the specific steps for conducting an assessment. A good deal of the material is a
25 summary of long-extant guidance, and so will be familiar to most readers. The Preamble purpose does
26 require setting out the processes and analyses, but perhaps this could be done somewhat more briefly.
27 On the other hand, the aspects that are under revision need to be flagged or expressed in more general
28 terms. In particular, the Preface notes that Phase 3 of the IRIS revision process is yet to happen, and this
29 will include review of current methods for weight-of-evidence analysis. The methods for abstracting
30 data, systematically considering study quality and interpretation issues, and ranking relative study
31 impact are also in transition.

32
33 Section 5.5 references the carcinogen classification scheme of the 2005 Carcinogenicity Assessment
34 guidelines, which presumably are part of what is being reconsidered. The section also cites the
35 Integrated Science Assessment criteria for causality (applied in the evaluation of criteria air pollutants)
36 as "another example" and, further on in the Preamble notes that the agency is investigating what
37 descriptors to use and may use these or others. This is confusing as to what applies to the current
38 trimethylbenzenes assessment. The SAB finds that discussing the intent of descriptors is probably more
39 useful than recounting definitions that may or may not be used and may or may not be seen as in
40 keeping with the spirit of the overall revision process.

41
42 It should be clear that the Preamble itself is not guidance; it only summarizes guidance that is set out
43 elsewhere; an unambiguous statement to this effect should be added. This is especially critical because -
44 - being only summaries and explanations -- the treatment in the Preamble is less developed and

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unaccompanied by the fuller discussion about motivation, meaning, interpretation, and scientific justification of the briefly described analyses, presumptions, standards, or judgments. Without reference to the fuller treatment, 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. Citations are 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 are 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 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.

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.

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1 In short, the reader should easily find the conclusions, the choices and reasoning, and the justifications
2 of choices, respectively.

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

6
7 The second way of reading -- for the choices and reasoning -- has also markedly improved, though some
8 suggestions can be made, as discussed below.

9
10 The third way of reading -- the examination of particulars and the ability efficiently to find them
11 documented with sufficient detail, is much improved from former IRIS documents. The relegation of a
12 lot of the details to well-structured appendices is helpful. The set of focused appendices helps a reader to
13 find the place where particular study aspects or analyses of issues are to be found. The organization of
14 the appendices -- and the consistency of presentation across IRIS documents -- are important in making
15 the place to find details clear. Although the general structure of the appendix entries can be discerned,
16 the plans for the structure and consistency have not been provided, so it will take some time and
17 examination of other documents following the same plan for readers to find things easily. The use of
18 appendices simultaneously allows presentation of more detail than may have been captured in earlier
19 generations of IRIS documents and also avoids cluttering the main body of the IRIS document -- where
20 interpretation and evaluation are considered. The appendix approach also frees the main document from
21 seeming to need to present all the details before drawing any interpretation.

22
23 ***Consistent Presentation of the Studies Considered***

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

29
30 It would also be useful for each study to have a short overview section (also in its appendix listing, not
31 repeating tabulated details) of the nature of the study, its examined endpoints, and relevant findings. The
32 goal of the overview is to provide context for the tabulated details, so that the details need not be read in
33 full to gain an idea of the general nature of the study and its importance to the assessment as a whole.
34 This overview should not discuss interpretations.

35
36 It is clear that the intent of this structure is to free the main document to focus on choices that were made
37 in the analysis (selection of possible endpoints, selection of studies to represent and characterize those
38 endpoints, and analyses and interpretations of their bearing on human risk estimation). The challenge is
39 to bring the appropriate data and level of detail from the appendices into the main body, so that the
40 interpretations and choices can be justified and documented, without overwhelming the interpretation
41 discussion or leaving out potentially relevant information. Sorting this out is the essence of the
42 systematic review process, and though clear strides have been made, more work is left to be done. The

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1 key to this process is to be transparent regarding both studies chosen for inclusion and those chosen for
2 exclusion; not just what supports an interpretation, but also what seems unexplained or even
3 inconsistent. Studies results should be cited when they are as consistent with a hypothesized potential
4 for human risk, and also when they have apparently contrary results with different implications for
5 scientifically supportable inference about human risk impacts.

6
7 The SAB finds that the overall structure of the main report provides a good framework -- with sections
8 on literature search, hazard identification for the various candidate endpoints, and dose-response.

9 ***Describing the Literature Search***

10 The Literature Search Strategy section is brief and focuses only on identification of pertinent studies
11 from the literature. The general description of the process and the specific implementation for TMBs
12 may be too exclusive, missing potentially informative ancillary studies that could help in interpretation
13 or evaluation of those studies strictly observing toxicity outcomes of the TMBs alone in controlled
14 settings. It should be clear that literature search is only the first step of systematic review, which needs
15 to be followed by evaluation of each study in terms of design, quality, shortcomings, main findings
16 (including both positive and negative findings), evaluation of the reliability of individual study results,
17 and identification of other studies, particularly on mechanisms, that could address uncertainties in the
18 primary database. This supports a further process of comparing results across studies to assess both the
19 consistency of specific effects, and also the manifestation of related effects that would be expected from
20 hypothesized underlying causative processes, both of which bear on the use of specific study results as
21 evidence regarding the existence and nature of hazards in human target populations.
22

23 ***Describing the Hazard Identification Steps***

24 The individual endpoint sections of the Hazard Identification have some discussion about interpretation
25 across studies and evaluations of bearing and relevance, though further discussion of interpretation
26 rationales and consideration of alternatives would be beneficial. It is the middle section of systematic
27 review -- after the studies are chosen but before the interpretation of their overall bearing gets
28 considered -- that does not have a clear home in the current document structure. As the agency develops
29 its approach for systematic review, including defined ways for abstracting data, judging study quality,
30 documenting factors bearing on interpretation and its limits, and considering the impact of related
31 studies, it will be important to develop the document structure that encompasses all aspects for
32 consideration. It is noteworthy that the Preamble has a section (Section 5) on evaluation of causality,
33 which depends on the existence of such a documented review and evaluation process, but the present
34 document has no particular place where the Preamble's named considerations -- strength, consistency,
35 specificity, temporal relationship, biologic plausibility, coherence, natural experiments, and analogy --
36 are systematically considered or documented.
37

38 It may be useful to consider adding a brief summary of the main features of pharmacokinetics and
39 metabolism before the section on Hazard Identification. The aim is not to replace the fuller treatment of
40 these issues in the appropriate appendix, but rather to set the context for the interpretation of studies
41 bearing on hazard, and the main presentation of pharmacokinetic details should continue to reside in the
42 appendix. The main text's section would note such things as extent of absorption, rapidity of elimination,

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1 main metabolic processes, main means of clearance (and what part of that is by metabolism), indications
2 whether metabolic saturation or enzyme induction might play a relevant role in toxicity studies, and any
3 notable unusual differences between experimental animals and humans. Again, the point would not be
4 just to list specifics (which can remain in an appendix) but to provide the basic insights that might bear
5 on how toxicity data are interpreted or on the limits to such interpretation.

6
7 A noteworthy change from earlier IRIS assessments is that the Hazard Identification section is separated
8 into assessments of each endpoint, with relevant data for that endpoint being reviewed within the
9 section. The SAB finds that this approach is a great improvement over the past practice of summarizing
10 study by study, with all the endpoints clustered study. The endpoint-by-endpoint analysis permits the
11 examination of consistency and sufficiency of data to draw hazard conclusions about each effect.

12
13 This being said, there are possible overarching ties among endpoints that would help in evaluation of the
14 hazard characterization of each (say, commonalities of dosimetry or mode-of-action) that should be
15 discussed in an appropriate place. It would be useful to include considerations that might indicate a
16 study as the critical study.

17
18 The tabulation of studies is useful, and the dose levels and dose-specific responses are important details
19 to include. The hyperlinks to the detailed description of studies in the appendices helps to make those
20 appendices directly supportive and makes finding of relevant information more efficient. The exposure-
21 response arrays are useful summary devices to aid communication, though they should not be read as
22 meta-analysis forest plots or otherwise be used as the primary basis of conclusions. Nonetheless, they
23 provide a valuable overview of the data. It is perhaps unfortunate that it is difficult to preserve the
24 distinction between studies on a given effect (especially if the studies appear to disagree) and also that
25 the dose-levels shown are only the extremes, the NOAEL and the LOAEL.

26
27 ***Describing the Dose-Response Steps***

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

31
32 It represents an important advance that the Hazard Identification sections for each endpoint have specific
33 places for discussion of consistencies and inconsistencies among data, on the relevance of studies for
34 human risk evaluation, on the knowledge of mode of action (even if it must say that little is known), and
35 on alternative interpretations of the available data on potential causation. The format that addresses each
36 of these issues in an orderly way for each endpoint is important to advancing the explanation of the basis
37 for conclusions and enhancing transparency. However, these interpretation passages in each Hazard
38 Identification section are somewhat too concise. It would be good to find a consistent way (perhaps
39 more appendices) to document the arguments without unduly distracting from the main discussion.

40 ***Presenting the Outcomes***

41 As it stands, both the Hazard Identification and Dose-Response sections simply dive in to the first
42 endpoint or analysis to be considered, and then have separate sections on each. There is little overview

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

12 **3.1.3. Standardized Evaluation of Critical Studies**

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

18
19 In general, a great deal of progress has been made in restructuring the document to focus the main body
20 on documenting and explaining the interpretations, choices, and analyses, and relegating the supporting
21 information to appendices. The use of links to the appendices aids in using them as support, without
22 encumbering the flow of the main arguments. At the same time, the details of studies are important
23 when the study results are used in constructing arguments. It will be an ongoing challenge to bring
24 enough into the main text to document the reasoning, to avoid leaving important aspects hidden in the
25 appendices, and still to have a readable document that fully explains the choices and conclusions made.

26
27 The process of systematic review still needs development. Documentation of the process of identifying
28 literature has progressed, but further development is needed in establishing standard practices for
29 abstracting relevant data, for evaluating study quality, strengths and shortcomings, and for integration of
30 evidence across studies. This includes the phases of evaluating individual studies, of comparing the
31 results of studies of similar objective into characterizations of their joint bearing in a way that addresses
32 discordant results, and of the overall integration across lines of evidence to form and justify judgments
33 about causality and appropriate dose-response analyses.

34
35 In this development, it should be borne in mind that the process of systematic review is not solely one of
36 identifying the “right” or the “best” data, with the interpretation and bearing on risk evaluation
37 becoming clear once the right choices are made. The integration and weight-of-evidence evaluation
38 process requires accepting that multiple interpretations are always possible, especially in different
39 contexts, and that consistency of causal interpretations with available data should be considered across
40 all applicable studies, bearing in mind the possible role of study quality limitations in generating
41 apparent discordances. This process should consider how results of particular studies are to be
42 generalized to apply to other situations (especially to actual human exposures); it needs to account for

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1 why other study results might disagree; and it needs to consider how other interpretations would have
2 different consequences for risk estimation.

3
4 An important challenge is that assessments must go ahead even as this further development proceeds
5 and before all aspects are complete. The strategy of working on the structure of the assessment, and
6 focusing the main text on documentation of the process and its choices and analytical options, is a good
7 way to begin.

8
9 The recommendations for revision of the IRIS process come from the NRC “Roadmap” (Chapter 7 of
10 the Formaldehyde review) and other sources. A good principle to follow in conducting assessments
11 during the process of revision is to consider the reasons behind the recommendations, and to make good
12 faith efforts to address the issues and to explain how the chosen approaches seek to reflect the NRC
13 recommendations, although the methods may not yet be fully developed and agreed upon. That is, trying
14 to address as well as one can the issues behind the recommended methodological and procedural
15 changes is a good way to make assessments as reformed as they can be, and improve acceptance as the
16 overall IRIS process continues to advance.

17 **3.1.4. Addressing Public Comments on the Draft *Toxicological Review of Trimethylbenzenes* (May**
18 **2012)**

19 *Charge Question: EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes*
20 *[May 2012] and has revised the assessment to respond to the scientific issues raised in the comments. A*
21 *summary of the public comments and EPA’s responses are provided in Appendix F of the Supplemental*
22 *Information to the Toxicological Review of Trimethylbenzenes. Are there scientific issues that were*
23 *raised by the public as described in Appendix F that may not have been adequately addressed by EPA?*
24

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

34
35 The SAB finds that Appendix F did address issues raised in public comments and that explanations were
36 furnished for the agency's stance on the issues and their disposition. That is, the issues were all
37 addressed according to the agency's judgments, and those judgments were transparently discussed.

38
39 The TMB Review Panel was divided, however, on the adequacy of the responses and the advisability of
40 the dispositions that were made as presented in the summary. In particular, there was a variety of views
41 on the role that testing of the C-9 fraction should have in the assessment, with some Panelists accepting
42 the reasons for omission of this from the main evaluation and others feeling that these results had a role
43 that had not been adequately explored. The discussion of the C-9 fraction in the August 2013 draft of the

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1 TMB Assessment is further discussed in section 3.2.3 of this report. There was also disagreement among
2 the TMB Panelists related to the interpretation of the pain sensitivity data, with some members
3 questioning whether the document adequately examined the question of reversibility following
4 termination of exposure, which further bears on whether ongoing or repeated exposures to TMBs should
5 be deemed to have accumulating toxicity beyond effects evident in shorter-term exposure; other panel
6 members believed that the data was consistent with cumulative toxicity and lack of reversibility. The full
7 discussion of these issues and their treatment in the TMBs assessment is covered in the responses to the
8 specific charge Questions in Section 3.2 of this report.

9
10 The SAB recommends that EPA provide a more robust discussion of the data and studies considered in
11 the TMB assessment including the C-9 fraction and mixtures. More detail is provided in the responses to
12 Charge question directed to the specific TMB assessment (See section 3.2.3)

13 **3.2. Toxicological Review of Trimethylbenzenes**

14 **3.2.1. Executive Summary**

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

19
20 The Executive Summary condenses the large amount of information presented in the draft TMB
21 Assessment and the Supplemental Information. Individual conclusions regarding RfC and RfD values,
22 as well as other relevant information (e.g., carcinogenicity) for each of the TMB isomers of concern are
23 clearly described. The SAB recognizes that there is always some tension to find the appropriate level of
24 detail to include in the Executive Summary. The draft TMB Assessment presents somewhat detailed
25 information on the data used to develop the RfC and RfD for each of the three isomers and that detail
26 may detract from the Executive Summary's intended purpose of brevity. As discussed in Section 3.1.1,
27 the Executive Summary should emphasize the major conclusions of the assessment and provide the
28 specific details of the critical studies in the main text of the assessment.

29
30 Recommendations to improve the Executive Summary include:

- 31
32 • The Summary should be truncated to emphasize the major conclusions. Specifically, citations
33 should be removed from the summary unless they are absolutely essential. Whole sections of the
34 Executive Summary are devoted to elaborating on "Confidence"; for example, the last
35 paragraphs in Sections 3 and 5 are identical except for the compound being discussed. The SAB
36 recommends that the EPA consider treating "Confidence" as a single very succinct section
37 toward the end of the Executive Summary. Issues pertaining to the use and rationale for
38 assigning confidence for each isomer should be relegated to the corresponding sections in the
39 main text.
- 40 • An example of other sections that present far too much detail is the middle paragraph on page
41 xxxvi. The text and table both describe the calculations for the RfC, where as an interested reader
42 can find the details in the main body of the text.

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- 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). A flow chart was provided (Figure LS-1) to tabulate the studies that were included and excluded.

The flow chart (Figure LS-1) indicated that the initial search identified approximately 4,300 papers, of which approximately 200 were used in the Draft TMB Assessment. The SAB finds that the description of the study selection is not transparent and should further clarify the criteria for omitting studies needs further clarification. 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 construct an on-line database that includes citations for the 4300 papers and group them according to the main reason as to why they were excluded. This could be accomplished by linking the document to the Medline search that was used.

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. A portion of the 65 papers were also 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 if only certain in vitro studies were included and what factors were used to determine if these studies should be 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 painter's 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

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1 Ruijten et al. (1994), Qian et al. (2010), Tang et al. (2011), and Hassan et al. (2013), are closely linked
2 to xylene but not cited in the document. The overall association of the effects reported in these studies in
3 painters with exposures to aromatic solvents like the TMBs is much stronger than the associations
4 reported by Chen et al. 1999, and Lee et al. 2005.

5
6 Section B4 in the Supplemental Information provides details on each of the human studies. While not
7 directly pertinent to the search strategy, the SAB recommends the inclusion of a summary table of the
8 studies related to each health effect: for example, a table with the 9 studies on neurotoxicity in humans,
9 reporting study design, inclusion, exclusion criteria, number of subjects, and main results. This is
10 common practice in epidemiologic reviews and meta-analyses. The current way of presenting the study
11 has some advantages because it is very analytical, but it is also hard to summarize.

12
13 Additional reference that should be considered by the EPA include:

- 14
- 15 • Chapter 8: Trimethylbenzenes in Acute Exposure Guideline Levels for Selected Airborne
- 16 Chemicals (NRC 2013),
- 17 • Health Hazards of Solvents Exposure among Workers in Paint Industry. (El Hamid Hassan et al
- 18 2013)
- 19 • Xylene-induced auditory dysfunction in humans (Fuente et al. 2013)
- 20 • Hearing loss associated with xylene exposure in a laboratory worker. (Fuente et al .2012)
- 21 • Visual dysfunction in workers exposed to a mixture of organic solvents. (Gong et al. 2003)
- 22 • Ototoxicity effects of low exposure to solvent mixture among paint manufacturing workers.
- 23 (Juárez-Pérez et al. 2014)
- 24 • Short latency visual evoked potentials in occupational exposure to organic solvents. (Pratt et al.
- 25 2000)
- 26 • Auditory brainstem response in gas station attendants. (Quevedo et al. 2012)

27 **3.2.3. Hazard Identification**

28 *Synthesis of Evidence*

29 *Charge Question: A synthesis of the evidence for trimethylbenzene toxicity is provided in Chapter 1,*
30 *Hazard Identification. Please comment on whether the available data have been clearly and*
31 *appropriately synthesized for each toxicological effect. Please comment on whether the weight of*
32 *evidence for hazard identification has been clearly described and scientifically supported.*
33

34 The synthesis of evidence for the three TMB isomers is nicely divided up into the various target organs
35 or forms of toxicity, as well as by exposure route and by human versus animal studies. The studies
36 chosen for review are clearly described and the summary tables and figures supplement the text
37 effectively. The tables are clear and useful, and the figures nicely summarize the available data for each
38 effect by each isomer. An introductory paragraph describing the section layout, including the summary
39 tables for each endpoint, would improve readability.
40

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1 However, the decision to disregard or limit consideration of the studies on solvent mixtures containing,
2 but not limited to, the TMBs appears to have affected the synthesis of evidence. Because important
3 toxicological observations have been made in both animal and human studies involving exposure to
4 aromatic solvent mixtures, an important toxicological perspective may have been lost. While the IRIS
5 Preamble, in Section 3.1 on identifying studies (lines 44-47) specifically states, "In assessments of
6 chemical mixtures, mixture studies are preferred for their ability to reflect interactions among
7 components." The SAB notes that the TMB assessment was prepared in response to the occurrence of
8 TMBs at Superfund sites and that detection of TMBs at sites are reported as individual isomers. The
9 solvent mixtures studies may not be representative of the contamination at the Superfund sites. The
10 SAB finds that the EPA needs to identify and characterize the available information on mixtures,
11 including the C-9 fraction and white spirit, and clarify why the studies on the individual isomers are
12 more appropriate than the studies on mixtures.

13
14 In addition, synthesis of available data appears to have been impaired by the decision not to include
15 literature on other closely related aromatic solvents. Toluene is briefly mentioned, but the potentially
16 relevant literature on ethylbenzene, xylenes, and styrene is largely excluded. This eliminated supporting
17 toxicological information on neurological endpoints which could have helped clarify potential
18 mechanisms of action. Such information is clearly supported in the IRIS Preamble, section 3.1 (lines 11-
19 15), and "Searches for information on mechanisms of toxicity are inherently specialized and may
20 include studies on other agents that act through related mechanisms." This is further supported in
21 Section 5.4, p. xxiii (lines 18-21), "Pertinent information may also come from studies of metabolites or
22 of compounds that are structurally similar or that act through similar mechanisms." It is therefore
23 recommended that additional animal and human studies on related aromatic solvents be considered in
24 the qualitative and mechanistic interpretations of TMB toxicity. Examples of such studies are included
25 in comments on the literature review.

26
27 The testing of the C-9 fraction reveals another important point. Because this mixture, as tested, was
28 about half TMBs, much of the observed effects could have been due to the TMBs. Competition for
29 metabolic clearance would likely have increased duration of exposure to the TMBs, so the minimal
30 observed toxicity in several C-9 studies provides important perspective to the TMB evaluation (although
31 both positive and negative interactions are possible). One application of the IRIS assessment for TMBs
32 may be for evaluating potential risks from exposure to the C-9 solvent and related aromatic mixtures, the
33 observation of effects of such mixtures is relevant, and needs further discussion.

34
35 More specifically, in another subchronic evaluation of the C-9 mixture, Douglas et al. (1993) found no
36 persistent neurotoxicity. In light of the concern or uncertainty involving the neurotoxicity endpoints used
37 in the draft TMB assessment, this possible discrepancy needs to be addressed in detail. Such a
38 discussion might involve considerations of the potential for chronic neurotoxicological effects of TMBs
39 alone, versus when exposure is to TMBs in mixtures. Data are available from many additional mixture
40 studies to provide further perspectives on this question, as reviewed, for example, by Richie et al.
41 (2001).

42
43 Discussion of the individual endpoints is flawed by questionable statistical statements or inferences. In
44 several places (pp. 1-3, 1-4, 1-5, 1-7, 1-36), the descriptions of non-statistically significant results infer

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1 that effects have been observed. The panel recommends that descriptions of results more closely adhere
2 to the rule that statistical significance provides the *definition* of whether an effect has occurred (although
3 data trends can be cautiously noted).

4
5 For neurological effects, which are the most consistently observed, the document clearly explains that
6 although mechanistic data are lacking for the TMBs, there is good rationale for making analogies with
7 toluene, for which much more information is available. This could have been greatly strengthened, as
8 mentioned above, by including supportive studies on the three xylene isomers as well as ethylbenzene
9 and other related solvents and mixtures.

10
11 This is all the more important because the neurological effect data that was presented is largely from a
12 single laboratory. Although the quality of the studies seems very high, the functional significance of the
13 observed effects is not as clear, nor is the extrapolation to humans. A more comprehensive discussion of
14 the evidence for prolonged effects of aromatic solvents would likely provide more confidence in the use
15 of these endpoints.

16
17 The discussion of respiratory effects should be strengthened by further consideration of the relevance of
18 the effects observed in the high-dose animal studies to humans. While it's clear that respiratory effects
19 are observed and are a relevant endpoint in humans, the distinction between the high-dose animal effects
20 and the human effects could have been made more clearly. The limitations of the human evidence for
21 hematological and clinical chemistry effect, based on the uncertainties in exposures (mixture
22 components, doses) should be more clearly described. With regard to carcinogenicity as an endpoint, the
23 document clearly communicates the inadequacy of the database, including the minimal genotoxicity
24 database.

25
26 The SAB recommends that EPA expand on the discussion of the similarities among methyl-substituted
27 aromatic compounds. The subsections in the hazard synthesis considering the similarities between the
28 three TMB isomers are very important but would be improved by further perspectives on related
29 solvents. This is critical with regard to the decision to base some of the RfDs on extrapolations among
30 the isomers. The evidence for similar effects and endpoints among methyl-substituted aromatic
31 compounds seems to be much stronger than what has been presented so far. The summary table (Page 1-
32 49, Table 1-7) is very helpful in understanding the points made with regard to toxic effects. A summary
33 table or scheme regarding toxicokinetics and metabolism would also be useful.

34 ***Noncancer Health Effects***

35 *Charge Question: Does EPA's hazard assessment of noncancer human health effects of*
36 *trimethylbenzenes clearly integrate the available scientific evidence (i.e., human, experimental animal,*
37 *and mechanistic evidence) to support the conclusions that trimethylbenzenes pose potential hazards to*
38 *the nervous system, respiratory system, the developing fetus, and the circulatory system (i.e., blood)?*
39

40 Section 1.2.1. Weight of Evidence for Effects Other Than Cancer of the TMB Assessment contains a
41 summary description of the toxicological evidence of effects of the TMBs on the nervous, respiratory,
42 hematological and developmental systems. The information summarized in this section should not only

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1 identify organ system effects to focus on in the subsequent dose response step but also provide a
2 conclusion that communicates the limitations and uncertainties associated with the data.

3
4 A short summary on the toxicokinetic similarities and differences among the three isomers should be
5 included early in the section to provide context to the subsequent effects summaries. In addition the
6 discussion regarding the neurologic effects should clearly acknowledge the varying limitations in the
7 available data set. The lack of chronic duration data, and therefore cumulative effects, for all three
8 isomers as well as the existence of only acute and short-term data for 1,3,5-TMB should be clearly
9 stated. The summaries for the respiratory, hematological and development effects already make these
10 distinctions.

11
12 Currently Section 1.2.1. of the TMB assessment does not contain a summary of mechanistic evidence. A
13 short summary of potential mechanisms or an acknowledgement that the mechanism of action is
14 unknown should be included within each effects summary discussion.

15
16 EPA defines hazard characterization as the description of the potential adverse health effects attributable
17 to a specific environmental agent, the mechanisms by which agents exert their toxic effects, and the
18 associated doses, route, duration, and timing of exposure. The summary and evaluation section of the
19 hazard identification section should include a conclusion paragraph(s) which states that the effects on
20 the nervous system, respiratory system, the hematological system, and developing fetus identified in the
21 hazard identification step will be the focus of the subsequent dose response evaluation as well as
22 describing the relative importance of the different health effects. The conclusion should also include
23 statements regarding the adequacy of the limited data set available for hazard identification. This
24 statement should address the question: based on the sensitivity of endpoints assessed and effects
25 observed with other similar compounds but not assessed for TMBs, what is the confidence that the
26 hazards, i.e., sensitive health endpoints, have been adequately identified?

27 ***Carcinogenicity***

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

32
33 As noted in the detailed response to charge question on carcinogenicity, 1,2,4-TMB has been assessed in
34 only one study (See section 3.2.11). The EPA found that there were a number of deficiencies
35 concerning this bioassay and the SAB agrees with the agency's finding. The EPA also noted that no
36 carcinogenicity bioassays have been conducted with 1,2,3-trimethylbenzene or 1,3,5-trimethylbenzene.
37 As such, the SAB concludes that EPA's hazard assessment of the carcinogenicity of trimethylbenzenes
38 did integrate all available scientific evidence and agrees with the EPA that there is "inadequate
39 information to assess the carcinogenic potential" of trimethylbenzenes.
40

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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 (Jarnberg 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 were similar to those for blood. Marked over prediction of high-dose data necessitated omission of the highest dose for benchmark dose (BMD) modeling. 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 & 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 Zahlsten 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

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1 domain. This is necessitated by the fact that the 100-ppm dose is in the middle of the rat dose-response
2 range used for benchmark dose modeling. Over predicting rat dosimetry in this range thus has the
3 potential to influence the results of dose-response modeling and extrapolation of potency to humans.
4

5 The EPA should attempt to provide better model predictions to the rats' venous blood data. This may
6 require recalibration of some type, such as the addition of a first-order metabolic pathway consistent
7 with the PBPK model of Jarnberg and Johanson (1999), or changing hepatic blood flow to 25% instead
8 of 17% of cardiac output, which is a more common physiologic parameter value.
9

10 Alternatively, the EPA could conduct BMD modeling of the Korsak and Rydzynski (1999) data using
11 air TMB concentration as the dose metric to derive the point of departure. Subsequently, the PBPK
12 model would be used to convert the POD to the weekly average blood concentration. This alternative
13 approach yields a BMDL of 84 mg/m³ (17ppm), which would be predicted by the PBPK model to yield
14 a blood concentration of 0.087mg/L in rats. The result is identical to the values derived by EPA,
15 suggesting the approach of dropping the high-dose group used by EPA is fine. EPA can use this
16 alternative approach to support their BMD modeling approach.
17

18 The SAB conducted a quality control quality assurance review and confirmed the model simulations
19 presented in Appendix B of the IRIS document draft. No fundamental flaws or issues were found. The
20 independent review is provided in Appendix B.
21

22 Despite the foregoing, the SAB finds that the model modified by the EPA is adequate for predicting the
23 weekly average blood level of TMB in humans, the dose metric advocated by EPA.
24

25 The SAB found that the PBPK modeling by EPA was appropriate and quite useful in simulating the
26 kinetics of TMB in the blood of humans repeatedly inhaling vapor levels within anticipated ranges of
27 environmental exposures. The SAB notes that given the available data the weekly average blood TMB
28 (i.e., parent compound) level was the most appropriate dose metric, given the lack of certainty about
29 TMB's mode of neurotoxic action and the identity of the toxic moiety.
30

31 The SAB notes that it was necessary for EPA to make a number of modifications of model input
32 parameters (e.g., reduction of liver blood flow, refinement of V_{max}, and increase in total cardiac output).
33 This numerical optimization resulted in better fit of simulated and empirical TMB kinetic data. By
34 modifying the model of Hissink et al. (2007), EPA has created a model different enough that it should be
35 reviewed. The EPA might also consider publishing the modified PBPK model, possibly adding the
36 route-to-route extrapolation. Submission for publication assures an additional review and "quality
37 certification" upon acceptance, however the panel recognizes this is not necessary for use of the model
38 in the IRIS document.
39

40 EPA's assumptions, in modifying the Hissink et al. (2007) model to predict the kinetics of inhaled TMB
41 for repeated exposure scenarios, were reasonable and appropriate. The major caveats, however, were not
42 identified up-front on page B-20 (e.g. the original model and its parameters were for TMB and white
43 spirit, lack of parameters for the oral route, lack of parameters for pregnancy). The SAB recommends
44 that EPA expand the explanation and justification for the modifications of model parameters.

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1 Specifically, the discussion of the input parameters (e.g., human tissue:blood partition coefficients
2 (PCs), cardiac output, liver blood flow) should be justified. Additionally the use of scaled-up rat V_{max}
3 values, when human values were available, requires further explanation. Metabolic constants could be
4 questioned, as they summarily reflect the rate of TMB metabolism during mixed exposures to white
5 spirits, rather than exposure to TMB alone. The use of a liver blood flow of 17.5% of cardiac output
6 should be justified, as it differs substantially from the traditional value of 25%. The EPA did not attempt
7 any re-estimation or adjustment of parameters for chronic exposure (e.g., enzyme induction, dose-
8 dependency, growth dilution, etc.). Results of sensitivity analyses can be used to respond to related
9 concerns. It was noted that human tissue: blood PCs used in modeling were twice those for rats.
10 Muhlenberg and Vijverberg (2000) estimated human brain:blood, fat:blood and kidney:blood PCs that
11 were higher for rats than for humans. It was suggested that 1st-order and saturable metabolism be
12 incorporated into the model, and the model run to explore the impact of the change.

13
14 The SAB did not find a specific discussion of the uncertainties in the model's structure. Therefore, these
15 uncertainties were not adequately characterized or discussed. While these uncertainties may be
16 implicitly included in the uncertainties discussion, they are not specifically discussed in reference to the
17 PBPK model.

18
19 The SAB notes that the PBPK model of Jarnberg and Johanson (1999) is a human PBPK model. The
20 model is for TMB alone, and thus avoids the complications and uncertainties of: (1) concurrent exposure
21 to other components in white spirit; and (2) species-to-species extrapolations. Empirical human kinetic
22 data are available from the same laboratory for model parameterization and validation. Human
23 neurobehavioral data are also available in the literature from other research groups. The results of these
24 studies identify human NOAELs/LOAELs for acute irritation and central nervous system (CNS) effects
25 by TMB and white spirit. It is EPA policy to consider utilization of human data and validated human
26 models when they are available. The SAB recommends that EPA evaluate the Jarnberg and Johanson
27 model and at a minimum discuss the model selection in future drafts of the assessment.

28
29 The SAB provides these recommendations:

- 30 • In light of the poor fits of the model outputs to the toxicokinetic profiles of TMB in rats, the rat
31 model should be adjusted and the outcomes assessed. Suggested modifications include:
 - 32 ○ Recalibrate by potentially adding a first-order metabolic pathway, consistent with the
33 PBPK model of Jarnberg and Johanson (1997), by adjusting hepatic blood flow, and/or
34 by other scientifically-defensible and reasonable means to improve fit.
 - 35 ○ Conduct BMD modeling of the Korsak and Ryzdzynski (1999) data using air TMB
36 concentration as the dose metric to derive the POD. Subsequently use the PBPK model to
37 convert the POD to the weekly average blood TMB concentration.
- 38 • Reconsider the accuracy and assess the influence of modification of certain model input
39 parameters, including: K_m , V_{max} , work load, tissue:blood PCs and liver blood flow. It may be
40 worthwhile reestimating selected parameters that change with growth/long-term exposure (e.g.,
41 growth dilution, enzyme induction, and dose-dependency).
- 42
- 43
- 44

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- 1 • Discuss the uncertainties inherent in the recalibrated rat and human models, and consider
2 whether an independent peer review of the revised PBPK model would be helpful in view of the
3 extent of the revisions and its intended use in the support of IRIS risk assessments. Also consider
4 publication of the revised model and its extended applications.
5
- 6 • Consider exploring the utility and relative merits of the revised Hissink et al. (2007) model
7 versus the human model of Jarnberg and Johanson (1999), if the code for the latter model can be
8 obtained.
9

10 *Charge Question: The internal dose metric selected for use in the derivation of the RfC and RfD for*
11 *1,2,4-TMB was the steady-state weekly average venous blood concentration (mg/L) of 1,2,4-TMB for*
12 *rats exposed for 6 h/day, 5 days/week. Please comment on whether the selection of this dose metric is*
13 *scientifically supported and clearly described. If a different dose metric is recommended for deriving the*
14 *RfC, please identify this metric and provide scientific support for this choice. Are the uncertainties in the*
15 *selected dose metric adequately characterized and discussed?*

16 The use of any dose metric should be guided by the mode of action of the chemical being examined.
17 However, for the TMBs, there is a paucity of information on their mode of action, and the agency has
18 inferred the mode of action to be similar to that for chemicals such as toluene. Given that the critical
19 effects upon which the RfC is being determined are neurological and, therefore, are extrapulmonary
20 effects due to inhalation of the TMBs, the selection of the internal dose metric comes down to either the
21 maximum venous concentration or the steady-state weekly average venous blood concentration. While
22 there are acute effects of 1,2,4-TMB that might bring into play the maximum blood concentration, there
23 were also effects with 90 days of exposure. However, given the uncertainties in the mode of action
24 (MOA), the SAB finds that the selection of the internal dose metric of the venous blood concentration
25 averaged over a week of exposure is reasonable.

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

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

40 Using the PBPK model-estimated internal dose metrics as the dose inputs for BMD modeling required
41 the agency to drop the high dose exposures from all modeling efforts because the venous blood dose
42 metrics consistently over-predicted experimental results for high exposures. This overestimation may

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1 be due in part to the agency using minute ventilation as the driver function for internal dose rather than
2 decomposing minute ventilation into its two components, namely tidal volume and breathing
3 frequency. While the exposure level is high and that may lead to a 50 % reduction in respiratory rate,
4 respiratory irritants such as the TMBs cause subtle shifts in the breathing pattern while maintaining the
5 same overall minute ventilation. Shallower breathing leads to a shift upward in the respiratory tract for
6 the site of deposition. In addition, the PBPK modeling for humans did not take into account the periods
7 of exercise the subjects underwent, which may explain the model's greater deviations from
8 experimental results at high exposure levels. While the high doses would not need to be dropped if the
9 agency added an exponential rising model to their suite of models to be fit, the SAB notes that external
10 air can be used as the dose metric and then the PBPK model used to back calculate the appropriate
11 venous blood levels, arriving at the same result that the agency obtained. If the SAB's suggestions for
12 improvements in the PBPK model do not lead to a better agreement with the high dose exposures, the
13 agency would be well advised to include the external air dose metric and corresponding venous blood
14 back calculations.

15
16 While uncertainties concerning model parameters, potential for kinetic changes with repeated exposures,
17 and model estimates of internal dose are discussed, the uncertainties in the selected dose metric (weekly
18 average venous blood concentration) are not adequately characterized or discussed. On page 2-7, the
19 document simply states "Weekly average venous blood 1,2,4-TMB concentration was chosen as the
20 internal dose metric on which to base the POD as it is assumed that the parent compound is the toxic
21 moiety of interest and that average blood concentration of 1,2,4-TMB is assumed to adequately
22 represent the target tissue dose across the multiple tissues of interest". At a minimum, the agency could
23 add that in the absence of knowing the mode of action, use of the area under the curve is not uncommon
24 to utilize, which leads to supporting the average venous blood concentration as a viable dose metric. The
25 document cites the effects of toluene in the brain as an example of a chemically-induced toxic endpoint
26 for which the venous blood concentration is a relevant dose metric.

27 **3.2.5. Inhalation Reference Concentration (RfC) for 1,2,4-TMB**

28 *Charge Question: A 90-day inhalation toxicity study of 1,2,4-TMB in male rats (Korsak and Rydzynski,*
29 *1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection*
30 *of this study is scientifically supported and clearly described. If a different study is recommended as the*
31 *basis for the RfC, please identify this study and provide scientific support for this choice.*

32
33 The SAB generally agrees with the choice of the Korsak and Rydzynski (1996) study as the basis for
34 derivation of the RfC for 1,2,4-TMB. The study utilized a 90-day exposure period and, thus, the longest
35 duration exposure study available in the literature; in addition, it included multiple exposure levels. It
36 was well-conducted and utilized adequate sample sizes of rats such that it was not underpowered. In
37 addition, it was based on widely-used behavioral assays. An examination of the study indicates these
38 behavioral studies were carefully carried out and data from control animals were consistent with
39 previously published observations.

40
41 Clarification of this choice, however, could be significantly improved in the document in several ways:
42

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- 1 • The rationale for the choice of Korsak and Rydzynski (1996) is not specifically described and the
2 reasons for its choice over other studies, e.g., the 4-week exposure studies, need to be more clearly
3 stated.
- 4 • As currently written, there is confusion over chronicity of exposure vs. effects. It would be helpful
5 to modify the terminology particularly related to outcome measures, perhaps as acute effects vs.
6 long-term effects/irreversible effects and retain the use of the word chronic/subchronic etc. to
7 descriptions of statements related specifically to exposure.
- 8 • Separate the write-up into sections that specifically elaborate on the acute effects and provide a
9 separate section related to effects observed post-exposure. Given the commonality of even the
10 trends in data across these studies, some mention of the biological significance in the absence of
11 statistical significance ($\alpha = 0.05$ as an arbitrarily chosen value) should be mentioned.
- 12 • The text, where applicable, could include additional qualifications as to “reversibility of effects” at
13 the 2-week post-exposure time-point as this assessment of reversible effects is based on statistical
14 rather than biological significance, with statistical reversal for one but not the other isomer, and a
15 minimal difference in values from the last time point during exposure.
- 16 • It was recommended that the EPA re-calculate the RfC as if the study were subchronic (i.e., UF
17 converts to 1 from 3) and report this value as well.
- 18 • Include more specific mention of the potential cumulative neurotoxicity that is suggested by the
19 repeated measurement finding of rotarod performance failures across the course of exposure.
- 20 • Include more specific descriptions of the similarity of the animal behavioral endpoints to what has
21 been observed in humans.

22
23 *Charge Question: Decreased pain sensitivity (measured as an increased latency to pawlick response*
24 *after a hotplate test) in male Wistar rats was concluded by EPA to be an adverse effect on the nervous*
25 *system and was selected as the critical effect for the derivation of the RfC. Please comment on whether*
26 *the selection and characterization of this critical effect is scientifically supported and clearly described.*
27 *If a different endpoint(s) is recommended as the critical effect(s) for deriving the RfC, please identify*
28 *this effect and provide scientific support for this choice.*
29

30 The SAB agrees that the reduction in pain sensitivity as indicated by an increased latency to pawlick
31 response in a hotplate test is a valid adverse nervous system effect and appropriately selected as a
32 critical effect for the derivation of the RfC. This effect was variously seen in response to short-term, 4-
33 week, and 90-day studies. The associated U-shaped dose-effect curves seen with these isomers,
34 moreover, are highly consistent with the effects of various other pharmacological agents (e.g., opioids)
35 on this response and likely reflective of the mechanisms by which these isomers act. This assay is
36 widely used in the behavioral pharmacology literature and particularly in the study of pain nociception
37 and opioid pharmacology.
38

39 The SAB agrees that the observation of prolonged latency in the hot plate test 24 hour post-footshock
40 delivery that was observed in studies by Gralewicz and colleagues (e.g., 1997, 2001) also constitutes an
41 adverse effect. The administration of footshock immediately after the hot plate test trial essentially
42 maximizes the capabilities of the nervous system and, thus, provides a type of nervous system probe that

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1 then unmask a prolonged latency to a hot plate stimulus 24 hours later. It shows that when the nervous
2 system is maximally stressed, it cannot respond/recover in a normal timeframe.

3
4 In addition to the recommendations above for the document related to the nervous system effects, this
5 section could also benefit from some additional description of the hotplate procedures, including the
6 rationale/approach for using the footshock intervention in the post-exposure behavioral assessments
7 carried out after the 4-week exposures.

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

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

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

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

27
28 The SAB recommends that EPA provide better justification for applying the "one standard deviation"
29 from the mean of the control group for the neurotoxicological endpoint. EPA should also provide better
30 explanation of the issues associated with the homogeneity of variance across dose groups in the Korsak
31 and Rydzynski (1999) study, its implications for BMD modeling, and how EPA addressed this in their
32 BMD modeling.

33
34 *Charge Question: Please comment on the rationale for the selection of the uncertainty factors (UFs)*
35 *applied to the POD for the derivation of the RfC for 1,2,4-TMB. Are the UFs appropriate based on the*
36 *recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference*
37 *Concentration Process (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are*
38 *proposed, please identify and provide scientific support for the proposed changes.*

39
40 Consistent with guidance provided in *A Review of the Reference Dose and Reference Concentration*
41 *Process* (U.S. EPA, 2002), five possible areas of variability and uncertainty were considered by the EPA
42 in deriving the proposed RfC for 1,2,4-TMB. This consideration is reflected in choices regarding five
43 specific uncertainty factors, namely:

- 44 1. UF_A – an interspecies uncertainty factor;

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2. UF_H – an intraspecies uncertainty factor;
3. UF_L – a LOAEL (lowest observed adverse effect level) to NOAEL (no observed adverse effect level) uncertainty factor;
4. UF_S – a subchronic to chronic uncertainty factor; and
5. UF_D – a database uncertainty factor. In responding to this charge question, the Panel evaluated the choice and rationale for each of these UFs, reaching the following conclusions.

UF_A. The SAB agreed 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 agreed with the UF_H of 10 and its rationale, although one TMB Review Panel member suggested that a UF_H of 3 would be appropriate. 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. There was discussion on the human susceptibility to general anesthetics only varies by a factor of about 2, and on this basis a UF_H of 3 could be selected 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 EPA choices for UF_L values, i.e., a UF_L of 1 for all endpoints except increased BAL cells, for which a UF_L of 10 was selected. However, the SAB recommends 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 BMR equal to one standard deviation change in the control mean for modeled endpoints was selected. Explanation of the reasoning for selection of one standard deviation (versus one-half standard deviation) should be added to the document 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 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

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

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

33 **3.2.6. Inhalation Reference Concentration (RfC) for 1,2,3-TMB**

34
35 *Charge Question: A 90-day inhalation toxicity study of 1,2,3-TMB in male rats (Korsak and Rydzynski,*
36 *1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection*
37 *of this study is scientifically supported and clearly described. If a different study is recommended as the*
38 *basis for the RfC, please identify this study and provide scientific support for this choice.*
39

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

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1
2 *Charge Question: Decreased pain sensitivity (measured as an increased latency to pawlick response*
3 *after a hotplate test) in male Wistar rats was concluded by EPA to be an adverse effect on the nervous*
4 *system and was selected as the critical effect for the derivation of the RfC. Please comment on whether*
5 *the selection and characterization of this critical effect is scientifically supported and clearly described.*
6 *If a different endpoint(s) is recommended as the critical effect(s) for deriving the RfC, please identify*
7 *this effect and provide scientific support for this choice.*

8
9 The SAB agrees that, as with 1,2,4-TMB, reduction in pain sensitivity as indicated by an increased
10 latency to pawlick response in a hotplate test was a valid adverse nervous system effect and
11 appropriately selected as a critical effect for RfC derivation. The SAB notes that the agency
12 appropriately uses the same rationale to derive the RfC for 1,2,4-TMB. A detailed response is in Section
13 3.2.5 and the SAB refers the reader to that section rather than reiterate the response to the Charge
14 question.

15
16 *Charge Question: In order to characterize the observed dose-response relationship comprehensively,*
17 *benchmark dose (BMD) modeling was used in conjunction with default dosimetric adjustments (U.S.*
18 *EPA, 1994b) for calculating the human equivalent concentration (HEC) to identify the point of*
19 *departure (POD) for derivation of the RfC. Please comment on whether this approach is scientifically*
20 *supported for the available data, and clearly described.*

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

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

26
27 The SAB response to this charge question deals with the same issues as charge question for 1,2,4-TMB
28 and did not identify any issues specific to 1,2,3-TMB and refers the reader the section 3.2.5.

29
30 *Charge Question: Please comment on the rationale for the selection of the uncertainty factors (UFs)*
31 *applied to the POD for the derivation of the RfC for 1,2,3-TMB. Are the UFs appropriate based on the*
32 *recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference*
33 *Concentration Process (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are*
34 *proposed, please identify and provide scientific support for the proposed changes.*

35
36 The SAB notes that the UF values selected by the EPA for 1,2,3-TMB are identical to those selected for
37 1,2,4-TMB, and that the justifications are essentially the same. Thus, the SAB response to this charge
38 question and recommendation are the same as the response to Charge Question for 1,2,4-TMB and
39 refers the reader to Section 3.2.5

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

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

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

The inhalation-based developmental toxicity study (Saillenfait et al. 2005) was considered as a potential study to define a critical effect for 1,3,5-TMB RfC derivation. The SAB is not aware of chronic or subchronic studies to support an RfC derivation for 1,3,5-TMB similar to the Korsak Rydzynski (1996) used for 1,2,4-TMB. The SAB notes that the endpoints and approach used by Saillenfait et al. differ from those Korsak and Rydzynski (1996) used to develop the RfC for the other two isomers and recommends that the agency conduct additional evaluation of the study before relying on the 1,2,4-TMB data to extrapolate the RfC.

The Saillenfait study was well-conducted and followed the appropriate European Union guidelines and experimental methods for that (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 notes 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 SAB notes that the EPA's evaluation of the Saillenfait et al. study and conclusion to not base the RfC derivation on the study was not sufficiently described (2005). The SAB recommends that the EPA revise and expand the discussion section to consider calculating fetal and maternal endpoint-based candidate RfCs for a comparison to the neurotoxicological endpoint used for the other isomers. The SAB also notes that the Saillenfait study has two major limitations: (1) no neurotoxic endpoints were collected (decreased pain sensitivity had been determined by 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 too short (GD 6-15; only 10 days) to be considered useful for deriving a chronic RfC.

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1
2 Saillenfait et al. (2005) could be used to derive an inhalation subchronic candidate RfC based on
3 fetal/maternal effects. Those authors selected 100 ppm (492 mg/m³) for the maternal NOAEL for
4 mesitylene (1,3,5-TMB isomer) with 300 ppm (1476 mg/m³) as the maternal LOAEL based on
5 decreased maternal weight gain and food intake. The developmental NOAEL in the study was 300 ppm
6 (1476 mg/m³) and the developmental LOAEL was 600 ppm (2952 mg/m³) based on decreased mean
7 male fetal body weights. Using the above NOAELs as the PODs and the same combined UF (UF_A, UF_H,
8 UF_L, UF_D) that will be used for the proposed chronic RfC determination, a subchronic candidate RfC for
9 fetal and maternal effects could be determined.

10
11 In the draft TMB Assessment, EPA set the maternal NOAEL at 300 ppm (1476 mg/m³) and the maternal
12 LOAEL at 600 ppm (2952 mg/m³) based on decreased corrected body weight gain, higher exposure
13 levels than Saillenfait et al. The SAB finds that this is not a correct interpretation of a maternal NOAEL
14 for the Saillenfait et al. paper. Decreased corrected body weight gain was measured only at one time
15 point (C-section) one day after cessation of exposure. Statistically significant decreased maternal
16 weights were observed at gestational days (GDs) 13-21 when the fetuses would be contributing far less
17 to the mother's weight and at GDs 6-21 (entire treatment period). Reduced maternal body weights
18 correspond exactly with the statistically significant decreased food consumption values recorded at GDs
19 6-13, 13-21 and 6-21 (entire treatment period). An evaluation of statistical methods used in the study
20 may also be appropriate

21
22 In Section 2.3.2 of the TMB Assessment [Methods of Analysis for 1,3,5-TMB (p. 2-36)] used a NOAEL
23 of 300 ppm (1476 mg/m³) was used as the POD for the developmental endpoint (decreased male fetal
24 body weight) that was not the NOAEL (POD = 2974 mg/m³) listed in Table 2-13 (p. 2-38). As 1476
25 mg/m³ should have been used for the POD, this will change the whole calculation for POD_{HEC} presented
26 in the table.

27
28 In addition to the above analysis and considerations, the SAB noted that there are minor errors in the
29 description of the 1,3,5-TMB inhalation data. In Section 2.3.1. Identification of Studies and Effects
30 Other Than Cancer for 1,3,5-TMB, there were errors in Table 2-12 that need to be addressed:

- 31 • The female fetal body weight average for the 100 ppm (492 mg/m³) group should be 5.47
32 ± 0.21 and not 5.74 ± 0.21 (it is correct in other tables of the document).
- 33 • The level of significance for decreased maternal body weight gain for the 600 ppm
34 (2,952 mg/m³) group should have two (**) and not one (*) asterisk to indicate p < 0.01.
- 35 • The table also states with a footnote (b) that numbers of live fetuses was not explicitly
36 reported. However, Saillenfait et al. did report them in Table 3 of their manuscript. The
37 total numbers of fetuses were 297, 314, 282, 217, and 236, for the control and exposure
38 groups, respectively, and should be included in Tables 2-2 and 2-12 of the draft TMB
39 Review document.

40
41 *Charge Question: Please comment on whether EPA's approach to developing the RfC for 1,3,5-TMB is*
42 *scientifically supported for the available data and clearly described.*
43

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1 The SAB recognizes that the agency's general approach to developing the RfC using isomers is
2 scientifically appropriate. However, the description of the agency rationale and discussion of the
3 Saillenfait et al (2005) did not address several concerns. Therefore, the SAB recommends that the
4 agency reconsider and strengthen the justification for using this approach for 1,3,5-TMB. The SAB
5 notes that in addition to points previously identified, there are differences in physical and toxicological
6 parameters (i.e., Henry's Law constant and toxicokinetics) for 1,3,5-TMB as compared with the other
7 isomers and provide sufficient uncertainty to set an RfC for this isomer based on 1,2,4-TMB and/or
8 1,2,3-TMB. These difference create an additional concern that the RfC was not based on the available
9 isomer specific study. An alternative approach to developing the RfC could be based on using the
10 fetal/maternal data. The SAB notes that approach may not adequately address neurotoxicity endpoint
11 used for the other isomers, but the TMB report should at a minimum conduct a comparative analysis and
12 improve the justification for using the extrapolation from the other isomers.

13
14 **[Note to Authors: The discussion for using the oral toxicological data for the 1,3,5-TMB is
15 inconsistent between sections 3.2.8, 3.2.9, and 3.2.10. Please consider this in your review.]**
16

17 **3.2.8. Oral Reference Dose for 1,2,4-TMB**

18
19 *Charge Question: The oral database for 1,2,4-TMB was considered inadequate for derivation of an*
20 *RfD. However, available evidence demonstrates similar qualitative profiles of metabolism and patterns*
21 *of parent compound distribution across exposure routes (i.e., oral and inhalation). Furthermore, there is*
22 *no evidence that would suggest the toxicity profiles would differ to a substantial degree between oral*
23 *and inhalation exposures. Therefore, route-to-route extrapolation, from inhalation to oral, using the*
24 *modified Hissink et al. (2007) PBPK model was used to derive a chronic oral RfD for 1,2,4-TMB. In*
25 *order to perform the route-to-route extrapolation, an oral component was added to the model, assuming*
26 *a constant infusion rate into the liver. Specifically, in the absence of isomer-specific information, an*
27 *assumption was made that 100% of the ingested 1,2,4-TMB would be absorbed by constant infusion of*
28 *the oral dose into the liver compartment. The contribution of first-pass metabolism was also evaluated.*
29

30 *Please comment on whether EPA's conclusion that the oral database for 1,2,4-TMB is inadequate for*
31 *derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral*
32 *data are available to support the derivation of an RfD for 1,2,4-TMB. If so, please identify these data.*
33

34 The SAB agrees that the primary toxicological endpoints for 1,2,4-TMB (neurotoxicity, hematotoxicity)
35 can be extrapolated across dose routes from the inhalation data with the assistance of PBPK modeling.
36 There is ample precedent within the IRIS system for this approach to derive a reference value for a
37 chemical with missing data by a particular dose route.
38

39
40 The SAB is not aware of adequate repeat dose studies for 1,2,4-TMB via the oral dose route. The
41 available acute exposure studies offer limited support in developing an RfD. The SAB recognizes that
42 this represents a data gap. One potential way to fill this data gap is to use oral data for a closely related
43 TMB. The Koch Industries (1995) oral gavage studies for 1,3,5-TMB are of potential utility in this

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1 regard as this was a repeat dose subchronic gavage study with suitable numbers of animals and adequate
2 reporting of results. However, there were limitations in the Koch Industries study (primarily that it
3 didn't involve neurotoxicity endpoints) and the study does involve an extrapolation across congeners.
4 Presented with those limitations, the Koch Study does not provide a superior alternative to the PBPK
5 approach EPA implemented. However, to the extent that the Koch Industries studies are used to derive
6 RfDs for other endpoints for 1,3,5-TMB (e.g., hepatic, renal), these RfDs could be considered at a
7 minimum for comparative purposes for 1,2,4-TMB as well.

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

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

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

33
34 The SAB agrees with the UFs selected in the development of the oral RfD for 1,2,4-TMB, but
35 recommends that the discussion of uncertainty be strengthened with respect to bioavailability
36 assumptions. As discussed in the previous response, the oral RfD for 1,2,4-TMB was derived by
37 incorporating an oral intake component into the PBPK model for 1,2,4-TMB to obtain a human
38 equivalent oral dose POD. The EPA used the same UFs for the oral RfD as were used in the
39 development of the inhalation RfC. Given that the oral RfD was based upon the same endpoint and
40 derived from the same study as the RfC, the SAB agrees that it is logical to use the same UFs. Thus, the
41 comments and recommendations regarding UFs are applicable to this charge question as well (see
42 Section 3.2.5). There was discussion regarding whether there is additional uncertainty associated with
43 incorporation of the oral intake component in the PBPK model, and specifically regarding assumptions
44 made with that component regarding oral absorption of 1,2,4-TMB and first-pass metabolism. Unlike

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1 modeling of internal concentrations from inhalation exposure that can be verified with existing
2 experimental data, there are no data with which to assess model predictions of internal doses following
3 oral 1,2,4-TMB exposures. The SAB does not consider this additional uncertainty sufficient to increase
4 the composite UF for the oral RfD, largely because the nature of the uncertainty (possible lower
5 absorption by the oral route), would add extra health protection. The SAB recommends that the potential
6 uncertainties associated with oral bioavailability of 1,2,4-TMB be discussed more clearly in the
7 document.

8
9 **[Note to Authors: The discussion of the oral toxicological data for the 1,3,5-TMB is inconsistent**
10 **between sections 3.2.8, 3.2.9, and 3.2.10. Please consider this in your review.]**
11

12 **3.2.9. Oral Reference Dose (RfD) for 1,2,3-TMB.**

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

17
18 *Please comment on whether EPA's conclusion that the oral database for 1,2,3-TMB is inadequate for*
19 *derivation of an RfD is scientifically supported and clearly described. Please comment on whether oral*
20 *data are available to support the derivation of an RfD for 1,2,3-TMB. If so, please identify these data.*
21

22 The SAB is not aware of adequate repeat dose studies for 1,2,3-TMB via the oral dose route. The
23 available acute exposure studies offer limited support in developing an RfD. There are two subchronic
24 gavage toxicology study of 1, 3, 5-TMB (Koch Industries, 1995; Adenuga et al., 2014). This GLP study
25 was reviewed by the three senior scientists under a contract between Versar, Inc. and the EPA. The
26 conduct and reporting of the 90-day dosing study were judged by the peer reviewers to be consistent
27 with GLP requirements. While the standard 90-day protocol was followed and neurological endpoints
28 were not assessed in either study. Manifestations of CNS depression following inhalation exposures
29 have been seen in rats and humans. EPA chose not to use the Koch et al. (1995) study for derivation of a
30 RfD, because it did not assess the potential for neurological effects. EPA should consider deriving
31 RfD(s) for endpoints developed in the Koch et al. (1995) and Adenuga et al. (2014) study, such as liver
32 and kidney weight changes, which were not seen in inhalation studies. This would be consistent with
33 EPA's desire to derive RfDs for multiple endpoints. Such orally-based RfDs for 1, 3, 5-TMB could then
34 be considered for extrapolation to 1, 2, 3-TMB. Upon doing so, EPA can consider the appropriateness of
35 applying a database uncertainty factor to the oral point of departure to compensate for the data gap of
36 not having an oral neurotoxicity endpoint in the current approach. This option is commonly utilized for
37 derivation of RfDs in these situations. By comparing the RfDs generated from the oral studies and from
38 the extrapolation from the RfC through using route-to-route extrapolation, EPA can provide a clear
39 explanation for why the use of the PBPK route-to-route based RfD for 1, 2, 4-TMB may be preferable to
40 application of a database uncertainty factor to an orally-based point of departure.
41

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

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1
2 The SAB is not aware of oral exposure data or PBPK model that are available for the 1,2,3-isomer. The
3 derivation of an RfD value is scientifically supported and clearly described. Because of uncertainty in
4 the extent of first-pass metabolism and oral bioavailability, the effective dose reaching target tissues
5 might be less than estimated. Hence, no changes in the UF_D or composite UF are recommended.
6 Reference values and composite UF values should be the same across isomers.
7

8 **Note to Authors: The discussion of the oral toxicological data for the 1,3,5-TMB is inconsistent**
9 **between sections 3.2.8, 3.2.9, and 3.2.10. Please consider this in your review.]**
10

11 **3.2.10. Oral Reference Dose (RfD) for 1,3,5-TMB.**

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

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

18 *Please comment on whether oral data are available to support the derivation of an RfD for 1,3,5-TMB.*
19 *If so, please identify these data.*
20

21 The Koch et al study (1995) was the only isomer-specific and route-specific study available in the peer-
22 reviewed literature for oral exposure to 1,3,5-TMB when the TMB Assessment was drafted. The SAB
23 finds that the concerns expressed by the EPA do not rise to a sufficient level to reject this study from
24 consideration. Therefore, the Koch study should be carried through the process for comparative
25 purposes, and the results reported (i.e., POD or RfD). The EPA should make use of the Koch Industries
26 study and a recently published study by Adenuga et al. (2014) for comparative purposes.

27 These subchronic gavage toxicology studies of 1, 3, 5-TMB (Koch Industries, 1995; Adenuga et al.,
28 2014) conduct and report of the 90-day dosing were found to be consistent with good laboratory
29 practices and requirements. The Koch study was submitted for an EPA Office of Water test rule was
30 reviewed by three senior scientists under a contract letter review (Versar 2013). Manifestations of CNS
31 depression following inhalation exposures have been seen in rats and humans. EPA chose not to use the
32 Koch 1995 (see the TMB report appendix F) study for derivation of the RfD, because it did not assess
33 the potential for neurological effects and “presented limited toxicological information” other than that
34 EPA considered in the TMB assessment.
35

36 EPA should consider deriving RfD(s) for endpoints in the Koch et al (1995) and Adenuga et al. (2014),
37 such as liver and kidney weight changes, which were not seen in inhalation studies. This would be
38 consistent with EPA's desire to derive RfDs for multiple endpoints. Such orally-based RfDs for 1, 3, 5-
39 TMB could then be considered for comparison and possibly extrapolation to the other isomers. Upon
40 doing so, EPA can consider the appropriateness of applying a database uncertainty factor to the oral
41 point of departure to compensate for the data gap of not having an oral neurotoxicity endpoint. This
42 option is commonly utilized for derivation of RfDs in these situations. By comparing the RfDs generated

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1 from the Koch et al (1995) and Adenuga et al. (2014) and from the RfC using route-to-route
2 extrapolation, EPA can then provide a clear explanation for why the use of the PBPK route-to-route
3 based RfD for 1, 2, 4-TMB may be preferable to application of a database uncertainty factor to an
4 orally-based point of departure.
5

6 **3.2.11. Carcinogenicity of 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB**
7

8 *Charge Question: The draft Toxicological Review of Trimethylbenzenes did not conduct a quantitative*
9 *cancer assessment for any isomer due to the lack of available studies. Please comment on whether data*
10 *are available to support the derivation of a quantitative cancer risk estimate.*
11

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

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

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

31 The SAB is not aware of any human studies on carcinogenicity of trimethylbenzenes, and notes that a
32 number of biomarker studies and their association with cancer of various sites have been published.
33 These biomarker studies should be reviewed and included. Some examples are:
34

- 35 • Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of
36 potential urinary cancer biomarkers--a powerful strategy for breast cancer diagnosis. (Silva et al
37 2012)
- 38 • Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-
39 phase microextraction in combination with gas chromatography-mass spectrometry. (Silva et al.
40 2011)

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- 1 • Cellular responses after exposure of lung cell cultures to secondary organic aerosol particles.
2 (Gaschen et al. 2010)

3
4 Based upon the deficiencies of the Maltoni et al. (1997) study, the lack of bioassays with 1,2,3-
5 trimethylbenzene and 1,3,5-trimethylbenzene, and the lack of human studies, the SAB agrees that the
6 EPA could not conduct a quantitative cancer assessment for any isomer due to the lack of appropriate
7 studies.
8

9 **3.3. Additional Recommendations**

10 The SAB identified two additional topics not addressed directly in the Charge that warrant additional
11 consideration by the agency: (1) an expanded discussion of sensitive life stages and vulnerable
12 populations, and (2) deriving the subchronic RfC and RfD for the TMB isomers.
13

14 **3.3.1. Susceptible Populations and Lifestages**

15
16 The draft *TMB Assessment* provides one paragraph on this subject, spanning pages 1-54 to 1-55. It
17 correctly identifies various types of immaturity (metabolism, renal clearance) as potentially leading to
18 greater vulnerability in early life. However, this section could provide a better outline of the kinds of
19 information needed to understand the potential vulnerabilities in early life, including key aspects of
20 TMB mode of action and key developmental features.
21

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

- 23 • whether it is the parent compound or metabolites (or both) that contribute to toxic effect;
- 24 • which metabolic systems are responsible for removing parent compound and creating important
25 metabolites; and
- 26 • what is the role of distributional phenomena (e.g., uptake into brain; partitioning into fat) and
27 other clearance mechanisms in determining chemical fate and access to target sites.
28

29 Based upon the mode of action information available, the developmental factors which may influence
30 toxicokinetics can be discussed. For example, with TMBs the draft document assumes that the parent
31 compound is responsible for toxicity with modeling assuming that a saturable Phase I oxidative
32 Cytochrome P450 (CYP) process is responsible for decreasing parent compound levels in venous blood.
33 This section should state whether it is known which CYP(s) are responsible for TMB saturable
34 metabolism as different CYPs have different developmental patterns. Also it may be possible to state
35 whether it is likely that altered percent body fat in early life, slower renal clearance and immature blood
36 brain barrier would have any implications for a fat seeking neurotoxic compound such as TMB. Analogy
37 may be drawn with other alkylbenzenes which do have toxicokinetic modeling data in early life such as
38 toluene. Toluene has already been referred to in the mode of action section of the document; it is also
39 neurotoxic and its mode of action is the parent compound with the level getting to the brain determined
40 by saturable CYP metabolism. If the EPA determines these parallels to provide a useful analogy, then

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1 early life modeling papers for toluene by Pelekis et al. (2001) and Nong et al. (2006) may be useful for
2 describing the degree of toxicokinetic uncertainty presented by early life stage exposure to TMBs.

3
4 Regarding toxicodynamic vulnerability, perhaps the most relevant data would be developmental
5 neurotoxicity information for the TMBs themselves. This has been acknowledged as a data gap by EPA
6 but a Hungarian study (Lehotsky et al. 1985) did test a C-9 mixture containing trimethylbenzenes
7 (Aromatol) for developmental neurotoxicity in rats. That study had minimal reporting of results, simply
8 stating that there were no effects of Aromatol on dams or offspring at any time point (Lehotsky et al.
9 1985). This is in spite of the fact that the high dose of Aromatol was 2000 mg/m³, a dose that one would
10 expect to have a neurotoxic effect in dams during and after exposure, based upon results of other testing.
11 The lack of any toxicity in dams or offspring combined with the lack of reporting of any data (including
12 Aromatol treatment group neurological testing or Aromatol composition) and the fact that it was a
13 mixture and not a specific TMB makes this study of minimal utility. Therefore, the developmental
14 neurotoxicity data gap remains.

15
16 Some discussion is warranted concerning what is known about early life vulnerability to aromatic
17 solvent neurotoxicity. Several studies are available suggesting a vulnerable window of brain
18 development in mice to the neurotoxic effects of toluene (Win-Shwe et al. 2010, 2012). The USEPA
19 should evaluate this evidence relative to other developmental neurotoxicity studies that may be available
20 for toluene and other related alkylbenzenes to determine whether this data gap represents a large
21 uncertainty.

22
23 This section should conclude with a statement as to the potential vulnerabilities which could be present
24 in early life and whether any specific data exist for TMBs that would show the extent of such variability.
25 Data gaps should be described both in terms of TMB MOA and early life information that limit EPA's
26 ability to develop a more quantitative assessment of vulnerability. Potential options for decreasing the
27 uncertainty created by these data gaps can be considered to include life stage specific PBPK modeling to
28 address toxicokinetic uncertainty as has been done for toluene. With respect to toxicodynamic
29 uncertainties, the EPA can consider the potential utility of early life developmental neurotoxicity data
30 for toluene as a way to understand the magnitude of uncertainty present for the TMBs in this area.

31 **3.3.2. Developing Subchronic RfCs and RfDs**

32
33 The SAB notes that the agency has chosen an approach to developing RfCs and RfDs for the TMBs that
34 also develops subchronic values. In addition to responding to the charge questions related to
35 development of chronic toxicity values for 1,2,4-, 1,2,3-, and 1,3,5-TMB, the TMB Review Panel
36 discussed using the analysis presented in the TMB Assessment to support development of subchronic
37 toxicity values (i.e., subchronic RfCs and oral RfDs) for these chemicals. The EPA and other
38 environmental regulatory agencies are frequently required to address the risks associated with exposures
39 lasting less than a lifetime. Because the toxic endpoint(s) of concern for a given chemical, as well as
40 threshold doses or concentrations for toxicity, can change with exposure duration, the toxicity value
41 used in risk assessment should be matched to the extent possible to the length of exposure associated
42 with the scenario of interest. Recognizing the need for toxicity values for less-than-lifetime exposures,

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1 the EPA Risk Assessment Forum recommended that the agency develop such values and incorporate
2 them into the IRIS database (U.S. EPA, 2002).

3
4 For the TMBs, the principal studies used to create the proposed RfCs and RfDs are all subchronic in
5 duration, and the analysis needed to support a robust set of subchronic toxicity values has in effect
6 already been done. The toxic endpoints and dose-response relationships are clearly relevant for
7 subchronic exposure, and the same PODs and the same UFs — except UFs_s, which are used to generate
8 a chronic toxicity value from subchronic study data — would apply to the development of a set of
9 subchronic RfCs and RfDs.

10
11 Given the potential usefulness of these toxicity values for risk assessment, the importance of having the
12 values available on IRIS, and the very small amount of additional work required to add them to the
13 TMB Assessment, the SAB recommends that the review be expanded to include the presentation of
14 subchronic RfCs and RfDs for 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB. These values should be
15 calculated using the same inputs as for the chronic toxicity values, but omitting the UFs_s. The SAB
16 anticipates that incorporation of these values will require minimal edits to existing tables and text.
17

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22 [586DE985257B65005D37E7/\\$File/TMBs_Draft_TR_IRIS_HeroPublic_Aug_16_2013.pdf](http://yosemite.epa.gov/sab/sabproduct.nsf/02ad90b136fc21ef85256eba00436459/EE1E280E77586DE985257B65005D37E7/$File/TMBs_Draft_TR_IRIS_HeroPublic_Aug_16_2013.pdf)
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33

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APPENDIX A

NCEA Charge to the Science Advisory Board for the
IRIS Toxicological Review of Trimethylbenzenes
August 2013 (Updated May 2014)¹

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

¹ The charge for TMBs was updated to include general charge question #4 requesting comment from the external peer review panel on the adequacy of EPA's assessment revisions and response to the public comments. The CAAC Augmented for the TMB Panel discussed and revised this charge question on the May 22, 2014 teleconference.

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

5 **Charge Questions**

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

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

30 **General Charge Questions:**

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

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

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

10
11 **Chemical-Specific Charge Questions**

12 **A. Executive Summary**

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

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

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

24 **C. Hazard Identification**

25 ***Synthesis of Evidence***

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

30 ***Summary and Evaluation***

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

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D. Toxicokinetics and Pharmacokinetic Modeling

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

16
17
18

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APPENDIX B

External Review of Timethylbenzene

PBPK Model Internal Metrics

June, 2014

Model Overview

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

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

Rat Internal Dose Metrics

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After implementing the modest model corrections, the Agency numerically optimized metabolic parameters (V_{max} and K_m) to fit the rodent data. The Agency chose the repeat dosing data of Swiercz et al. (2003) to calibrate the model and optimized parameters are shown in Table 1. The model fits to the data sets from Hissink et al., 2007 and Swiercz et al., 2003 are shown in Figures 1 and 2 and a comparison of predicted blood concentrations to study-specific end of exposure measures concentrations for these two studies are shown in Table 3.

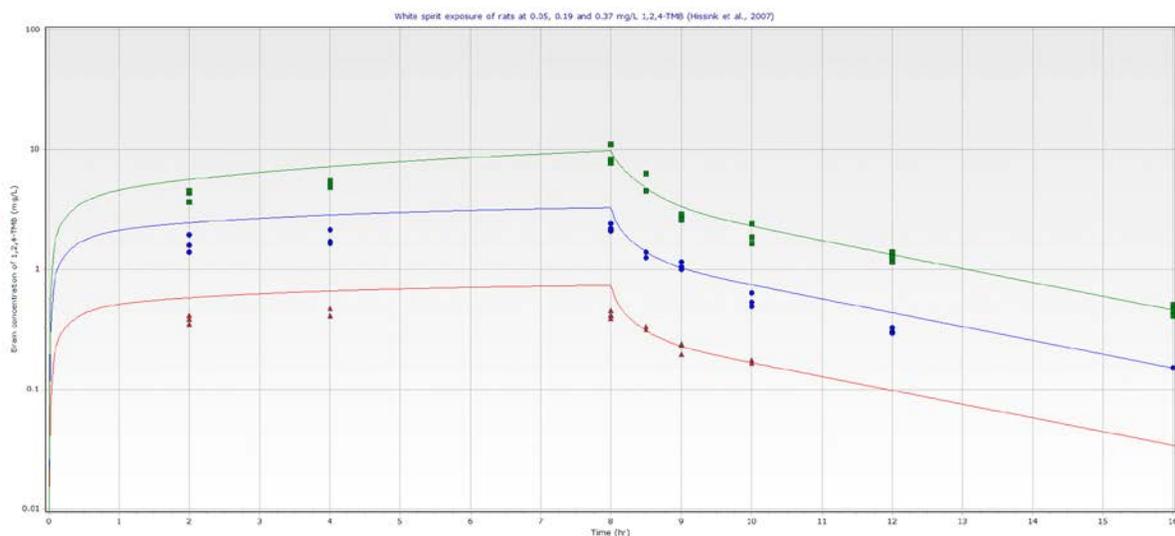
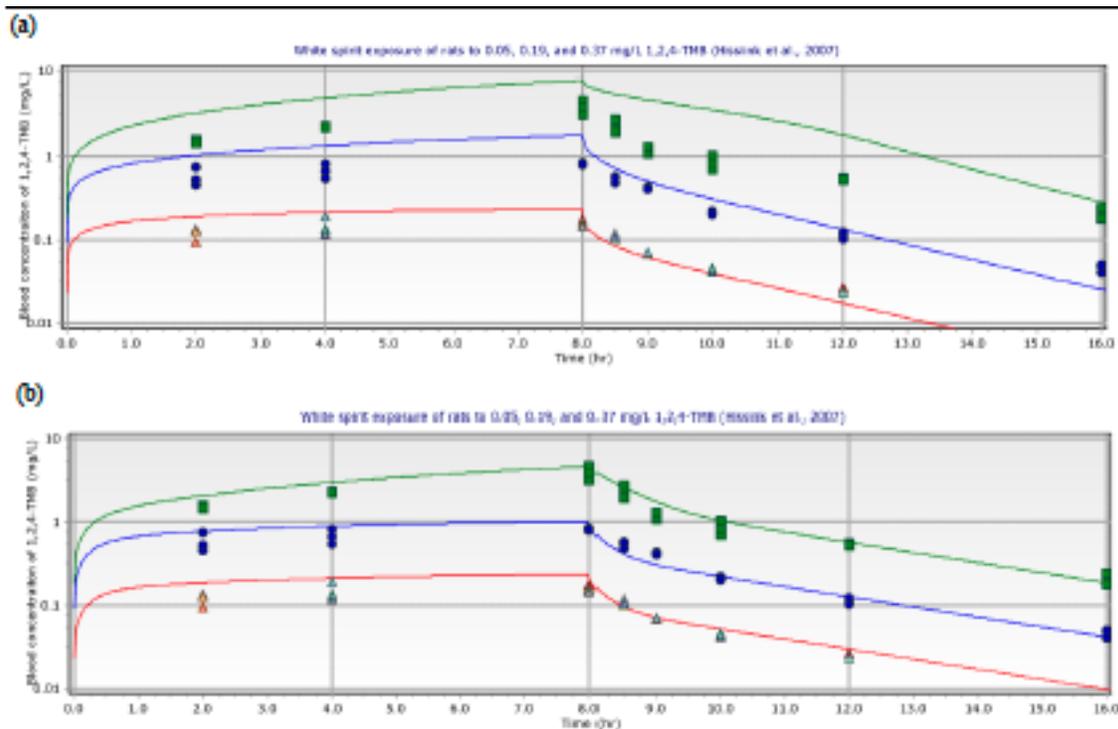


Figure 1. Model predicted blood concentrations for the study described in Hissink et al., 2007. Compare this figure to B-10(b) of U.S. EPA (2013). This figure represents the fit to the final model parameters and thus replicates Figure B-10(b).

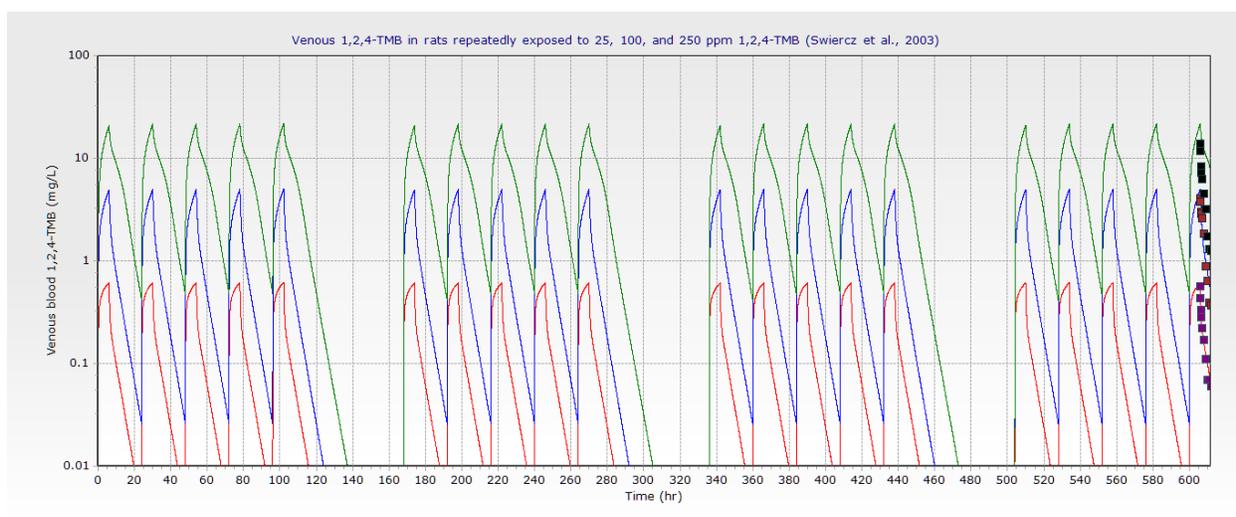
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Note: Rats exposed to 1,2,4-TMB in white spirit (WS) (Hissink et al., 2007) (a) before and (b) after numerical optimization. See Legend, Figures B-7 and B-8.

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



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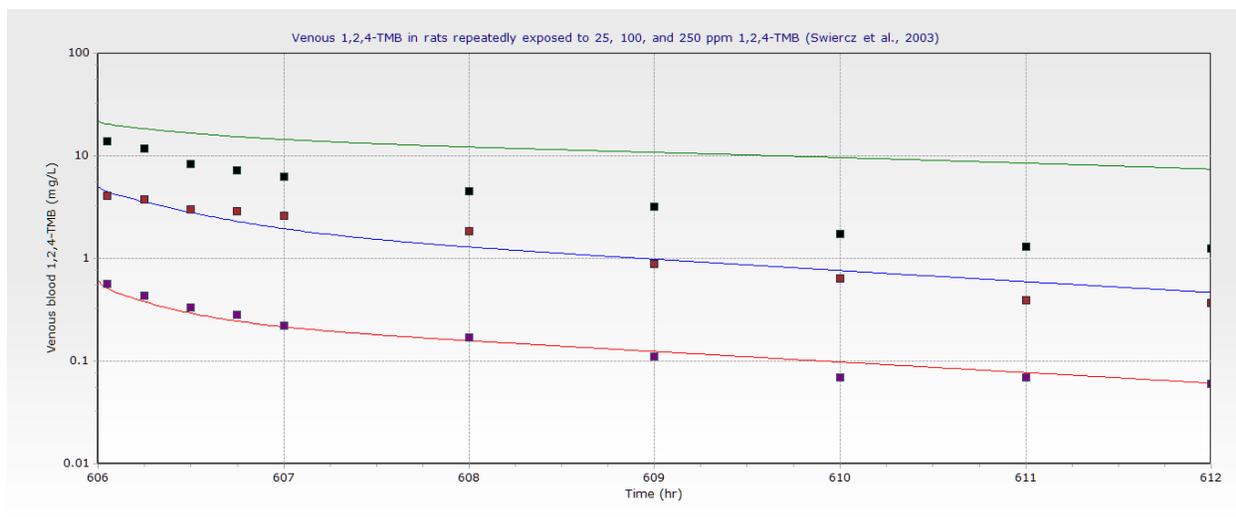
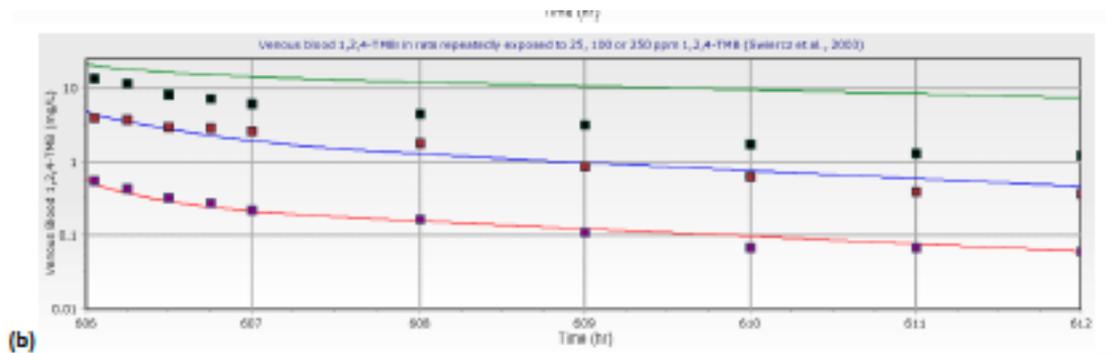


Figure 2. Model predicted blood concentrations for the study described in Swiercz et al., 2003. Rats were exposed to TMB 6 hr/day, 5 days/wk for 4 weeks. Blood was collected from the tail vein after the last exposure. Top) whole timecourse, Bottom) last 6 hr. Compare this figure to B-12 of U.S. EPA (2013).



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

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

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

Human Internal Dose Metrics

In the report (U.S. EPA 2013), the human exposure data of Hissink et al., 2007 was shown with the 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).

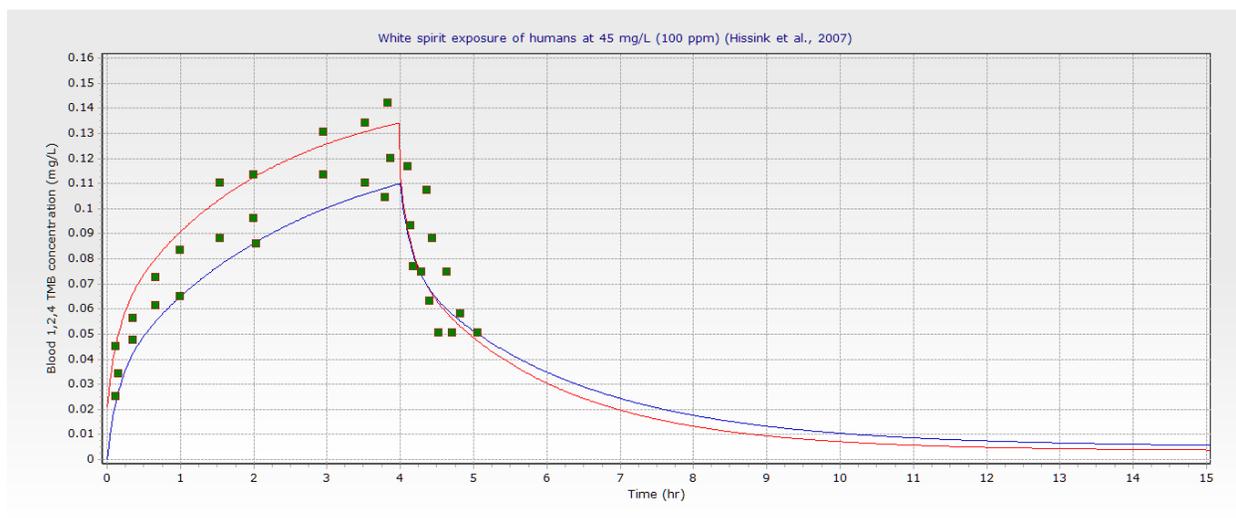


Figure 3. Comparisons of model predictions to measured human venous blood in human volunteers exposed to 100 ppm WS with 7.8% 1,2,4-TMB (39 mg/m³ 1,2,4-TMB). The red line shows the fit when the metabolic parameters optimized to fit rat data

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

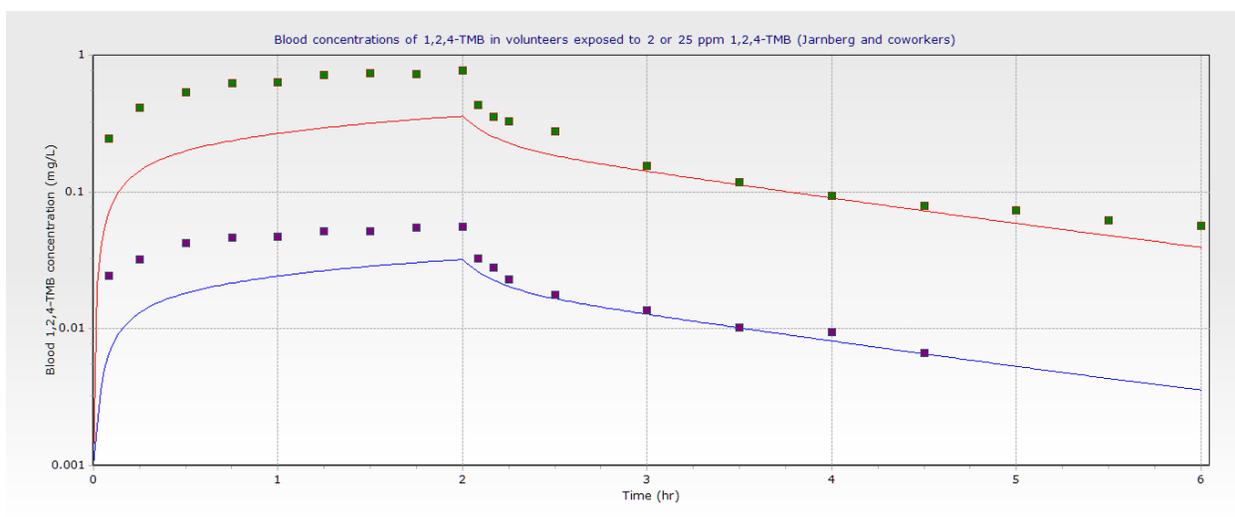
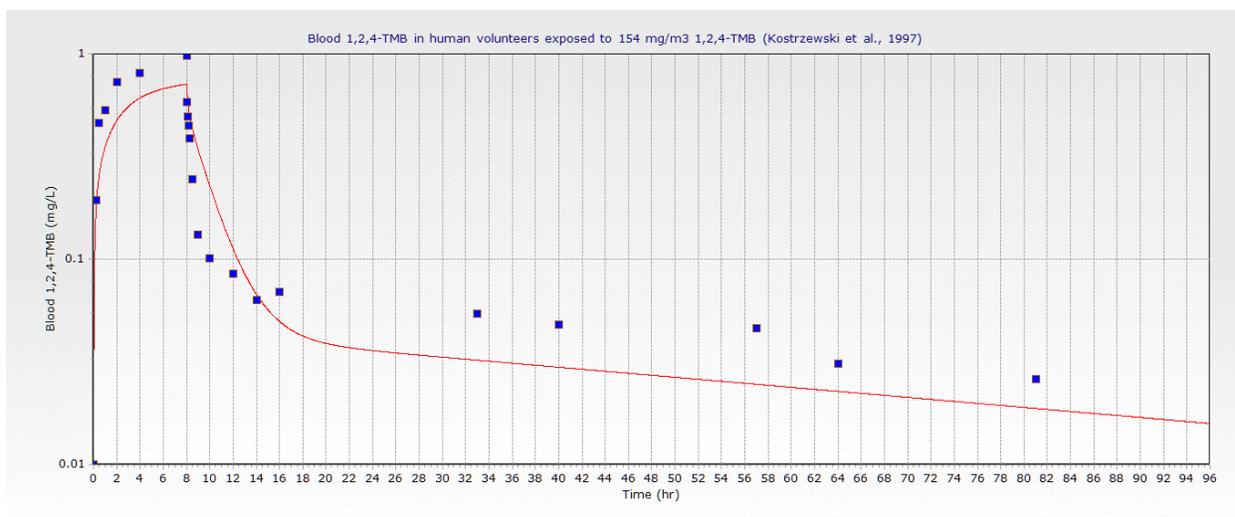


Figure 4. Comparisons of model predictions to measured human venous blood concentrations of Järnberg et al. (1998, 1997a; 1996) in volunteers exposed to 2 or 25 ppm (~10 or 123 mg/m³) 1,2,4-TMB for 2 hours while riding a bicycle (50 W).



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Figure 5. Comparisons of model predictions to measured human venous blood concentrations in Kostrzewki et al. (1997) in human volunteers exposed to 154 mg 1,2,4-TMB/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 overpredicts rat data. According to U.S. EPA 2013:

The measured Wistar rat arterial blood and tissue concentrations were consistently overpredicted by the model, suggesting collection delays in the studies. The model also consistently overpredicted the measured Sprague-Dawley rat tissue and blood concentrations, including the “recovery” (12 hr post-exposure) samples, which should not be subject to collection delays. Many of the “validation” comparisons were made at exposure concentrations (250 ppm [1,230 mg/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

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

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

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Suggested Conventions to Facilitate PBPK Model Review

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

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

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

References

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

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

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

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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)

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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. $QP = QPC \cdot BW^{0.74}$, $QC = QCC \cdot BW^{0.74}$. Since this is generally thought of as a “body surface area” correction, either is acceptable, the use of a different power is noted in footnote of table B-13. In addition, the EPA version of the model uses two different QPC values to correct for alveolar volume ($QC/QC2=0.7$).

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

§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, $QR = QRTOTC \cdot QC - QL - QBR$. Where QC is total cardiac output, QL and QBR are liver and brain

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flows, respectively. The EPA did this to correct mass-balance issues. Therefore, a direct comparison cannot be made to the values from Hissink et al.

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

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

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

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TABLE 2. COMPARISON OF HUMAN MODEL INPUT PARAMETERS

Parameter	(Hissink et al., 2007)	Transmitted 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/kg ^{0.7})	3.5	4.17	Scaled from rat Optimization

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Km (mg/L)	0.25	0.322	Scaled from rat Optimization
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* Within EPA version of model code, this is raised to the 0.74 power, not 0.7. $QP = QPC * BW^{0.74}$, $QC = QCC * BW^{0.74}$. Since this is generally thought of as a “body surface area” correction, either is acceptable, the use of a different power is noted in footnote of table B-13. In addition, the EPA version of the model uses two different QPC values to correct for alveolar volume ($QP/QP2=0.7$).

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

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

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

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

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

6 **TABLE 4. MODEL SIMULATED AND EXPERIMENTAL MEASURED CONCENTRATIONS OF 1,2,4-TMB IN**
7 **MALE SPRAGUE-DAWLEY RATS EXPOSED TO 1,2,4-TMB AT THE END OF 12 HOUR EXPOSURE**
8 **(ZAHLEN, 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

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1.5	14	48	3.5
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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

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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

9 Column 1 is the data taken from U.S. EPA, 2013 Table C-1 (Korsak et al., 2000a). Column 2
10 are the weekly average blood concentrations produced using average exposures and body
11 weights from that study in this assessment. Column 3 shows the same assessment using the

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1 rat parameters from Hissink et al, 2017 (Table 1). Column 4 shows the difference between the
2 Hissink and U.S. EPA, 2013 parameterization.

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5 *HUMAN INTERNAL METRIC COMPARISON AFTER CONTINUOUS INHALATION EXPOSURE: VENOUS TMB*
6 *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

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