

6/12/14 Preliminary Comments for review and deliberations by the CAAC Committee Augmented for the Review of the EPA's Draft IRIS Trimethylbenzenes Assessment. Do Not Cite or Quote. These preliminary comments are draft and a work in progress. They do not reflect consensus advice or recommendations, have not been reviewed or approved by the chartered SAB and do not represent EPA policy.

**Preliminary Comments from Members of the Chemical Assessment Advisory
Committee Augmented for the Review of the
EPA's Draft IRIS Trimethylbenzene Assessment
(June 17, 2012)**

Comments Received as of June 12, 2014

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Comments from Dr. Beland

General Charge Questions:

Question 1. NRC (2011) indicated that the introductory section of IRIS assessments needed to be expanded to describe more fully the methods of the assessment. NRC stated that they were "not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear, concise statements of criteria used to exclude, include, and advance studies for derivation of [toxicity values]." Please comment on whether the new Preamble provides a clear and concise description of the guidance and methods that EPA uses in developing IRIS assessments.

As explained in Appendix D, the EPA elected to include a Preamble that "describes the application of existing EPA guidance and the methods and criteria used in developing the assessment." The advantage of such an approach is that it can be applied across all IRIS reviews and any additions or changes to the process can be readily ascertained. The Preamble clearly delineates the scope of the IRIS program, how reviews are developed, and the multiple layers of review. The document also includes the program's approach to identifying and selecting pertinent epidemiologic and experimental animal studies, the methods used to evaluate the quality of the data from the studies, and the approach for evaluating the evidence for specific effects in both humans and experimental animals. The Preamble also describes the EPA's approach for selecting studies for deriving toxicity values, the methods used for deriving the toxicity values, and a description of the overall confidence in the values obtained.

I believe the Preamble appropriately address the concerns expressed in NCR (2011) and provides a concise and logical description of the assessment process.

Question 2. NRC (2011) provided comments on ways to improve the presentation of steps used to generate IRIS assessments and indicated key outcomes at each step, including systematic review of evidence, hazard identification, and dose-response assessment. Please comment on the new IRIS document structure and whether it will increase the ability for assessment to be more clear, concise and easy to follow.

The IRIS assessment of trimethylbenzenes begins with a description of the literature search strategy. The text is accompanied by a table of key search words and a flow chart that indicated the reasons for inclusion or exclusion of specific studies. The section on the search strategy is followed by a section on hazard identification that summarizes the key responses (neurological, respiratory, reproductive and developmental, hematological and clinical chemistry, and carcinogenicity). The text is accompanied by liberal use of summary tables and figures that assists the reader in understanding the strength and significance of the effects. The main document is accompanied by appendices that present the major findings, primarily through the use of figures and tables, of the cited literature. Each section on key responses (e.g., neurological) includes a summary of the strengths and weakness of the evidence. The section on hazard identification is followed by a section on dose-response analysis. The section begins with a rationale of the studies selected for the analysis and then the methods used for conducting the analyses. Again, liberal use is made of tables and figures, and the appendices give specific details for each of the analyses.

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I believe the current structure is a rational approach to address the criticisms of NRC (2011). The sections flow in a logical manner and the EPA has gone to great lengths to specify the strengths and weaknesses of the data used for the dose-response assessment.

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

This has been addressed to some extent already. The document is organized so the responses of the greatest magnitude and consistency are discussed first. The EPA clearly describes which studies are given the greatest weight and the reasons for their selection. A common format is used for the tables, which strengthens the presentation, and appendices provide data from the original studies (primarily in the form of tables and figures) to improve further the presentation.

Question 4. EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes and has revised the assessment to respond to the scientific issues raised in the comments. A summary of the public comments and EPA's responses are provided in Appendix F of the Supplemental Information to the Toxicological Review of Trimethylbenzenes. Has EPA adequately addressed the scientific issues?

In Appendix F the EPA considers the public comments, which in this instance were from a single source, the Hydrocarbon Solvents Panel of the American Chemistry Council (ACC). It is my opinion that the EPA carefully considered each of the comments made by the ACC and, where appropriate, modified the draft IRIS assessment to address the concerns of the ACC. In instances where the draft assessment was not modified, the EPA provided a clear explanation as to why the original text was maintained.

A. Executive Summary

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

The Executive Summary does summarize the major conclusions pertaining to hazard identification and the dose-response analysis. Nonetheless, I feel the level of detail contained within the summary detracts from its intended purpose. I recommend that the summary be truncated to emphasize the major conclusions. Specifically, I suggest that citations be removed from the summary unless they are absolutely essential. Much of Section 15 (Susceptible Populations and Lifestages) is speculative. While the concepts may be correct, I do not feel they are appropriate in an executive summary. This section could be truncated after the first sentence, which is a clear summary of what is known. The last paragraphs in Sections 3 and 5 are identical except for the compound being discussed. I wonder if these sections could somehow be combined to avoid redundancies. Other sections (e.g., the middle paragraph on page xxxvi) present far too much detail: this section is supposed to be an Executive Summary and interested readers can find the details in the main body of the text.

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B. Literature Search Strategy/Study Selection

Question 1. The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the Literature Search Strategy/Study Selection section. Please comment on 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 search strategy is clearly articulated. The databases are clearly defined, as are the search terms (Table LS-1). A flow chart is provided (Figure LS-1) to tabulate the studies that were included and excluded. One concern I have is the exclusion of studies that were "not available in English". This strikes me as unacceptable and leads to the question of how many of the 65 references that were "excluded based on manual review of papers/abstracts" were eliminated because they were not written in English.

I am not aware of 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-, 1,2,4-, or 1,3,5-trimethylbenzene.

C. Hazard Identification

Synthesis of Evidence

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

As noted earlier, the section on hazard identification summarizes the key responses (neurological, respiratory, reproductive and developmental, hematological and clinical chemistry, and carcinogenicity). The text is accompanied by liberal use of summary tables and figures that assists the reader in understanding the strength and significance of the effects. The main document is accompanied by appendices that present the major findings, primarily through the use of figures and tables, of the cited literature. Each section on key responses (e.g., neurological) includes a summary of the strengths and weakness of the evidence.

I do have some specific comments regarding some of the data. On page 1-5, the EPA states "it is unclear whether the tests were performed sequentially in the same cohorts of animals". Was any consideration given to contacting the investigators for clarification? Other examples of this exist. At times there are curious statistical results. For example on page 1-10, under 1,2,4-trimethylbenzene pain sensitivity, 191% is significant, but 206% is not. Likewise, on page 1-12 under 1,2,3-trimethylbenzene pain sensitivity, 22 and 78% are significant, but 68% is not. A similar trend occurs for 1,3,5-trimethylbenzene pain sensitivity (246% is significant but 215% is not; page 1-14). On page 1-16, 1,2,3-trimethylbenzene electrocortical activity, three doses are listed, but four responses are given. Under Mode of Action on page 1-22, the EPA suggests that hydroxylated trimethylbenzene metabolites (catechols?) are responsible for the perturbations in normal neurotransmission. Did the EPA uncover any direct evidence for this? On page 1-33, the EPA presents maternal and fetal body weights. While

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the body weight changes for the dams are rather substantial, the changes in fetal body weights strike me as being rather modest. Likewise, while some of the hematology and clinical chemistry values may be statistically significant (Tables 1-5 and 1-6), the changes strike me as being rather modest and probably within the normal range of control animals. On page 1-46, the EPA indicates that 1,2,3-trimethylbenzene is a direct acting mutagen. I can think of no plausible mechanism for this. For Table 1-7, I suggest adding a column to indicate if the exposure was by inhalation or oral.

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

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

The carcinogenicity of 1,2,4-trimethylbenzene was been assessed by Maltoni et al. (Ann NY Acad Sci 837: 15-51, 1997). The compound was administered by oral gavage to male and female Sprague-Dawley rats (50 per sex) 4 times per week (Monday, Tuesday, Thursday, and Friday) at a dose of 800 mg/kg body weight in 1 ml olive oil beginning at 4-5 weeks of age and continuing for 104 weeks. A control group containing an equal number of rats was administered olive oil. The experiment was terminated after 123 weeks and an extensive histopathological analysis was conducted.

The only remarkable finding from the study was neuroesthesioepitheliomas, a tumor arising from the olfactory neuroepithelium, which occurred in treated but not control animals, with an incidence of 2% in treated male rats and 4% in treated female rats compared to 0% in control rats. No statistical analyses were presented in the paper but a Fishers Exact test conducted by the EPA indicated that the incidence as not significant. These tumors are very rare in rats and it is noteworthy that in the same study ethylbenzene also induced neuroesthesioepitheliomas.

The Maltoni et al. study has a number of shortcomings. It is unclear how the dose was selected and because only one dose was used nothing can be said about dose-response. The dosing schedule was quite unusual and the authors stated that a more frequent schedule (i.e., 5 or 6 days per week) would have resulted in unacceptable toxicity. Survival was affected by treatment but quantitative data were not presented. Body weights were collected, but the data were not reported, and, as noted above, there were no statistical analyses presented.

Carcinogenicity bioassays do not appear to have been conducted with 1,2,3-trimethylbenzene or 1,3,5-trimethylbenzene.

Trimethylbenzenes do not appear to be genotoxic when assessed in a standard battery genotoxicity assays. The one exception was 1,2,3-trimethylbenzene in the Ames assay in the absence of S9. The significance of the finding is uncertain because it is not clear (at least to me) what mechanism could lead to such a response.

The NTP has conducted chronic inhalation bioassays in rats and mice with toluene and xylenes and did not detect an increase in tumors.

Based upon the deficiencies of the Maltoni et al. study and the lack of bioassays with 1,2,3-trimethylbenzene and 1,3,5-trimethylbenzene, I agree that the EPA could not conduct a quantitative

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cancer assessment for any isomer due to the lack of appropriate studies. Furthermore, based on the lack of genotoxicity of all three isomers, and the similarity in structure to toluene and xylenes, which do not appear to be carcinogenic in experimental animals, I suspect that the trimethylbenzenes will not be carcinogenic. The only caveat to the conclusion is the unusual finding of neuroesthesioepitheliomas.

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Comments from Dr. Bruckner

Charge Question D.1.

The PBPK model of Hissink et al. (2007) was clearly shown to reliably predict blood 1,2,4-trimethylbenzene (TMB) concentration time-profiles in rats that inhaled 600, 2400 and 4800 mg white spirit (WS)/m³, as well as in humans who inhaled 600 mg WS/m³ for 4 hours. The modeling was clearly described in Appendix B of the document. The model appears to have been properly utilized by Hissink et al. to forecast blood TMB levels in rats and humans during and following the inhalation exposures. The model also appears to have been properly used by EPA staff to forecast average weekly venous TMB concentrations. Is there a reason why the model was not employed to forecast exposures necessary to produce equivalent target organ (brain) TMB doses?

Most of the assumptions and parameters used in model development and optimization seem to be logically and physiologically sound. Why were rat V_{\max} values scaled to humans, when the V_{\max} value derived by Janberg and Johanson (1998) in humans could have been used? This is probably not too important, as the extent of TMB's metabolism is relatively modest and sensitivity analyses showed metabolic parameters had a modest to moderate impact on model output. Why are most of the human tissue:blood partition coefficients (PCs) twice those for rats? Meulenberg and Vijverberg (2000) estimated human fat:, brain: and kidney:blood PCs for TMB and the other two isomers that were higher for humans than for rats. Calculated liver:blood and muscle:blood PCs were comparable for the two species.

The authors of the EPA document went to great length to verify the accuracy of the model code of Hissink et al. (2007), and to check the accuracy of the researchers' calculations and the impact on model outputs. EPA staff also devoted considerable effort to model optimization and validation. The model was run a number of times to assess the fit of its simulations to empirical rat and human time-course data published by several groups of investigators. There was generally reasonable to excellent concordance of simulated and published data. It is noteworthy, however, that the model consistently underpredicted blood TMB levels of persons inhaling TMB alone (i.e., no WS). This was the case for the intra- and post-exposure blood profiles of both Kostrzewski et al. (1997) and Jarnberg et al. (1998).

It is not clear to this reviewer why EPA chose a CNS depression end-point in rats and extrapolated from a rat PBPK model to humans, when quality human neurobehavioral and kinetic data are available, as is a validated human inhalation PBPK model for TMB. Although I am not an authority in animal behavioral testing, it is my impression that rodents are not particularly sensitive models of CNS depression, and that pain avoidance is not too sensitive a measure. Human no- and low-effect vapor exposure levels for irritation and CNS effects for TMB and WS have been reported by Jarnberg et al. (1996), Jones et al. (2006), Jarnberg et al. (1998) and Lammers et al. (2007). Jarnberg et al. (1996), for example, reported no discomfort or CNS effects in subjects inhaling 25 ppm (123 mg/m³) 1,2,4-, 1,2,3- and 1,3,5-TMB for 2 hours. Those subjects were exercising at 50W, which enhanced their absorbed dose and the expected adverse effects, in any. Students at rest inhaling 100 ppm (492 mg/m³) of WS, reported no subjective symptoms (Milling-Pederson and Cohr, 1984), while other students experienced symptoms only at 200-

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400 ppm (Cohr et al., 1980). Thus, there is a reasonable body of human evidence that 100 ppm TMB or WS is a LOAEL or NOAEL.

It is usually far preferable to utilize a fully validated human model, if one is available for the chemical of interest. This, of course, avoids the uncertainties of interspecies extrapolations and the considerable time and effort EPA staff spent checking, optimizing and validating the model of Hissink et al. (2007). The PBPK model of Jarnberg and Johanson (1999) is for TMB alone. Using it also avoids the complications of interactions of TMBs with the myriad of other hydrocarbons in WS. Jarnberg et al. (1998) clearly showed that blood levels of TMB were markedly elevated both during and post exposure of humans to WS, compared to the same vapor level of TMB alone. This phenomenon was apparently due to competitive metabolic inhibition by the other hydrocarbons present in WS. Urinary excretion of dimethylhippuric acid was significantly different in the WS and TMB (alone) subjects.

The empirical blood TMB time-profile data, used by Jarnberg & Johanson et al. (1999) to develop and validate their PBPK model for TMB, are superior to those utilized by Hissink et al. (2007). The former research group obtained data from 9-10 subjects, 2 TMB exposure levels, 2 workloads and different exposure regimens. In contrast, the data of Hissink et al. (2007) came from experiments with just 3 subjects who inhaled one vapor concentration of WS. Microsomal enzymes were likely substantially induced in their participants, as all 3 drank alcohol regularly each week. One person also smoked. It is very important to have quality empirical data, on which to base models and judge model performance. A good deal of my time in research has been spent generating experimental/laboratory time-course blood and tissue data for this purpose.

Charge Question E. 3.

The benchmark dose (BMD) modeling approach appears to have been appropriately conducted and adequately described and referenced. The duration-adjusted point of departure (POD) was converted to a POD_{HEC} by use of the PBPK model of Hissink et al. (2007). I have commented previously on preference for use of human CNS-effect data and the human PBPK model of Jarnberg and Johanson (1999) versus the rat to human extrapolation model of Hissink et al. (2007).

I have also commented on the outdated, over-simplified methodology of EPA (1994b) for calculation of a human equivalent concentration (HEC), using only the ratio of human and animal blood: air partition coefficients (PCs). Blood: air PC is just one determinant of systemic absorption of volatile organic chemicals (VOCs). Alveolar ventilation rate, pulmonary blood flow rate, tissue: blood PCs, and TMB metabolic rate are also important factors. All are significantly higher in rats than in humans. Muhlenberg and Vijverberg (2000) calculated higher fat: blood and brain: blood PCs for rats than for humans. Thus, it is not surprising that Yoshida et al. (2010) estimated that rats inhaling $50 \mu\text{g}/\text{m}^3$ of 1, 2, 4-TMB for 2 hours would absorb $6.6 \mu\text{g}/\text{kg}$, while humans inhaling $24 \mu\text{g}/\text{m}^3$ of 1, 2, 4-TMB for 2 hours would absorb only $0.45 \mu\text{g}/\text{kg}$. This important species difference should be, but apparently is not taken into account in the EPA's methodology for calculating HECs.

Charge Question F. 3.

See comments above in response to question E. 3.

Charge Question H. 1.

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It is clearly stated that no subchronic or chronic 1, 2, 4-TMB oral dosing studies were found. Therefore, use of a PBPK model for route-to-route extrapolation is necessary.

Charge Question H. 2.

The use of a validated model, such as that of Hissink et al. (2007) for extrapolation from inhalation to oral exposure, is appropriate and clearly described. It is stated in lines 7-9 of page 2-46 that one source of uncertainty is the assumption of 100% bioavailability of ingested 1, 2, 4-TMB. This will not occur, except at high (metabolically saturating) doses. As described in lines 1-6 of page 2-45, relatively low oral doses of 1, 2, 4-TMB will result in 4-fold lower steady-state blood concentrations (than equivalent an inhalation exposure), due to first-pass uptake/elimination.

Charge Question I.1

There are few inhalation studies of 1,2,3-TMB, but no applicable oral studies I am aware of. I agree with EPA that 1,2,3- and 1,2,4-TMB are similar enough, that the 1,2,4-TMB oral reference dose should be adopted for 1,2,3-TMB.

Charge Question J. 1. EPA's conclusion, that the oral database for 1, 3, 5-TMB is an adequate for derivation of a RFD, is clearly justified.

SPECIFIC COMMENTS

p. B-2, lines 14-17: It is stated that maximum blood concentrations of 1,2,3-TMB were higher than those of 1,2,4- and 1,3,5-TMB. This apparent difference is very likely not statistically significant, as what are apparently standard deviation bars clearly overlap.

p. B-2, line 23: It is stated here that no SDs for blood 1,3,5-TMB concentrations were given by Jones et al. (2006). The authors included SDs in Figure 3 of their paper.

p. B-2, line 25: "Blood:fat" partition coefficient (PC) should be "fat:blood."

p. B-3, lines 14-21: It is noted that the blood: air PCs for the 3 TMB isomers calculated by Meulenberg and Vijverberg (2000) are quite similar for rats and humans. The documents' authors conclude in lines 20 and 21 that "patterns of absorption would be similar across species." This is not necessarily true. The blood:air PC is just one determinant of systemic absorption of volatile organic chemicals (VOCs) such as TMBs. Alveolar ventilation rate, pulmonary blood flow rate, tissue:blood PCs, and TMB metabolic rate are also important. All are significantly higher in rats than humans. Meulenberg and Vijverberg (2000) calculated higher fat:blood and brain:blood PCs for rats than for humans. Thus, it is not surprising that Yoshida et al. (2010) estimated that rats inhaling $50 \mu\text{g}/\text{m}^3$ 1,2,4-TMB for 2 hours would absorb $6.6 \mu\text{g}/\text{kg}$, while humans inhaling $24 \mu\text{g}/\text{m}^3$ 1,2,4-TMB for 2 hours would absorb only $0.45 \mu\text{g}/\text{kg}$. This important species difference should be, but apparently is not taken into account in the EPA's methodology for calculating human equivalent concentrations (HECs).

p. B-5, 1st pgr. : It should be pointed out that the systemic disposition of VOCs is largely dependent upon the neutral lipid content of tissues, although determinations of tissue:blood PCs for highly lipid-

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soluble chemicals take other properties of tissues and blood into account (Parham et al., 1997; Peyret et al., 2010).

B-6, line 2: Which publication(s) addressed quantitative species differences in TMB metabolism? What were the findings/conclusions?

B-11, pgr. 1: It should be pointed out here that half-lives of TMB isomers are much shorter in rats than in humans. As I noted previously, rats receive a higher systemic dose than humans upon equivalent inhalation exposures, but eliminate the bioactive (i.e., CNS depressant) parent compounds more rapidly.

p. B-12, line 8: 150W should be 100W.

ADDITIONAL REFERENCES

Cohr, K-H, Stockholm, J and Bruhn P (1980). Neurological response to white spirit exposure. *Dev. Toxicol. Environ. Sci.* 8: 95-102.

Milling-Pedersen, L and Cohr K-H (1984). Biochemical pattern in experimental exposure of humans to white-spirit. I. The effects of a single dose. *Acta Pharmacol. Toxicol.* 55: 317-324.

Parham, F M, Kohn, M C, Matthews, H B, DeRosa, C and Portier, C J (1997). Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* 144: 340-347.

Peyret, T, Poulin, P, and Krishnan, K (2010). A unified algorithm for predicting partition coefficients for PBPK modeling of drugs and environmental chemicals. *Toxicol. Appl. Pharmacol.* 249: 197-207.

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Comments from Dr. Cohen

Chemical-Specific Charge Questions A. Executive Summary

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

Comment: The Executive Summary clearly and sufficiently condenses the large amount of information presented in the IRIS document and the Supplement. Individual conclusions regarding RfC and RfD values, as well as other relevant information (e.g., carcinogenicity, susceptible populations and lifestages) for each TBM isomer of concern is clearly and (moreover) succinctly described. It is less clear why whole sections of the Summary need be dedicated to elaborating on "Confidence" of the conclusions as these can be/addressed more fully in the document. Overall, the Executive Summary clearly and sufficiently condenses the IRIS document and Supplement into a concise summary.

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.

Question 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?

Comment: The chosen dose metric was based on models ultimately derived from the Hissink et al. (2007) study that sought to characterize internal exposure parameters following white spirit (WS) inhalation for a single 8-hr period. The rat models were developed to predict levels of 1,2,4-TMB (and n-decane) in blood and brain, and then the model was scaled allometrically to obtain estimates for human blood following inhalation. Ultimately, the models were used to estimate an air concentration that would produce human brain concentrations similar to those in rats at the no-observed-effect-level (NOEL) for central nervous system effects. The Supplement of the IRIS document provides an adequate review of all the potential 1,2,4-TMB PBPK models that were evaluated for potential use in the assessment, reasons for final inclusion or exclusion, and the basis for ultimate selection of Hissink et al.

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(2007). Details are also provided about verification of the model (i.e., accuracy of model code) and model parameter plausibility (across rats and humans). Drawbacks (uncertainties) associated with the use of Hissink et al. to generate the model/metric, i.e., use of parameters from co-exposed hosts to predict kinetics of 1,2,4-TMB in hosts with no co-exposures, potential for kinetic changes with repeated exposures, etc., are also discussed. The document also indicates limitations (i.e., exposure ranges) to which the model is seemingly restricted and provides indications that potential use of a different study (Swiercz et al., 2003) might be required when repeated exposures to 1,2,4-TMB atmospheres at levels above those in the accepted model are evaluated. In conclusion, the dose metric is scientifically supported and clearly described, and uncertainties about the metric are adequately characterized/discussed.

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

Question 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.

Comment: The Korsak and Rydzyński (1996) study sought to characterize potential neurotoxicities in rats that underwent repeated exposures to 1,2,4-TMB atmospheres for 90 days. Specifically, the study evaluated shifts in pain sensitivity (hotplate test) immediately and 2 wk after the final TMB exposure. The IRIS document also reports other studies that examined hematologic and respiratory endpoints in rats that faced similar exposure regimens; other studies that are noted assessed maternal/developmental endpoints in rats exposed to much higher 1,2,4-TMB levels. Reasons for exclusion of many of the non-neurotoxicity studies as a basis for the RfC derivation are clearly described (i.e., lack of dose-response or consistency of response, etc.). Reasons for exclusion of "hotplate + foot shock"-based studies in the available cluster of 1,2,4-TMB neurotoxicity studies are also outlined in the document. While there is some concern a subchronic exposure study is being used to derive a "chronic inhalation reference concentration" and pain sensitivity effects dissipate within 2 wk, these issues are ultimately addressed by use of an uncertainty factor that specifically accounts for this during calculation of the RfC. Thus, selection of the Korsak and Rydzyński (1996) study appears to be scientifically supported and clearly described.

Question 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.

Comment: The literature indicates there is significant translocation of 1,2,4-TMB from the lungs to the brain post-exposure. Thus, the Korsak and Rydzyński (1996) were justified in evaluating changes in pain sensitivity to reflect potential neurotoxicities in exposed rats. While the IRIS document reports on other endpoints that could be used to reflect 1,2,4-TMB-associated neurologic changes (i.e., in neuromuscular function/coordination, motor and/or cognitive function), it also describes why those studies and their endpoints were not ultimately selected to define the critical effect for derivation of the

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RfC. The document also indicates why measures of changes in pain sensitivity based on a “hotplate only” approach are preferable to those from studies using a “hotplate + foot shock” protocol. While the IRIS document also notes studies by the same investigators that examined hematologic and respiratory endpoints in rats that faced similar exposures, the reasoning underlying exclusion of these non-neurotoxicity studies as the source of the critical effect are clearly described (i.e., lack of dose-response or consistency of response, etc.). In accepting neurotoxicity as the class of toxicity most reflective of adverse effects from subchronic 1,2,4-TMB exposure, and by showing that among the various studies of 1,2,4-TMB-induced neurotoxicity there was only a clear dose-related change in latency to paw-lick response (thereby allowing a NOEL/LOAEL to be derived had no model been available to generate BMR/BMDL values), the choice of decreased pain sensitivity (i.e., increased latency to pawlick response in hotplate test) to reflect 1,2,4-TMB-induced effects on the central nervous system as the critical effect for the RfC derivation is scientifically sound and clearly described.

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

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

Comment: The Korsak and Rydzyński (1996) study sought to characterize potential neurotoxicities in rats that underwent repeated exposures to 1,2,3-TMB atmospheres for 90 days. Specifically, the study evaluated shifts in pain sensitivity (hotplate test) immediately and 2 wk after the final TMB exposure. The IRIS document also reports other studies by these investigators that examined hematologic and respiratory endpoints in rats that faced similar exposures. Unlike with 1,2,4-TMB, other studies to examine maternal/developmental endpoints in rats exposed to the same or much higher levels of 1,2,3-TMB are not reported. Reasons for the exclusion of the hematologic and respiratory studies as a basis for the RfC derivation are clearly described (i.e., lack of dose-response or consistency of response, etc.). Reasons for exclusion of one “hotplate + foot shock”-based study of 1,2,3-TMB neurotoxicity or another “hotplate only” study (Gralewicz and Wiaderna, 2001) are clearly outlined in the text. Again, there is concern about a subchronic exposure study being used to derive a “chronic inhalation reference concentration” and that pain sensitivity effects dissipate within 2 wk. However, again, these issues are ultimately addressed by use of an uncertainty factor to account for this during the RfC calculation. Another concern about the study (as reported in document) is that while there is a clear 1,2,3-TMB-induced effect on pain sensitivity after the 90 days of exposures, the data in Table 1-1 suggest a loss of significance at the middle of the three doses tested. It is not until further in the text this ‘error’ is explained and the statistical concern mitigated. An apparent loss of dose-responsivity between the two highest test doses does not appear to be discussed; the impact of this on the BMD modeling only becomes clear later when it is noted that the data from the high exposure group was dropped to facilitate model fitting. Thus, the selection of the Korsak and Rydzyński (1996) study appears to be scientifically supported and clearly described.

Question 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

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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.

Comment: The literature indicates there is significant translocation of 1,2,3-TMB from the lungs to the brain; therefore, Korsak and Rydzyński (1996) were justified in evaluating changes in pain sensitivity to reflect potential neurotoxicities in exposed rats. While the IRIS document reports other endpoints that could be used to reflect 1,2,3-TMB-associated neurologic changes (i.e., in neuromuscular function/coordination, motor/cognitive function), it also describes why those studies and their endpoints were not ultimately selected as critical effect for derivation of the RfC. The document also indicates why measures of changes in pain sensitivity based on a "hotplate only" approach are preferred to those using a "hotplate + foot shock" protocol. While the IRIS document also notes studies by the same investigators that examined hematologic and respiratory endpoints in rats that underwent the same exposures, the reasons for exclusion of these non-neurotoxicity studies as the source of the critical effect are clearly described (i.e., lack of dose-response or consistency of response, etc.). In accepting neurotoxicity as the class of toxicity most reflective of adverse effects from subchronic 1,2,3-TMB exposure, and by showing that among the various studies of 1,2,3-TMB-induced neurotoxicity there was only a clear dose-related change in latency to pawlick response, the choice of decreased pain sensitivity (i.e., increased latency to pawlick response in hotplate test) to reflect 1,2,3-TMB-induced adverse effects as the critical effect for the RfC derivation seems justified. However, there is a concern that tempers the choice of this study and this endpoint as the critical effect. Specifically, the study - as reported in document - reported there was a clear 1,2,3-TMB-induced effect on pain sensitivity after the 90 days of exposures, but with a loss of significance at the middle of the three doses tested. While this 'error' is explained and the statistical concern mitigated later in the document, the impact of this on the BMD modeling only becomes clear later when it is noted the high exposure group data was dropped to facilitate model fitting. If the other reviewers can justify this approach as it pertains to generation of the final RfC value, then the choice of decreased pain sensitivity to reflect 1,2,3-TMB-induced adverse effects on the nervous system as the critical effect for the RfC derivation can be deemed scientifically sound.

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

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

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

Comment: Unlike for 1,2,4- and 1,2,3-TMB, data about potential neurotoxicities from inhalation exposure to 1,3,5-TMB are limited. In the few relevant studies, the utility of a pain sensitivity parameter was compromised by use of the "hotplate + foot shock" approach. An option to derive a critical effect from respiratory-related changes was abrogated by the limitation of the only available data being derived from a 6-min exposure of mice (not rats). While there is some data on hematologic/clinical chemistry endpoints, the outcomes do not show consistent significance among any 1,3,5-TMB-induced changes. A study by Saillenfait et al. (2005) appears to be the only one: using rats exposed to 1,3,5-TMB atmospheres for more than one time (i.e., up to 15 days, in series); using several isomer dose levels; and, noting dose-related impacts on fetal body weight or maternal weight gain. Of the two parameters, the latter would best serve as the critical effect to be used to define an RfC for 1,3,5-TMB. Based on standard calculations, the data ultimately yielded an RfC of 1 mg/m³ (after accounting for uncertainty factors inherent to all the isomer studies). This value is ≈20-times that of the RfC of the other two isomers.

The IRIS document indicates this value is questionable. The text notes that a basis to reject this value is that the studies were done using maternal/fetal toxicities – and not neurotoxicity – as the critical effect. It is also noted "there are important similarities in the two isomers [1,2,4 and 1,3,5] neurotoxicities" that would suggest the two should have 'similar' RfC values. Justification for this conclusion is then bolstered by the isomers having "important similarities in regard to chemical properties and toxicokinetics" etc. that would likely also give rise to similar blood dose metrics. From these assumptions, it was concluded the 1,2,4-TMB RfC should be 'adopted' as that for 1,3,5-TMB.

This approach and conclusion appear to lack merit. In the case of the 'similar neurotoxicities', it is not clear which neurologic parameter is being used to justify this statement. It could not be the pain sensitivity values, as for 1,3,5-TMB these were based on a hotplate/foot shock protocol the document previously indicated was not the best indicator of isomer effect (details can be found in early parts of the IRIS document and in the Supplement). As a result, either the IRIS document should accept the value calculated from the maternal/fetal general effect OR in the absence of conclusive data at this time, no RfC should be proposed until further studies are performed.

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

Comment: As stated above, the approach used to define the 0.05 mg/m³ RfC for 1,3,5-TMB appears to lack merit. The approach and calculations used with the original maternal/fetal endpoints data (as critical effect) were, on the other hand, better justified and clearly described. As such, the document should accept the value calculated from the maternal/fetal general effect OR in the absence of conclusive data at this time, not propose an RfC for 1,3,5-TMB until further studies are performed. .

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General Charge Question

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

Comment: Many of the scientific issues raised by the public and reported in Appendix F are in line with comments of this Reviewer. There are others submitted by the public as well that are reported in Appendix F. In each case, it appears that the EPA has adequately addressed each of the comments/opinions/issues.

From an editorial point-of-view, much of the same information is repeated over and over, albeit in response to individual public comments/issues. It would seem that this portion of the Supplement could be streamlined/edited down.

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Comments from Dr. Cory-Schlecta

General Charge Questions

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

In general, the document does quite a good job in meeting the NRC 2011 recommendation of providing more expansive methods of assessment. It does so, however, from a totally generic context. It seems that it would be more appropriate to post this section (preamble) on the web site, but to present a version of this as it specifically relates to the chemical that is the topic of the document, in this case TMB. In a way, this is a bit confusing, since much of this information is then elaborated in the document itself. Perhaps the approach that should be used in the preamble may be a topic for discussion of the CAAC committee. In fact, it would almost seem more appropriate to include this whole section, given its generic nature, in the Appendix material.

There were however, some areas where the guidance could be further clarified or elaborated:

- p. xxiii, line 17, clarify what 'both' refers to here.

- p. xvii, lines 23-27 seems to contradict earlier statements relating to the requirement for published data.

- p. xx, lines 1-2 could be extended to include the potential for transgenerational effects

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

The new IRIS structure does provide an assessment that is more clear and easy to follow. It seems to provide the necessary information, i.e., it is not too concise. The summary tables are also helpful as the reader proceeds through the document.

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

The current document as the new model for assessments seems to actually already embody much of what was recommended by the NRC 2011 document. This document included the discussion of how it evaluated what would be included as critical studies and what studies were not so considered. The

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discussion included both the strengths and weaknesses of those studies, consistency across studies and recognized that all pertinent studies were from a single lab (related to the neurotoxicity endpoint). Further, this approach was used for all three TMBs under evaluation in this document.

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 Rydzyski, 1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.***

The selection of the Korsak and Rydzyski 1996 paper for the derivation of the RfC is scientifically justified; it is the study that involved the most protracted exposure, subchronic exposure, by the most relevant route (inhalation) and examined multiple organs and examined exposure–response. Thus, this study included more of the criteria for an ideal study than any other extant study. Further, the weaknesses/limitations of the study are also noted. From this study the nervous system endpoints were found to be most sensitive.

In general the study is clearly described although there was at some points confusion for me as a reader as to the exact experimental design in terms of confusion as to actual exposure days and post-exposure timing of some of the assessments, i.e, were they actually number of days post-termination of exposure or of the overall experiment?

- 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.***

The hotplate test and its outcome, here time to pawlicking after administration of an aversive stimulus, is a scientifically valid outcome measure for derivation of the RfC. This test and variants thereof, has been widely used and remains so to evaluate nociception pathways, mechanisms and potential interventions and their relation to nervous system function. They are more typically used to evaluate acute pain, but in some variants, delayed pain or neuropathic pain can be evaluated. This is an appropriate critical effect for deriving the RfC.

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

- 1. A 90-day inhalation toxicity study of 1,2,3-TMB in male rats (Korsak and Rydzyski, 1996) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is***

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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.

As with 1,2,4-TMB, the use of the Korsak and Rydzyski 1996 study as the basis for the derivation of the RfC is warranted for the same reason, i.e., it is the study that met more of the criteria for an ideal study than any others in the literature.

- 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.***

The hotplate test and its outcome, here time to pawlicking after administration of an aversive stimulus, is a scientifically valid outcome measure for derivation of the RfC. This test and variants thereof, has been widely used and remains so to evaluate nociception pathways, mechanisms and potential interventions. They are more typically used to evaluate acute pain, but in some variants, delayed pain or neuropathic pain can be evaluated.

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

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

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

The decision not to base the RfC for 1,3,5-TMB on the Saillenfait et al., 2005 study, which would ignore the other comparable neurotoxicities of this compound with 1,2,4-TMB as well as with the similarities in its toxicokinetics is persuasive and provides an adequate

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justification for the approach chosen. Furthermore, the study of Saillenfait et al., 2005 relies on very crude measures of effect in both dam and fetus.

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

The approach used to derive the RfC for 1,3,5-TMB is logical and based on the similarities of its toxicokinetics and other neurotoxicity outcomes to 1,2,4-TMB, using the same reference value as for 1,2,4-TMB is the sounder and safer approach. This rationale is clearly described in the document.

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Comments from Dr. Ginsburg

Charge Question #4. Appendix F: Resolution of Public Comments

Comment on Page F-1 – Information Quality Guidelines – USEPA's assessment justifies its selection of RfC/RfD studies based upon reasonable standards of evaluation (numbers of animals, consistency in the data, evidence of a dose response) although it would have been helpful to have some analysis of whether there are any inconsistencies in the data (e.g., non-monotonic dose response) or any unusual features of study design (e.g., differential results in foot shock vs non-foot shock studies) that would affect interpretation of hazard or dose response. It appears that some of the design elements in the neurotoxicity test battery were non-standard. While EPA talked about these elements as to how they might have impacted the results, further discussion would be helpful in terms of what these results mean (with vs without foot shock for example) for human risk to TMBs. In fact, the document could have done a better job of describing the various design permutations in key studies (e.g., timeline for TMB exposure, pain exposure, foot shock, latency studies).

Overall, however, I believe that EPA's current document and response to this comment are in line with NAS recommendations for IRIS reform and data quality objectives.

Comment on Page F-2 to F-3 Regarding C9 Test Data not Used: it seems inconsistent that EPA is rejecting C9 toxicology data when it is accepting PBPK data (Hissink model) that is based on exposures to white spirits, a volatile mixture of which TMB is only a small portion. The fact that 1,2,4-TMB was specifically sampled for in the Hissink study facilitates use of these data but it remains that this was a mixture within which it is possible that the other ingredients in the mixture may have modified 1,2,4-TMB fate. In fact, Jarnberg et al. 1997 demonstrate that this is the case with 1,2,4-TMB clearance impeded when dosed in white spirits as compared to on its own. It appears that EPA is assuming that this wouldn't change modeling results because the current effort is a cross-species evaluation with both humans and rats exposed to white spirits in the underlying data, theoretically having a similar effect on TMB kinetics in rats and humans. However, this is unproven and creates an uncertainty which may be as large as the extrapolation from C9 toxicology results to TMB.

Comment on Page F-4 Regarding Reversibility of TMB Effect on Pain Sensitivity: EPA's description of the issues and defense of the position that there may be some latent neurotoxic effects from TMB is accurate and well done. However, as I point out elsewhere, its not obvious that reversibility in some studies warrants a 3 fold rather than 10 fold subchronic to chronic UF and there is some suggestion based upon analogy with toluene that 10 fold may be a reasonable time frame extrapolation. This thread continues with the comment from Page F-11.

Other Comments and Responses in App F: I generally concur with the manner in which these have been addressed by USEPA.

Charge Question D. Toxicokinetics and Modeling

Child/Adult Metabolism Differences: Page 1-54 outlines some of the toxicokinetic issues in extrapolating dose response from adults to young children and rightly points out that there may be meaningful differences across life stage. However, no context is provided with respect to what might

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be the active toxicant (parent compound or metabolite) which makes interpretation of metabolic differences across life stages difficult. The mechanism of action section draws heavily on analogy with what is known for toluene with suggestions that both parent compound and metabolites may have activity in various mechanistic pathways. However this discussion is vague and does not clearly state whether parent compound or metabolite (or both) are likely behind neurotoxic and other effects. Both this section (susceptible populations) and that on mode of action would benefit from the inclusion of statements regarding what is known about the role of parent compound vs metabolites in causing the neurotoxicity of TMBs and related alkylbenzenes. Regarding metabolic differences across life stages, the CYP isozyme(s) responsible for TMB metabolism should be stated if known as their developmental pattern can differ. Further perspective can be gained from toxicokinetic models of child/adult differences published for toluene (Pelekis et al. 2001; Nong et al. 2006).

Charge Question E.3 – Benchmark dose modeling for 1,2,4-TMB:

Model selection and evaluation of model fit to experimental dose response data appears to be reasonable, yielding BMDL values that are in line with the underlying dose response data.

The draft document uses a 1 SD response as the BMR according to USEPA (2012) guidance on benchmark response for continuous data. This BMR is recommended in the EPA guidance where there are no other biological or statistical arguments or methods for selecting a benchmark response (e.g., there is no a priori population distribution cutpoint in the continuous variable below which the response is obviously adverse). This level of response (1 SD change from control mean) is a large displacement; it signifies a 10% extra risk for being below the 1.4th percentile of the control population distribution of values, a cutpoint that would appear to be extreme relative to the amount of change one might expect from a typical low dose effect level.

For example if the control mean is 100 and the standard deviation is 30 for normally distributed data, the percent change from control at the BMR is 66% while for SD = 20 the percent change is 44% and for SD=10 the percent change is 22%. Thus even for a small SD the BMR is still a 22% decline in the parameter value. The benchmark dose methodology recommends 3 things about this choice of BMR that the TMB document does not do: 1) it recommends it to be considered a LOAEL as stated: “That is, a change of one SD in the control mean would be statistically significant in most studies with 10 or more animals per dose group, and the corresponding BMD would generally be interpreted as a LOAEL, depending, of course, on the biological significance of the outcome being measured. Thus, as previously discussed for quantal data, judgments about the biological and statistical characteristics of the data must be made. For example, for frank effects, a lower BMR may be warranted (e.g., 0.5 SD).” 2) As stated in the last quote consideration should be given as to whether the effect in question is a “frank” effect (elsewhere called “a more severe effect”) and if so a lower BMR may be warranted; and 3) whatever the choice of BMR, the rationale for this choice should be explained. Regarding these 3 points, the TMB document provides no particular justification for a 1 SD BMR for the neurotoxicity data treating it as a default approach, there is no discussion of how weak or severe the neurotoxic effect is to justify the 1 SD BMR, and the BMR effect level is treated as a NOAEL in not using a LOAEL to NOAEL UF approach for the BMR even though it calls the “BMR a minimal, biologically significant change”. In summary, greater justification is needed for choosing the BMR for neurotoxicity as a 1 SD change from the mean and consideration should be given to calling it a LOAEL rather than a NOAEL. However, the BMDL appears to fall into a range compatible with a minimal LOAEL based upon the dose response data.

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Charge Questions E. 4 and F.4. Size of the Uncertainty Factor for Subchronic to Chronic Extrapolation: The EPA assessment uses a 3 fold uncertainty factor for this extrapolation for both 1,2,3- and 1,2,4-TMBs whereas the uncertainty can be considered as large as 10 fold in some cases. EPA's basis for a smaller than 10 fold UF is the reversibility of the neurotoxic effect with the implication that this effect would not cumulate to any great extent with longer exposure. In contrast to this statement, the hazard review identified several neurological effects that were seen weeks to months after short-term exposure suggesting persistence of neurological effect (altered electrocortical activity, altered performance on passive avoidance test). On this point the document states that in one study effects after the high dose (1235 mg/m³) were reversible and so there may be less concern for cumulative neurotoxic effect. However, in the same paragraph it states that there is a persistence of neurotoxic effects (page 1-52). This discussion should be tightened up with a critical evaluation of the reversibility of neurotoxicity presented that details the studies, endpoints and timeframes over which reversibility was seen.

This evidence should then be carried over into a more convincing argument as to why the size of the subchronic to chronic uncertainty factor should only be 3 fold (page 2-12). The reversibility issue is brought up again on pages 2-17 to 2-18 with a back and forth of evidence but leaving the issue as uncertain. Given the evidence that TMB effects on neurological functioning can be detected long after its levels in the body have declined, there would appear to be a lasting effect that goes beyond simple solvent effects on the CNS. It is worth noting that the document draws analogies with toluene due to similar structure and neurological effects, hypothesizing that the neurotransmitter effects caused by toluene may underly TMB neurotoxicity as well (page 1-22). This reviewer notes that ATSDR MRLs for toluene are more than 10 fold lower when going from acute to chronic inhalation MRLs as well as when going from acute to intermediate oral MRLs (ATSDR 2000).

Another consideration regarding this extrapolation is whether increasing blood levels of parent compound or active metabolites might be expected from prolonged administration. Increasing blood levels over time would lead to an expectation of greater toxicity from chronic as opposed to subchronic effect. The fact that there is substantial TMBs storage in lipid and that a human PBPK model of 1,2,4-TMB (Jarnberg and Johanson, 1999) predicted increasing blood levels prior to each work shift over the course of the work week in spite of 16 hrs of no exposure in between shifts, suggests a potential for increasing body burden and blood level. However, toxicokinetic modeling has not documented how long it takes to reach steady state blood levels.

Database Uncertainty Factor: A database deficiency uncertainty factor (UF_D) of 3 fold is used for the assessed TMBs on the basis of datagaps in the areas of reproductive toxicity testing and developmental neurotoxicity testing. These datagaps are properly identified and I agree merit the application of a UF_D. However, the decision to use 3 fold rather than 10 fold for this UF is vague. Perhaps clarity can be enhanced by once again relying upon analogy with toluene. The developmental neurotoxicity database for toluene is extensive with the identification of windows of heightened vulnerability in utero and postnatal. For example, Win-Shwe et al. (2010, 2012) have found that exposure to a low level of toluene (5 ppm) during a window of postnatal vulnerability can lead to learning deficits in older (day 49) mice. Given that the IRIS RfC for toluene is based upon a human adult NOAEL that is approximately 10 fold higher than this candidate developmental LOAEL, the example of toluene may

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provide useful perspective on the importance of the neurodevelopmental datagap for TMBs. Taking a full 10 fold uncertainty factor for intra-human variability may somewhat mitigate the uncertainty presented by using only a 3 fold UF for subchronic to chronic extrapolation.

Charge Question F.3. Since USEPA benchmark dose methods were similar for 1,2,3-TMB as for 1,2,4-TMB my response above under E.3 applies here as well. Once again the calculated BMDL appears to be a reasonable derivation based upon model choice, fit to the underlying data and position of the BMDL relative to the dose response data for pawlick response.

Charge Question H.1. There are no repeat dose studies for 1,2,4-TMB via the oral dose route with the acute studies offering little assistance in devising an RfD. I have not read the Koch Industries oral gavage study for 1,3,5-TMB but another scientific review panel found it inappropriate for use in hazard assessment. Therefore, the inhalation to oral extrapolation based upon toxicokinetic modeling appears to be a reasonable approach and one for which there is ample precedent within the IRIS system.

Charge Question H.2. (also relevant to Charge Question D.1.)

The PBPK model developed by Hissink et al. for 1,2,4-TMB is not ideally suited to the current USEPA purpose because the exposure was not just to TMB but to “white spirits” a complex mixture of aliphatics and aromatics which may alter the disposition of TMB relative to a TMB-only exposure. The content of 1,2,4-TMB in the exposure substance (white spirits) was only 7.8%. Further, the Hissink et al. model does not simulate the decay phase of the human volunteer TMB data as closely as desirable (overpredicts TMB in exhaled breath possibly based upon an underprediction of metabolic clearance). Thus, USEPA was justified to modify the metabolic parameters (Appendix B) to achieve better fits and to explore additional validation data sets. However, the adjusted metabolic parameters did not yield a close fit to several data sets in rats (Zahlsen et al. 1996, Table B-11 – all 3 doses TMB overpredicted 3-4 fold – single 12 hr exposure 75-450 ppm; ; Zahlsen et al. 1992, Table B-10 5 fold overprediction of parent compound from repeated exposure to 100 ppm; Swiercz 2003, Table B-9 – doses as low as 25 ppm overpredicted 2-6 fold, repeated or acute exposure). Thus, the modified Hissink et al. model appears to underpredict TMB clearance across multiple doses and datasets, with some of this potentially due to repeat dose induction of enzymes, but this does not explain the overprediction even in single day studies. There is a greater tendency to underpredict clearance at high dose. It is noteworthy that Jarnberg and Johanson modeled TMB metabolic clearance via a saturable (roughly 80%) and a first order (roughly 20%) pathway to obtain reasonable fits to human volunteer data at 2 and 25 ppm exposures. Incorporation of an extra metabolic pathway that would be active at high dose due to first order kinetics (non-saturable) may have helped USEPA simulate the rat data. EPA's description of the validation data are that the overpredictions may be due to lower ventilation rate at high dose due to narcotic effect or “collection delays in the studies” (Page B-47). They conclude that model fits to lower dose rat data (below 100 ppm) and human data (below 30 ppm) yield reasonable fits. However, the overprediction of TMB blood levels occurs in these regions as well as described above.

The implication of this overprediction of parent compound in blood and brain in rats is to potentially underestimate potency as it associates a higher internal dose with a particular toxic effect. However, this may be balanced by a similar overprediction of human TMB dosimetry. Appendix B validation runs of the human model based upon 3 datasets and exposures from 2 to 30 ppm TMB suggests an uneven fit to the Kostrzewki 1997 data (some portions of curve overpredicted, others underpredicted, AUC probably

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close), an underprediction of the exposure phase Jarnberg et al. data and reasonable prediction of the Hissink et al. 2007 data. Thus, overall there appears to be less model accuracy issue with human as compared to rat modeling, leading to the possibility that the overprediction of rat internal dose may have a net effect on risk calculations. (The underprediction of human 1,2,4-TMB concentrations reported in Jarnberg et al. datasets (Figure B-15) is somewhat disconcerting because it may overpredict metabolic clearance opposite to the way the rat model appears to be behaving). The rat model accuracy issues raise the concern that removal of the TK portion of the across species uncertainty factor (3 fold) is not appropriate as the model may not fully address TK uncertainties. Alternative modeling approaches include basing the RfC on animal external dose and a full 10 fold across species UF or use of the RfC 1994 inhalation dosimetry approach (for TMB the cross-species TK extrapolation would boil down to whether there was any differences in partition coefficients because the more rapid inhalation rate in rats would be balanced by the more rapid metabolic clearance rate). Another consideration is that TMB may directly enter the CNS via retrograde axonal flow from nasal olfactory epithelium as has been documented for a number of toxicants and pharmaceuticals (,). However, the importance of this pathway relative to absorption in the pulmonary region and the potential across-species differences in this pathway have not been explored for TMB or related solvents.

The lack of oral toxicity information for TMBs has led to USEPA adopting a route-to-route extrapolation from inhalation data by adapting the Hissink et al. PBTK model to include the oral dose route. In theory this is a reasonable approach (target site systemic rather than local). However, one problem is the lack of any model calibration or validation for the oral route. Further, hepatic first pass metabolism is expected to play a large role in cross-route differences in parent compound distribution to CNS as hepatic intrinsic clearance is substantial - hepatic clearance was estimated to be roughly double hepatic blood flow in humans (Jarnberg and Johanson 1997). Therefore errors in model estimation of TMB metabolic rate will have a direct impact on the inhalation to oral extrapolation. However, the inhalation to oral dosimetry extrapolation is only performed in humans and the PBTK model validations appear to be somewhat more reliable in humans. The predicted 4 fold difference in internal steady state blood concentration between inhalation and oral exposure for an equivalent external dose does not seem unreasonable but might be checked by across route dosimetry comparisons for related alkylbenzenes (e.g., toluene, xylene) as available. This could potentially increase confidence in a modeling effort which is based, at this point, purely on first principles. Another important consideration is that if the key dose metric for neurotoxic effects was peak dose rather than weekly average dose, then the inhalation to oral comparison (4 fold higher steady state blood level by inhalation) may differ as oral exposure involves a difference dosing pattern with ingestion tending to occur in bolus doses (e.g., drinking a glass of water) throughout the day, while inhalation exposure is more uniform and thus has smaller peaks. Application of the oral model now has a simplistic uniform delivery pattern (constant infusion) over the course of the day. This is unrealistic but it may not matter if average blood level is the key dose metric. However, this aspect of dose route extrapolation (exposure pattern) should be considered more closely if peak dose instead of (or in addition to) average dose becomes an important dose metric.

In summary, application of PBPK modeling to hazard and dose response assessment of TMBs carries a variety of uncertainties and limitations including the fact that the key dose metric is not known (parent vs metabolite, peak dose vs average or AUC dose); the uncertainty that this creates should be explicitly discussed in the EPA assessment. Further, a consistent overprediction of TMB blood concentrations by

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2-3 fold, especially in rat simulations, may have an impact on the across-species extrapolation of potency. While several possible reasons for this overprediction are possible (narcotic effect on breathing rate, metabolism rate too high, experimental features not captured by the modeling such as sample timing issues, use of white spirit TK data may overpredict TMB concentrations because of impedance of TMB clearance as suggested by Jarnberg et al. 1997 in humans), an explanation has not been forthcoming. The inhalation-to-oral extrapolation involves unrealistic dose simulation (oral infusion) and there are no calibration or validation data for the oral route. These uncertainties and limitations should be better acknowledged in the assessment, their implications discussed and potential enhancements considered (e.g., adding a second metabolic pathway; consideration of oral to inhalation dosimetric differences for other alkylbenzenes, as available).

Charge Question I.1. There are no repeat dose studies for 1,2,3-TMB via the oral dose route with the acute studies offering little assistance in devising an RfD. I have not read the Koch Industries oral gavage study for 1,3,5-TMB but another scientific review panel found it inappropriate for use in hazard assessment. Therefore, the inhalation to oral extrapolation based upon toxicokinetic modeling appears to be a reasonable approach for 1,2,4-TMB with further extension reasonable to 1,2,3-TMB based upon similar toxicological and toxicokinetic properties.

Charge Question J.1. There are no repeat dose studies for 1,3,5-TMB other than the Koch Industries study. I have not read the Koch Industries oral gavage study for 1,3,5-TMB but another scientific review panel found it inappropriate for use in hazard assessment. Therefore, the inhalation to oral extrapolation based upon toxicokinetic modeling appears to be a reasonable approach for 1,2,4-TMB with further extension reasonable to 1,3,5-TMB based upon similar toxicological and toxicokinetic properties.

References

Nong et al. (2006) Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. TAP 214: 78-87.
Pelekis, M; Gephardt, LA; Lerman, SE. (2001) Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile compounds. Regul Toxicol Pharmacol 33:12-20.

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Comments from Dr. Goeden

General Charge Questions:

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

Preliminary comment:

EPA has done an admirable job in attempting to distill a multitude of guidelines down to approximately 20 pages, however, there are several locations where additional critical detail should be provided:

- *3.3 Selecting pertinent experimental studies. This section is overly simplistic. In addition to duration concepts such as timing and type of endpoint (e.g., immunotoxicity) assessed should be included. While a comprehensive chronic study is desirable well conducted less-than-chronic studies are extremely informative. In addition EPA 2002 recommended that less-than-chronic RfDs and RfCs be derived to facilitate risk characterization of less-than-chronic exposure durations such as short-term higher exposures to infants and children.*
- *Nonlinear carcinogens - utilization of the RfD approach to assess risk should be discussed.*
- *Animal-to-human extrapolation uncertainty factor – Section 7.6 states “. . . If a biological based model adjusts fully for TK and TD differences across species, this factor is not used. In most other cases, a factor of 10 is applied.” This statement is not consistent with US EPA 2011 which recommends the hierarchy of approaches: 1) PBTK modeling, 2) chemical-specific data and 3) default using body weight scaling and reduction of the uncertainty factor from 10 to 3.*

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

Preliminary comment:

The structure is clear and easy to follow; however, the content is still repetitive in some areas and lacks sufficient detail in other areas. See specific comments below, particularly for hazard identification.

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

Preliminary comment:

The current presentation format, which is organized by specific effect, is too fragmented to convey the existing toxicological data in a clear way. A complete concise summary of the toxicological database is essential and provides the foundation for weight of evidence discussions. The evidence tables must present the results of toxicological studies in a concise and informative way. The tabular study summaries should include information sufficient to convey the quality of the study (e.g., study design; N, age, sex, species/strain; chemical purity; dose regimen and dose levels), health effects

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evaluated and basic results (negative as well as positive). Limitations or concerns regarding the quality of the resulting data should also be included in the table. It is only after this information is compiled that the reviewer can draw conclusions regarding the quality of the dataset and the most sensitive effects associated with exposure. The current presentation format does not achieve this goal.

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

Preliminary comment:

EPA has adequately responded to the public comments summarized in Appendix F. It should be noted that the response to the second comment regarding insufficient detail in the Preamble is pending input from the peer review panel.

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Chemical-Specific Charge Questions

A. Executive Summary

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

Preliminary comment:

Page xxxv line 3: 1, 2, 4-TMB were observed to result in [an increase in self-reported] asthma (Billionnet et al., 2011).

Section 2. Table ES-2. A statement or footnote clearly noting that the proposed RfC values for hematological (decreased clotting time) and respiratory (increased inflammatory lung lesions) systems were only slightly higher than the RfC derived for neurological effects (8×10^{-2} vs 5×10^{-2} mg/m³), indicating that effects in these organ systems may also be of concern. This is useful and important information for conducting risk characterizations.

Page xxxvi, paragraph 1: context regarding dose level as well as duration should be added to this paragraph, e.g., were effects observed at higher concentrations following acute exposure than chronic exposure?

Page xxxvi, last paragraph:

Rationale for application of a subchronic-to-chronic uncertainty factor is relevant to derivation of a chronic RfC since there is no post-exposure period. More appropriate rationale would be indication of increasing severity of effect with increased exposure duration from occupational and/or animal studies.

Rationale for database uncertainty factor should include the need for evaluation of effects on inner ear function (e.g., hearing loss).

Section 4: Table ES-3. A statement or footnote clearly noting that the proposed RfC values for hematological (decreased segmented neutrophils) and respiratory (increased inflammatory lung lesions) systems were only slightly higher than the RfC derived for neurological effects (6×10^{-2} and 8×10^{-2} vs 5×10^{-2} mg/m³), indicating that effects in these organ systems may also be of concern.

Same comments regarding rationale for application of subchronic-to-chronic and database uncertainty factors as above.

Section 8. Key reasons for rejecting Koch Industries (1995b) study, the only subchronic oral study, should be stated (e.g., study did not include assessment of neurological parameters which have been found to be the most sensitive endpoint. Simply stating that it was not suitable and referring the reader to Appendix F (15 pages) to locate this information is not acceptable.

Section 8. Units are need for Reference Value in table ES-4.

Route-to-route extrapolation was utilized to extrapolate from the neurological based RfC to a neurological based RfD. As noted earlier the proposed RfCs for hematological and respiratory systems were only slightly higher than the neurological based RfC. Route-to-route extrapolation should also be used to generate corresponding 'proposed' RfDs for these health endpoints as well or an explanation of why this is not appropriate should be included.

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Section 15. The sensitivity analysis of the PBPK model identified QPC (ventilation rate) and metabolic rate among the more sensitive parameters. Higher (on a per body weight basis) inhalation rates and lower metabolic rates early in life should be discussed as well as whether the human variability factor of 10 is adequate to address these differences/concerns.

B. Literature Search Strategy/Study Selection

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

Preliminary comment:

The process used is clearly described. Description of the criteria used to determine relevance of a study for inclusion/exclusion needs to be expanded. Rationale for excluding a study simply because it is not available in English should be stated.

C. Hazard Identification

Synthesis of Evidence

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

Preliminary comment: The Executive Summary and the Dose-Response sections are organized by chemical then route of exposure. This organizational structure should also be followed for the Hazard Identification section. Currently the Hazard Identification section is organized by specific effect rather than by chemical, route or organ system. It is too fragmented to convey the existing toxicological data in a clear way. Rather than present a complete concise summary of the toxicological database EPA has presented a selection of the database.

Hazard identification involves answering the following question "does the agent cause the adverse effect?" The evidence tables must present the results (negative as well as positive) of toxicological studies in a concise and informative way. The tabular study summaries should include information sufficient to convey the quality of the study (e.g., study design; N, age, sex, species/strain; chemical purity; dose regimen and dose levels), health effects evaluated and basic results. It is only after this information is compiled that the reviewer can draw conclusions regarding the quality of the dataset and the most sensitive effects associated with exposure.

Presenting results for specific preselected toxicological effects in isolation rather than summarizing the results from the relevant toxicological studies is not transparent and does not achieve the intended purpose. Inclusion of observations regarding general health status provide needed context (e.g., neurological effects were or were not observed at dose levels associated with changes in clinical chemistry, organ weight/histology).

Through several years of trial and error staff at the Minnesota Department of Health have developed a template for concise study summaries that they have found useful. I have included an example of this template partially summarizing the inhalation studies for 1, 2, 4-TMB and the oral studies for 1, 3, 5-TMB (see attached table).

Extensive footnotes in Table 1-1 significantly limit the utility of the tabular summary and not all of the footnotes appear to be utilized within the table. The study summaries within Appendix B do not appear to have any

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particular order (e.g., route, date, authors name) making it difficult to readily locate information. The study summaries also appear to contain a select but not complete summary of the study results.

The narrative should complement the tables and contain a synthesis of the strength of the evidence presented by the inhalation and oral data summarized within the tables for each chemical. The narrative is repetitious of the information in the tables.

The Figures are a useful addition allowing for easy visual assessment of health effects.

Summary and Evaluation

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

Preliminary comment: Section 1.2.1. Weight of Evidence for Effects Other Than Cancer clearly summarize the available scientific evidence from studies involving TMBs. The available information is limited, especially studies involving the oral route of administration. It would be helpful to add summary information regarding structurally similar chemicals such as toluene and xylene.

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

Preliminary comment: Section 1.2.2. Weight of Evidence for Carcinogenicity clearly summarize the limited available scientific evidence and appropriately classifies the evidence as "inadequate information to assess carcinogenic potential". In light of the limited dataset for TMBs additional supporting summary information for structurally similar chemicals could be used to bolster the weight of evidence.

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?

Preliminary comment: This is not my area of expertise and I cannot comment on the appropriateness of the model inputs.

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The description of the modifications and modeling were clearly described. The optimized model produces acceptable simulations for low exposure levels ($\leq 492 \text{ mg/m}^3$ for rats and $\leq 147.6 \text{ mg/m}^3$ in humans) – is this limitation of concern?

The model assumptions and parameters were clearly described. I was pleased to see parameter sensitivity assessments included in Appendix B, however, this information did not appear to be utilized to any great extent. A brief discussion of the impact of parameters identified as moderately and highly sensitive should be included in sections of the document which discuss life-stage sensitivity and uncertainty.

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?

Preliminary comment: This is not my area of expertise however, based on the toxicokinetic properties the selection of this dose metric appears appropriate.

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.

Preliminary comment: Korsak et al 1997 reported pulmonary inflammation and clinical chemistry changes at lower dose levels (LOAEL of 123 mg/m^3), however, the document provided clear rationale for why these effects were not selected as the basis for the RfC. Selection of neurological effects reported in Korsak and Rydzyński is appropriate and adequately supported in the document.

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.

Preliminary comment: Rationale for not utilizing impaired neuromuscular function and coordination as measured by rotorod performance is clearly presented.

Table 2-2: recommend inclusion of the Reference with the Endpoint as was done for Table 2-3 and 2-4.

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)?

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Preliminary comment: Yes, the modeling has been appropriately conducted and clearly described.

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?

Preliminary comment: If no information is available regarding the change in response that would be considered biologically significant then 1 SD change is recommended as a 'default' BMR. However, the text should include a brief description as to what level of biological change the 1 SD corresponds to (e.g., ~38% decrease in pain sensitivity relative to controls).

Calculations for POD_{ADJ} and POD_{HEC} (pages 2-9 & 2-10) – recommend utilizing the same example throughout. The current POD_{ADJ} is based on decreases in male fetal weight resulting in a POD_{ADJ} of 410 mg/m^3 whereas the POD_{HEC} is based on decreases in female fetal weights starts with a POD_{ADJ} of 403.2 mg/m^3 .

Table 2-3. units (mg/m^3 or mg/L) are needed.

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

Preliminary comment:

- *Interspecies UF – is clearly described*
- *Intraspecies UF – there may be insufficient data to quantitatively inform variability. However, a short qualitative discussion regarding decreased metabolic capacity, renal function, inhalation rates, etc. is warranted along a statement of whether the factor of 10 is likely (or not) protective of these differences.*
- *LOAEL-to-NOAEL – clearly described. However, no rationale was provided for why a full factor of 10 was utilized for increased BAL cells.). The default value is 10, however, if the adverse effect observed is considered to be of minimal severity a default value of 3 may be appropriate.*
- *Subchronic-to-chronic –In determining whether to apply this factor, consider: 1) data indicating additional health effects as the duration of exposure increases, 2) data indicating that the critical effect(s) progress in severity as exposure duration increases, or 3) data indicating that the POD decreases in value as exposure duration increases. A default value of 10 is often applied to shorter-duration PODs to derive chronic values unless data suggest a lack of progression with increasing exposure duration. If data addresses only some of the considerations, a value of less than 10 (e.g., 3) may be used. I agree with magnitude of 3 for this UF factor however, inclusion of reversibility is not appropriate rationale since under chronic exposure there will be no 'post-exposure' period. There is evidence of increasing severity with increasing duration in human studies.*
- *Database UF - This factor accounts for uncertainty based on existing data or deficiencies in the available dataset, resulting in the potential for additional data to yield a lower reference value (e.g., additional studies may show the chemical to be more harmful). In addition to the concerns regarding the absence of a well conducted developmental/multigenerational study to assess potential developmental effects the absence of information on other potentially sensitive effects (based on human studies or structurally similar chemicals) should also be mentioned (e.g., dysfunction of the inner ear, hearing loss).*

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Table 2-4. footnote c. In addition to high level of uncertainty it would be helpful to note that the POD_{HEC} for this effect (23.2 mg/m^3) is ~440-fold lower than the selected RfC (0.0527 mg/m^3) and therefore the selected RfC would be protective of these effects. This 'protectiveness' is visually portrayed in Figure 2-1.

Uncertainties in the Derivation of the Reference Concentration for 1,2,4-TMB – this section should identify the key assumptions/decisions made in the derivation process and whether the assumption/decision qualitatively potentially under- or over-estimates (neither or both) the RfC. For example, the decision to not utilize the rotorod performance data or reliance upon a PBPK model based on adult parameter values.

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

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

Preliminary comment: Yes, selection of this study is clearly described and supported.

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.

Preliminary comment: Rationale for not utilizing impaired neuromuscular function and coordination as measured by rotorod performance is clearly presented. Rationale for excluding liver organ weights, clinical chemistry and hematological effects is also clearly stated. Note: this level of information should be but was not included in the Hazard Identification study summary tables. For example, changes in liver organ weight but no histological alteration was not included in the Korsak et al study summary thereby limiting the usefulness of the tabular study summaries.

Table 2-7: recommend inclusion of the Reference with the Endpoint as was done for Table 2-8 and 2-9.

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

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

Preliminary comment: Yes, the modeling has been appropriately conducted and clearly described.

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

Preliminary comment: If no information is available regarding the change in response that would be considered biologically significant then 1 SD change is recommended as a 'default' BMR. However, the

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text should include a brief description as to what level of biological change the 1 SD corresponds to (e.g., ~22% decrease in pain sensitivity relative to controls).

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

Preliminary comment:

- *Interspecies UF – is clearly described*
- *Intraspecies UF – Both toxicokinetic factors such as metabolic differences and toxicodynamic differences such as susceptible life stages should be mentioned as well as whether the default factor of 10 is sufficient to address these differences. If this not known than this should be noted.*
- *LOAEL-to-NOAEL – clearly described.*
- *Subchronic-to-chronic –In determining whether to apply this factor, consider: 1) data indicating additional health effects as the duration of exposure increases, 2) data indicating that the critical effect(s) progress in severity as exposure duration increases, or 3) data indicating that the POD decreases in value as exposure duration increases. A default value of 10 is often applied to shorter-duration PODs to derive chronic values unless data suggest a lack of progression with increasing exposure duration. If data addresses only some of the considerations, a value of less than 10 (e.g., 3) may be used.*

The rationale provided states that it is based on an assumption that effects would be observed at lower concentrations in a study of longer duration. It is not clear if the available human and animal data was examined. Human studies have reported increased severity of effect with increasing duration. Figure 1-2, for example, suggests that the LOAEL for pain sensitivity was lower in the subchronic study than in the short-term study. Inclusion of reversibility is not appropriate rationale since under chronic exposure there will be no 'post-exposure' period. There is evidence of increasing severity with increasing duration in human studies.

- *Database UF - This factor accounts for uncertainty based on existing data or deficiencies in the available dataset, resulting in the potential for additional data to yield a lower reference value (i.e., additional studies may show the chemical to be more harmful). In addition to the concerns regarding the absence of a well conducted developmental/multigenerational study to assess potential developmental effects the absence of information on other potentially sensitive effects (based on human studies or structurally similar chemicals) should also be mentioned (e.g., dysfunction of the inner ear, hearing loss).*

Uncertainties in the Derivation of the Reference Concentration for 1,2,3-TMB – this section should identify the key assumptions/decisions made in the derivation process and whether the assumption/decision qualitatively potentially under- or over-estimates (neither or both) the RfC.

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

One developmental toxicity study (Saillenfait et al., 2005) following inhalation exposure to 1,3,5-TMB was identified in the literature and was considered as a potential principal study for the derivation of the RfC for 1,3,5-TMB. However, the candidate RfC derived for 1,3,5-TMB based on this study (and the critical effect of decreased maternal weight gain) was 20-fold higher than the RfC derived for 1,2,4-TMB (based on decreased pain

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sensitivity). Given the available toxicological database for 1,2,4-TMB and 1,3,5-TMB, there are several important similarities in the two isomers' neurotoxicity that support an RfC for 1,3,5-TMB that is not substantially different than the RfC derived for 1,2,4-TMB. Additionally, the available toxicokinetic database for the two chemicals indicates that internal dose metrics would be comparable. Thus, EPA concluded that deriving such disparate RfCs for these two isomers was not scientifically supported. Rather, EPA concluded that given the similarities in toxicokinetics and toxicity between the two isomers, there was sufficient evidence to support adopting the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB.

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

Preliminary comment: The toxicological data for 1, 3, 5-TMB is quite limited and consists of two short-term (4 week) neurological assessment study (Gralewicz and Wiaderna 2001; Wiaderna et al 2002) and a developmental (GD6-20) study. All three of these studies provide evidence that 1,3,5-TMB exhibits that same toxicological profile as 1,2,3- and 1,2,4-TMB. Neurological effects were clearly demonstrated in the 4 week duration studies but were not assessed in the developmental study. The developmental study did not evaluate neurological parameters but rather evaluated comparably crude endpoints of viability, body weight and physical/structural abnormalities. For this reason (not because the developmental based candidate RfC is 20-fold higher) it would not be appropriate to derive a health-protective RfC on the developmental study. The dataset for 1,3,5-TMB does not include animal studies of durations beyond 4 weeks and therefore a chronic RfC cannot be derived from 1, 3, 5-TMB data alone.

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

Preliminary comment: Given the limitations of the 1,3,5-TMB toxicological dataset it is logical to utilize the toxicokinetic and toxicodynamic similarities to 1,2,3- and 1,2,4-TMB to derive an RfC. The approach outlined in the document is supported by the available data.

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

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

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

Preliminary comment: the oral database for 1,2,4-TMB is inadequate for deriving an RfD.

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

Preliminary comment: The description of the route-to-route modification consists of only 2 paragraphs in Appendix B (B.3.3.5.). This is not my area of expertise. The dose range over which the model can be used is limited. The assumption of constant oral exposure and 100% absorption is clearly stated but there is no discussion of the appropriateness/reasonableness of this assumption or the impact (e.g., over- or underestimation of internal dose) on the derivation of the RfD.

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

Preliminary comment: see comments on the UFs used for 1,2,4-TMB's RfC above.

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

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

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

Preliminary comment: the oral database for 1,2,3-TMB is inadequate for deriving an RfD.

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

Preliminary comment: rationale for adopting the 1,2,4-TMB RfD as the RfD for 1,2,3-TMB is clearly describe and supported. See above comments regarding derivation of the 1,2,4-TMB RfD.

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

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

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

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Preliminary comment: The oral dataset for 1,3,5-TMB is quite limited but does consist of one subchronic study. The study appears to follow a standard subchronic study design. Additional information regarding the peer review comments would be helpful. It is clear that the oral subchronic study did not include neurological assessment which have been identified as the most sensitive endpoints following inhalation exposure. However, it is not clear whether this data gap can appropriately be addressed through the application of a database uncertainty factor or whether the use of the route-to-route based RfD is superior.

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

Preliminary comments: The database is limited and does not contain a longer duration study that assesses neurological effects, the identified sensitive health effect. However, there is no discussion within the document explaining why the standard approach for addressing data gaps would not be sufficient/acceptable. For example, utilization of the NOAEL/LOAEL ($NOAEL_{HED}/LOAEL_{HED} = 36/107$ mg/kg-d) from the Koch Industries 1995 along with a total UF of 1,000 (3 interspecies, 10 intraspecies, 3 subchronic-to-chronic, and 10 database to address lack of developmental/multigeneration and neurological study information) would result in an RfD of 0.036 mg/kg-d. This option should be discussed and rationale for utilizing the route-to-route based RfD instead it should be presented (e.g., lower uncertainty in using route-to-route based RfD).

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

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

Preliminary comments: Data are not available to support derivation of a quantitative cancer risk estimate.

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1, 2, 4-TMB - Summary of toxicological effect observed in inhalation studies in animals

Study Description and Reference	Effect(s) Observed at each dose level (earliest time point observed noted)	NOAEL/LOAEL [HEC] (mg/m ³)	Comments
<p>90 day Neurotoxicity Study Wistar Rats, adult male, N = 10 0, 25, 100 and 250 ppm pseudocumene (6 hr/day, 5 days/wk), ≥97% purity (0, 123, 492, 1,230 mg/m³) (recovery: 1,230 mg/m³ at 2 wks post-exposure) (Adjusted for continuous exposure: 0, 22, 88, 220 mg/m³) Korsak and Rydzynski (1996) Table B-30</p>	<p>Limited observations beyond neurological parameters: all animals survived exposure, clinical observations were unremarkable, and no significant differences in final body weights were observed.</p> <p><u>Neuromuscular function/coordination:</u> Rotorod performance was tested prior to start of exposure, weekly during exposure period & 2 wks after termination of exposure. Animals were pre-trained and only rats exhibiting normal neuromuscular function (remained on rotating rod for 2 min) for at least 10 consecutive days were used. <i>Results:</i> ↑ failure (0, 10, 20, 40*%) after exposure. At 1230 mg/m³ ↓ performance noted as early as 8 wks of exposure. @2 weeks post-exposure (0, ND, ND, 30%)</p> <p><u>Pain sensitivity –</u> Hot plate behavior tested immediately after termination of exposure. Latency of 60 sec was considered as 100% inhibition of pain sensitivity. ↑ paw-lick latency (15.4±5.8, 18.2±5.7, 27.6±3.2**, & 30.1±7.9** sec; @ 2 wks post-exposure @ 1230 mg/m³ 17.3±3.9 sec)</p> <p>(**p<0.01)</p>	<p>Administered 123/492 (22_{adj}/88_{adj}) [??] pain sensitivity</p> <p>Administered 492/1230 (88_{adj}/220_{adj}) [??] motor function</p>	<p>Authors state that TMBs exhibit stronger neurotoxic effects than those of toluene and xylene.</p>
<p>90 day Respiratory Irritation study, ≥97% purity</p>	<p>Limited observations beyond respiratory parameters: all animals survived exposure, clinical observations were unremarkable, and no significant differences in final body</p>	<p>Administered NA/123</p>	<p>Observed changes were not dose dependent, showing no progression</p>

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Study Description and Reference	Effect(s) Observed at each dose level (earliest time point observed noted)	NOAEL/LOAEL [HEC] (mg/m ³)	Comments
<p>Rat, Wistar, male, N = 6-7</p> <p>0, 123, 492, 1,230 mg/m³ (6 hr/day, 5 days/week), (<i>Adjusted for continuous exposure: 0, 22, 88, 220 mg/m³</i>)</p> <p>Bronchoalveolar (BAL) lavage 24 hrs post-exposure. Determined total protein, mucoprotein, LDG & acid phosphatase levels in BAL supernatant.</p> <p>Korsak et al. (1997), Table B-31</p>	<p>weights were observed.</p> <p><u>Pulmonary inflammation/irritation.</u> ↑total bronchoalveolar cell (BAL) count (0, 202***, 208**, 131*%) and ↑ macrophage count (0, 107, 170**, 116**%) – both with evidence of attenuation at high exposure. No significant change in polymorphonuclear leucocytes, lymphocytes or cell viability.</p> <p><u>Clinical chemistry.</u> ↑acid phosphatase activity with evidence of attenuation at high exposure (0, 47*, 74*, 45*%); ↑lactate dehydrogenase activity (0, 170***, 79*, 57%); ↑total protein (0, 37*, 37*, 26%), ↓mucoproteins (0, 12.5*, 19, 25%)</p> <p>(*p≤0.05; **p≤0.01; *** p≤0.001)</p>	<p>(NA/22_{adj}) [??] ↑BAL</p>	<p>with increasing exposure concentration.</p>
<p>90 days (6 hr/day, 5 days/week),), ≥97% purity</p> <p>Rat, Wistar, male and female, N = 10/sex/dose, except high dose which included additional 10/sex for examination 2 wks post-exposure</p>	<p>No change in body weight gain, food consumption or treatment related changes in absolute or relative organ weights or histology were reported. Changes in hematological parameters and incidence of pulmonary lesions were observed.</p> <p><u>Pulmonary inflammation/irritation</u> ↑in number of pulmonary lesions. Incidences not reported, authors report statistically significant increases at 492 (88_{adj}) and 1,230 (220_{adj}) mg/m³.</p>	<p>Administered 123/492 (22_{adj}/88_{adj}) [??] ↑pulmonary lesions ↓clotting time (females)</p>	

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Study Description and Reference	Effect(s) Observed at each dose level (earliest time point observed noted)	NOAEL/LOAEL [HEC] (mg/m ³)	Comments
<p>0, 123, 492, 1,230 mg/m³, (Adjusted for continuous exposure: 0, 22, 88, 220 mg/m³)</p> <p>Following exposure animals were necropsied – blood chemistry, organ weights & histology conducted.</p> <p>Korsak et al (2000a) Table B-32</p>	<p><u>Hematological toxicity</u> (evaluated prior to & 1 wk before termination of exposure) – ↓ red blood cells in males only (0, -1, -15, -23***%; recovery = 24% ↓) ↑ white blood in males only (0, 2, 4, 80***%; recovery = 18% ↓). ↓ reticulocytes in females only (0, -51, -49, -71*%; recovery = 65% ↑) and ↓ clotting time in females only (0, -23, -37**, -27*%; recovery = 60% ↑).</p> <p><u>Clinical chemistry</u> (conducted 18 hr after termination of exposure) – Non-monotonic ↑ in sorbitol dehydrogenase in males only (0, 73**, 74*, 73***%).</p> <p>(*p≤0.05; **p≤0.01; *** p≤0.001)</p>	<p>Administered 492/1230 (88_{adj}/220_{adj}) [??] ↓RBC/↑WBC (males)</p>	
<p><i>Additional studies</i></p>			

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1, 3,5-TMB - Summary of toxicological effect observed in oral studies in animals

Study Description and Reference	Effect(s) Observed at each dose level (earliest time point observed noted)	NOAEL/LOAEL [HED] (mg/kg-d)	Comments
<p>90 day oral gavage study Rat, Sprague Dawley CD, male & female, N=10/sex/dose Assessed @ 30 days, end of exposure and after 28 day recovery (600 mg/kg-d grp only)</p> <p>0, 50, 200, 600 mg/kg-d [Adjusted for 5 day/week – 0, 36, 143, 429 mg/kg-d]</p> <p>Koch Industries 1995b, Table B-28</p>	<p><u>Hematological</u> – ↑ monocyte levels (Ms only) (0, 100, 200*, 100*%; recovery 100% ↑)</p> <p><u>Clinical chemistry</u> – ↑ phosphorus levels (Ms & Fs) (M/F 0/0, 3/0, 8/5, 17*/23*%; recovery 11/13% ↓); ↓ sodium levels (Fs only) (0, 0, 0, -2*%; recover = 1% ↓) ↓ chloride levels (Fs only) (0, 0, 0, -3*%; 1% ↑) ↑ cholesterol levels (Fs only) (0, -3, 7, 41*%; recovery = 21% ↓) ↓ glucose levels (Ms only) (0, -10, -9, -19*%; recovery = 12% ↑) ↑ alkaline phosphatase activity (Ms only) (0, 5, 13, 46*%; recovery = 28% ↓)</p> <p>600 (429_{adj}) - ↓ body weight gain (11%); ↑ absol* (females) and rel* (males & females) liver weights; ↑ rel* (males) kidney weight. No histological changes reported. (*p≤0.05)</p>	<p>Administered 200/600 (143_{adj}/429_{adj})</p> <p>[HED 33-36/99-107 (F/M) based on DAF 0.23/0.25)]</p> <p>Clinical chemistry changes indicative of liver & kidney effects with organ weight changes at next dose level</p>	

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Study Description and Reference	Effect(s) Observed at each dose level (earliest time point observed noted)	NOAEL/LOAEL [HED] (mg/kg-d)	Comments												
<p>Single oral gavage Rat, Wag/Rij, male, N = 6 per treatment group</p> <p>0, 240, 960, 3850 mg/kg, Tomas et al. (1999a), Table B-39</p>	<p>≥ 240 - <u>Electrocortical activity</u> – EEG recordings. Inhibition of the duration and number of high voltage spindle episodes (response relative to vehicle control):</p> <table border="1" data-bbox="495 500 1255 659"> <thead> <tr> <th></th> <th>20 min</th> <th>40 min</th> <th>60 min</th> </tr> </thead> <tbody> <tr> <td><i>Duration</i></td> <td>0, -76*, -79, -86%</td> <td>0, -85*, -97*, -95*%</td> <td>0, -66*, -94*, -88*%</td> </tr> <tr> <td><i>Number</i></td> <td>0, -57, -67,-77%</td> <td>0, -52*, -93*, -91*%</td> <td>0, -49*, -91*, -89*%</td> </tr> </tbody> </table> <p>(*p≤0.05)</p>		20 min	40 min	60 min	<i>Duration</i>	0, -76*, -79, -86%	0, -85*, -97*, -95*%	0, -66*, -94*, -88*%	<i>Number</i>	0, -57, -67,-77%	0, -52*, -93*, -91*%	0, -49*, -91*, -89*%	<p>Administered NA/240</p> <p>[HED NA/58 based on DAF 0.24]</p> <p>Abnormal EEG</p>	
	20 min	40 min	60 min												
<i>Duration</i>	0, -76*, -79, -86%	0, -85*, -97*, -95*%	0, -66*, -94*, -88*%												
<i>Number</i>	0, -57, -67,-77%	0, -52*, -93*, -91*%	0, -49*, -91*, -89*%												
<p>Single oral gavage Rat, Wag/Rij, male, N = 10 per treatment group</p> <p>0, 960, 1920, 3850 mg/kg Tomas et al. (1999b), Table B-40</p>	<p><u>Motor function and/or anxiety</u> - open field- transient. ↑locomotor activity @ 20 min after exposure relative to pre-injection controls (0, 0, 46.7*, 42.4*%; ↑ 65-70% @40-60 min @ 3,850 mg/kg). No significant changes were reported at 10, 30, or 70 min</p> <p>(*p≤0.05)</p>	<p>Administered 960/1920</p> <p>[HED 230/461 based on DAF 0.24]</p> <p>↑locomotor activity</p>													

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Comments from Dr. Howd

Responses to Charge Questions:

General Charge questions 1,2,3

1. The new preamble on EPA guidelines seems like generic filler, which it seems to me is more appropriate in the available guidance manuals. Although it may meet the NRC request and new statutory requirements, it doesn't add anything of value to this document. The only thing I noticed missing from this preamble discussion was the U.S. EPA recommendation of a maximum uncertainty factor of 3,000.
2. The presentation of steps appeared to me to be clear and concise, probably a bit better than the previous format.
3. It's very difficult to develop "standardized approaches" for review and evaluation of the myriad of study designs and results. In this case the lack of several standard toxicity tests for the chemicals evaluated resulted in use for risk assessment of non-traditional endpoints, and thus the discussion of results comes across as somewhat ad hoc. I thought the discussion of the toxicological significance of observations was generally well handled, and "transparent." Two things concerned me, however; the frequent discussion of non-significant changes as if they were meaningful, and the use of decreased fetal weight gain as a primary toxicity endpoint when the dams are not eating well and failing to gain weight. Perhaps a better discussion or standardized approach for these issues could be provided.

Literature Review, Question B1

The description of the search strategy does not mention xylenes or ethylbenzene. Because of the close similarity of xylenes to TMBs and the very similar toxicological effects, it seems to me that this resulted in important papers being excluded, and thus weakening the conclusions. For example, the papers 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 link to TMBs, in my opinion. The link appears stronger to xylene and to a mix of aromatic solvents including TMBs. Studies such as those of Ruijten et al., 1994, Qian et al., 2010, and Hassan et al. 2013 are more linked to xylene and are not cited in this document, but the overall association of these effects in painters with exposures to aromatic solvents like the TMBs is much stronger, I think, than is obvious when excluding these and several other related studies. Other relevant references are cited in the responses below.

The TMB discussion in the recent NAS report, Acute Exposure Guideline Levels for Selected Airborne Chemicals, Vol 13 (2013), Chap. 8, Trimethylbenzenes (http://www.nap.edu/catalog.php?record_id=15852) could also be reviewed.

Hazard ID Synthesis of Evidence, Question C1

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Neurological effects

p. 1-1. The summary of neurological effects would have been strengthened if other relevant papers on aromatic solvents had been reviewed. A good example is the brief mention of "damage or dysfunction of the inner ear" with a single citation, when there are dozens of studies on this phenomenon (as ably cited in that single reference to Sulkowski et al., 2002).

Pain sensitivity

p. 1-4. I agree that changes in the hot plate pain sensitivity test long after the foot shock may relate to cognitive changes instead of actual changes in pain sensitivity, and am not clear as to how this should be interpreted. The test paradigm looks OK. Perhaps acute effects from TMBs (sedation, decreasing pain sensitivity) are altering the association of the foot shock pain and hot plate pain. If an acute effect was responsible, then it may not really be a persistent deficit, and it is debatable whether it should be used for a subchronic endpoint or need an extra UF for a chronic exposure duration. On the other hand, persistent effects on rotorod performance were found by Korsak and Rydzynski, 1996, and a persistent neurological deficit from long-term exposure to aromatic solvents has been observed in the painter studies, so it is appropriate to make health-protective assumptions when various interpretations of observed effects are possible.

Neuromuscular function and coordination; Motor function and/or anxiety; Cognitive function
These discussions are good, but I had some difficulty with the separation into these three overlapping categories.

In several places there are questionable statements about non-significant effects. One can mention such effects in association with a trend test, and can make cautious references to p values just short of 0.05, but relevance of such changes is always tenuous. One example here of questionable use is on p. 1-7, line 32. "Decreases in latency were consistently observed and similar in magnitude across all studies at 7 days post foot shock, although the decreases were not statistically significant for 1,2,4-TMB or 1,2,3-TMB...." If the changes are not statistically significant, then it is specious to say they were consistently observed.

Electrocortical activity

Adequate discussion, although it might have profited by referring to changes in evoked potentials associated with solvent exposures, which also document neurological effects. Relevant studies include:

Quevedo Lda S, Tochetto T, Siqueira MA, Machado MS. Auditory brainstem response in gas station attendants. *Braz J Otorhinolaryngol.* 2012 Dec;78(6):63-8.

Gong Y, Kishi R, Kasai S, Katakura Y, Fujiwara K, Umemura T, Kondo T, Sato T, Sata F, Tsukishima E, Tozaki S, Kawai T, Miyama Y. Visual dysfunction in workers exposed to a mixture of organic solvents. *Neurotoxicology.* 2003 Aug;24(4-5):703-10.

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Pratt H, Karim N, Bleich N, Mittelman N. Short latency visual evoked potentials in occupational exposure to organic solvents. *Neurophysiol Clin.* 2000 Oct;30(5):306-12.

Mode of Action Analysis - Neurological Effects

This discussion seems fine. Although I've never been satisfied that the solvent effects are mediated through alterations of neurotransmitters, this discussion adequately summarizes the studies, I think.

Summary of Neurological Effects

This is adequate, although could have been helped by including other solvent literature.

Respiratory Effects

Good summary

Reproductive and Developmental Effects

Decent description of the Saillenfait et al. study, although at this time a discussion of the significance of a decrease in fetal body weight when the dam is failing to thrive could have been provided. The nutritional effects of the decreased food consumption in the higher-dose groups could be addressed.

Hematological and Clinical Chemistry Effects

Good summary.

Carcinogenicity

Good summary

Similarities Among TMB Isomers (toxicity)

Good discussions overall. However, on p. 1-48, summarizing effects on fetal body weight of Saillenfait et al. (2005), I would have preferred an acknowledgement here that these effects on fetal body weight occurred at levels \geq than the maternal LOAEL, and thus may be secondary to maternal effects. A non-specific maternally-mediated effect is substantially different than other forms of developmental toxicity, and the relevance of decreased fetal body weight when the dam isn't eating could have been discussed. Since the estimated RfC for this effect is higher than for the other endpoints, it's a somewhat moot point for this risk assessment, but still raises the issue of relevance and applicability. It seems to me that this is the type of issue that NRC was referring to in requesting more explicit documentation of methods and guidelines for the issues that have to be decided in any particular IRIS toxicity evaluation. Is this issue discussed in the reproductive and developmental toxicity guideline documents?

Other than the above quibble, I think the data have been fairly and accurately described and I agree with the use of the chosen toxicological endpoints for each of the isomers.

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Similarities Among TMB Isomers (toxicokinetics)

Good discussions. However, on p. 1-50, top, discussing absorption of the TMBs, it says that absorption was observed to be higher for 1,2,3-TMB based on a slightly higher mean blood level. Because absorption, distribution, and excretion are all involved in developing a particular blood level (and no data are available on 1,2,3-TMB distribution), this seems a specious statement. Since no statistics were provided, it's not clear that the slight differences are meaningful, but certainly this should not be ascribed to differences in absorption.

Details in Appendix B, p. b-2, say blood levels were $6.5 \pm 0.88 \mu\text{M}$ for 1,2,4-TMB, $6.2 \pm 1.6 \mu\text{M}$ for 1,3,5-TMB, and $7.3 \pm 1.0 \mu\text{M}$ for 1,2,3-TMB as estimated from digitized data (scanning figures in the Jarnberg et al. papers). It doesn't say whether the error ranges are SD or SE, but my interpretation is that these values are unlikely to be statistically significantly different. Note also that the next study described, Kostrewski et al., 1997, provides results implying that 1,2,3-TMB levels in blood would be about the same as the other two isomers at similar exposures.

Therefore any points made about this "difference" in the Jarnberg study deserve to be eliminated. p. 1-51, line 5, says that the final-phase half-life of 1,3,5-TMB is "much greater" than that of the other two isomers, although the values given do not appear to be statistically significantly different ($120 \pm 41 \text{ hr}$ vs. 87 ± 27 and $78 \pm 22 \text{ hr}$). It then goes on to say, starting in line 7, "The difference observed in half-lives between the three isomers in the last elimination phase may be due to small sample sizes and difficulties in measuring slow elimination phases rather than a true difference in half-lives." It seems to me that the original wording (line 5) should reflect this uncertainty, rather than claiming a difference in line 5 and then correcting it later. Same problem in the discussion p. B-10.

Hazard ID Summary and Evaluation

1. The hazard assessment adequately summarizes the scientific evidence.

Dose Metric, Question D2

The dose metric, of average rat venous concentration, seems to me to be a carefully thought out and well documented measure, and is appropriate to use for the intended purpose.

Inhalation 1,2,4-TMB Question E1

The 90-day toxicity study of Korsak and Rydzyński seems an appropriate choice, and is well-described.

Question E2

These are complicated effects, but my judgment is that the authors are using the techniques appropriately and interpreting the results fairly, thus lending good credibility to the selected endpoints. I agree that the EPA has made scientifically defensible conclusions about these data, and that they are appropriate to select for the critical effect.

Inhalation 1,2,4-TMB, Question E-4

The derivation of UFs seems appropriate. One quibble about the rationale, however. P. 2-13, line 21 refers to EPA guidelines addressing potential greater effects in the developing organism

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due to "an incomplete blood-brain barrier." This is perfectly correct, but doesn't appear relevant in this case since there is no blood-brain barrier for lipophilic organic solvents. They are similar to anesthetic gases in this regard. Perhaps this sentence should be reworded.

Inhalation 1,2,3-TMB, Question F-4

The derivation of UFs seems appropriate. The same questionable statement is made here about the BBB (p. 2-28) and for 1,3,5-TNB (p. 2-40).

Oral 1,2,4-TMB, Question H3

I agree with the selection of UFs for this chemical.

Oral 1,2,3-TMB, Question I2

I think the derivation of the RfD for 1,2,3-TMB is scientifically supported and clearly described.

Oral 1,3,5-TMB, Question J2

I think the derivation of the RfD for 1,2,3-TMB is scientifically supported and clearly described.

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Comments from Dr. Lash

C. Hazard Identification.

Synthesis of Evidence

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

The synthesis of evidence for TMB isomer toxicity is nicely divided up into the various target organs or forms of toxicity. These include: neurological effects, respiratory effects, reproductive and developmental effects, hematological and clinical chemistry effects, and carcinogenicity. For each effect, hazard assessment is further divided by whether exposure is by inhalation or the oral route and then by each of the three isomers. The availability of studies in humans is first considered in each case, followed by studies in experimental animals (generally rodents). For each effect, isomer, and route of exposure, the document clearly describes the availability of studies in each category, the exposure doses and length of exposure in animal studies, and the effects observed.

The descriptive text is nicely supplemented with summary tables and figures. The tables are divided according to effect, route of exposure, and TMB isomer. These are very clear and useful. The figures nicely summarize the available data for each effect by each isomer in what is called an exposure response array. These figures list effects with a line showing dose and indicating low dose, high dose, NOAEL, and LOAEL.

For neurological effects, which are the most consistently observed, there is a subsection on mode of action analysis (section 1.1.1.1). The document clearly explains that although mechanistic data are lacking for the TMBs, there is good rationale for making analogies with toluene, for which much more information is available.

The second effect considered, respiratory effects, is also commonly and consistently observed in animals. The document also notes that in several studies, observed effects are not concentration dependent. The importance of this point is not really made clearly. While the document suggests that adaptation may occur after longer or higher dose exposures, alternative explanations are not considered. Respiratory depression is not a very sensitive effect, requiring > 500 ppm (> 2500 mg/m³) to elicit 50% decrease for all 3 TMB isomers. However, the document really does not adequately consider the relevance to humans of these high doses. In general, dose relevance for humans is not adequately considered. Moreover, although it is mentioned that the specific respiratory effects observed in experimental animals and exposed humans are not the same, there is mention that there are respiratory effects in humans nonetheless.

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For the hematological and clinical chemistry effects section, the document clearly discusses the human exposure studies and the inherent problems in drawing conclusions from these studies. As with many occupational exposure studies, the lack of detailed exposure information or the known presence of multiple agents is a significant limitation. While the document clearly acknowledges this limitation, it does not clearly describe the human exposures except to state that effects cannot be attributed to a single TMB isomer (see comment #6 below). It is not clear whether the human exposures were to multiple TMB isomers or to multiple solvents. If the latter, then the conclusion should be revised to indicate that effects cannot be attributed to either a specific TMB isomer or to a different solvent altogether.

With regard to carcinogenicity as an endpoint, the document clearly communicates the inadequacy of the database, including the minimal genotoxicity database.

Finally, two subsections in the hazard synthesis section (sections 1.1.6 and 1.1.7) consider the similarities between the three TMB isomers with regard to observed toxic effects and toxicokinetics and metabolism. This is a very important issue that is brought up several times throughout the entire document. The importance lies in the fact that for some effects, observations are only available for one or two of the isomers. The documented similarities provide rationale for extrapolating from one isomer (for which adequate data are available) to another isomer for which little or no data may be available. A summary table (Page 1-49, Table 1-7) is provided and this is very helpful in understanding the points made with regard to toxic effects. With regard to toxicokinetics and metabolism, however, a summary table or scheme would be useful to clarify this aspect.

Other comments:

1. Page 1-3, line 19: Change to "this pain sensitivity-related effect" because only the latter one persisted.
2. Page 1-3, lines 29-30: Text is inappropriate; cannot have a tendency if there was no statistical significance.
3. Page 1-4, lines 30-33: You cannot draw a conclusion from something if there is no statistical significance!
4. Page 1-5, line 16: Another example where lack of statistical significance is not handled properly.
5. Page 1-7, lines 32-35: Confusing and not an accurate use of statistical results; cannot say that "decreases were not statistically significant" because no decreases existed.
6. Page 1-35, lines 5-11: Describes studies of workers exposed to solvent mixture; concludes that cannot attribute effects to one TMB isomer. However, if other solvents were present, effects may be due to a different solvent and not a TMB isomer.
7. Page 1-36, lines 17-18: The sentence "...but the increases in activity were not significantly higher when compared to controls" is not appropriate; if there is no statistical significance, then there are no increases.

Summary and Evaluation

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

The "Summary and Evaluation" section is divided into three subsections: 1) weight of evidence for effects other than cancer; 2) weight of evidence for carcinogenicity; and 3) susceptible populations and life stages. The preponderance and commonality of neurotoxicity across species is clearly explained as is the limitations in the available human studies. Nonetheless, the document states the conclusion that "the evidence for TMBs identifies neurotoxicity as a toxicity hazard based on consistency and coherency of effect across multiple studies and durations of exposure." This conclusion is appropriate.

The respiratory system is also concluded to be a target organ despite the specific effects in humans being different from those in experimental animals. As noted in the "Synthesis of Evidence" question above, respiratory depression is not a particularly sensitive effect and the relevance of doses used in animals to observe effects to doses that humans are typically exposed is unclear. Nonetheless, I agree with the overall conclusion that the available data support the respiratory system as a potential target of TMBs.

Maternal and developmental toxicity are also concluded to be a target. This conclusion is based primarily on biological plausibility because of the absence of human data. Further, the conclusion is only made for 1,2,4- and 1,3,5-TMB because no pertinent studies were conducted with the 1,2,3-TMB isomer. It is surprising in retrospect that the document does not make the same conclusion for the 1,2,3-TMB isomer based on the pharmacodynamics and pharmacokinetic similarities between the three isomers. While this small discrepancy may not become apparent at this point in the document, when one considers these types of statements regarding the three isomers that are made in the second section on dose-response analyses, it would seem to be just as plausible that developmental toxicity is also relevant for 1,2,3-TMB.

Similar conclusions are made for hematological effects, based on both the human and animal exposure databases and biological plausibility. These are made clearly and presented logically.

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

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

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The document nicely defines each uncertainty factor (UF) and the major considerations that are used to select each particular value. UF values are listed in Table 2-4 on page 2-14 for the 5 target organs and potential critical effects within each organ system. A total of 10 potential critical effects are identified. For all 10 effects, the same UF value of 3 is used for interspecies extrapolation (UF_A), the same UF_H value of 10 is used for potentially sensitive individuals or subpopulations, and the same UF_D value of 3 is used for database uncertainty. With regard to the UF_L value for LOAEL to NOAEL uncertainty, all the potential critical effects had a value of 1 except for increased BAL cells (respiratory toxicity), to which a UF of 10 was applied. Finally, with regard to the UF_S value for subchronic to chronic extrapolation, a value of 3 was applied to all the effects except the two developmental endpoints, to which a value of 1 was applied. The choice of each value is logically and clearly presented and is supported by the available data. I have no proposals for changing any UF values. These values are completely consistent with standard U.S. EPA practice.

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

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

The document chooses the 1996 Korsak and Rydzynski study as the basis for derivation of the RfC. Justification for this begins by first ruling out using any of the available human exposure studies based on their inadequacy for dose-response analysis. Also the selected study has some limitations or deficits, logical assumptions are made to account for these based on other studies by the same group. With these assumptions in place, the study would seem to meet the criteria needed for it to be appropriately used to derive the RfC for 1,2,3-TMB. No other published study meets the needed criteria or provides more confidence than the one selected.

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

The decreased pain sensitivity test satisfies the criteria for a study to be chosen as the basis for RfC calculation and gives the lower RfC value, thereby providing the greatest protection. While a couple of the other critical effects give RfC values that are very close to that derived with this effect, the RfC value is the lowest when calculated as done here. Hence, by both the level of

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support from scientific evidence and the convention followed by the U.S. EPA, decreased pain sensitivity is the most appropriate critical effect based on the available data.

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

The basis for each of the five UF values is clearly presented and summarized for all potential critical effects in Table 2-9 on page 2-29. The composite UF value of 300 is reasonable based on the levels of confidence and certainty. All choices, decisions, and the conclusions derived from these are clearly described.

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

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

Unlike inhalation exposure, the database for oral exposures to TMB isomers is very limited and judged to be inadequate to enable calculation of the RfD value. However, the document explains the justification for performing a route-to-route extrapolation so that the inhalation exposure database can be used to calculate the RfD. The primary criteria are the existence of data that shows similar effects by the two routes of administration in acute exposures, similar toxicokinetics, and the existence of a well-validated PBPK model. Regarding toxicokinetics, the document states that there is an assumption of "continuous oral ingestion and 100% absorption of the ingested 1,2,4-TMB by constant infusion of the oral dose into the liver. This is a common assumption when information about the oral absorption of the compound is unknown." Although the lipophilicity of 1,2,4-TMB is consistent with the assumption of 100% absorption, there is some concern about first-pass (i.e., hepatic) metabolism and oral bioavailability. There is some brief discussion of this limitation but it does not seem to be incorporated into the calculated RfD. On page 2-46, section 2.4.4, the document notes that there is uncertainty about bioavailability, which raises the question, why was this not included in the UFs used to calculate the RfD?

This reviewer's recommendation would be to increase the UF_D value from 3 to 10. Thus, the composite UF would be 1000, which is still at a reasonable level. This would change the RfD calculation to the following:

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$$\text{RfD} = \text{POD}_{\text{HED}} \div \text{UF} = 6.3 \text{ mg/kg-day} \div 1000 = 0.006 \text{ mg/kg-day} = 6 \times 10^{-3} \text{ mg/kg-day}.$$

The proposed increase in the UF_{D} value is based on the absence of toxicokinetics and metabolism data, which generates additional uncertainty in the bioavailability of orally administered 1,2,4-TMB.

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

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

As for the 1,2,4-TMB isomer, the oral exposure database for 1,2,3-TMB is not adequate for calculation of an RfD value. However, the document clearly explains the rationale for using the RfD for 1,2,4-TMB, which is based on the similarities in chemical properties, pharmacokinetics, and pharmacodynamics of the three TMB isomers. As explained above (answer to Q. H.3), I believe that the uncertainty in the extent of first-pass or hepatic metabolism that occurs in an oral administration results in uncertainty in oral bioavailability. Hence, I recommend increasing the UF_{D} value from 3 to 10, which would increase the composite UF from 300 to 1000. This would in turn decrease the RfD by a factor of 3 from 2×10^{-2} mg/kg-day to 6×10^{-3} mg/kg-day.

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

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

Even less data are available on oral exposure to the 1,3,5-TMB isomer than for the other two isomers. Nonetheless, based on the well-documented similarities in chemical properties, pharmacokinetics, and pharmacodynamics among the three isomers, the logical choice is to use the same RfD value as calculated or chosen for the other two isomers. This is completely reasonable and is clearly presented. As noted above (Questions H.3 and I.2), this reviewer recommends increasing the UF_{D} value from 3 to 10, thereby increasing the composite UF value from 300 to 1000, finally resulting in an approximate 3-fold reduction in the RfD, from 2×10^{-2} mg/kg-day to 6×10^{-3} mg/kg-day.

General Charge Questions.

4. EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes and has revised the assessment to respond to the scientific issues raised in the comments. A summary of the public comments and EPA's responses are provided in Appendix F of the Supplemental Information to the Toxicological Review of Trimethylbenzenes. Has EPA adequately addressed the scientific issues?

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In the extended public comment period, the EPA received a total of 15 comments. Some of the comments were general, relating to basic procedure and transparency of sources. Other comments were very specific regarding decisions such as the choice of principal study, the choice of principal effect, how certain uncertainty factors were calculated, why certain datasets were excluded from the IRIS assessment, and why in cases where data for a specific TMB isomer were lacking, RfC or RfD values for other isomers for which more data are available were used.

As an overall comment on the responses, the EPA was very thorough and clear in their explanations and responses. The responses also demonstrated flexibility and a lack of bias. In those cases where the criticisms raised were justified, the EPA explained how the document was changed to accommodate the correction. However, there were several comments that were not correct, and the EPA was very thorough and methodical in explaining why the comment was not correct.

Comments on Specific Responses:

- 1) The first comment made a broad criticism of the transparency with which the EPA explained its sources of data, assumptions made, analytical methods used, and statistical analyses conducted. The response was concise and appropriate, clearly explaining why this criticism is inaccurate.
- 2) The second comment critiques the Preamble for not providing sufficient information. The EPA response was very appropriate as the comment is implying that the Preamble should be more than what it was intended to be.
- 3) Another comment was made regarding the exclusion of data from so-called C9 mixtures. The comment also cited the 1985 TSCA 4(a) test rule as a reason to compel the EPA to add data from such studies. The EPA response was clear and appropriate, noting that this test rule is outdated and some of the information on which it is based is incorrect. Further, the response clearly explains the problem of using data from exposures involving complex mixtures, especially when the chemicals of interest account for no more than 55% of the total chemicals in the mixture.
- 4) One comment questions the statement that there are no chronic, subchronic, or short-term oral exposure studies for 1,3,5-TMB found in the literature. The EPA considered the comment and information and decided to include a discussion of some of the data noted. However, limitations were noted in these studies. This showed that the EPA was open to appropriate suggestions and is not acting with a bias.

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5) A detailed comment was presented about the rationale for selecting decreased pain sensitivity as the critical effect for the TMB isomers. Further reference was made to the appropriateness of the choice of the Korsak and Rydzyński (1996) study as the principal study and the assessment of neurotoxicity in the Douglas et al. (1993) study using exposures to C9 aromatics. The EPA recounted in a fair amount of detail their rationale for choosing decreased pain sensitivity as the critical effect, the limits of the assay, and the specific conditions under which they used the assay. Further, the EPA reiterated the issues with using exposures to C9 aromatics and why they restricted their database to those studies with defined and predominant TMB exposures. The comment also questioned the chosen critical effect because of the question of its reversibility. However, the EPA provided a cogent and straightforward explanation for why this question is really academic because the exposures are considered to be lifetime and reversibility is not a problem. Hence, the EPA provided a detailed and clear response to the concerns raised about the critical effect and principal study choices.

6) A follow-up comment was provided about potential inconsistencies with the studies that were chosen to be included in the IRIS assessment, including the principal study. Again, the EPA response demonstrated an open-minded and flexible attitude towards these criticisms. While acknowledging the inconsistencies, the EPA went on to explain how the document was modified to provide additional explanation. Where the comments about the pain sensitivity tests are valid, the EPA acknowledges those points and made appropriate changes. Where not valid, however, the EPA clearly explains why not.

7) A further comment critical of the pain sensitivity endpoint was provided. The comment notes a “lack of agreed guidelines for study conduct and rationale for administering foot shock were cited in the Draft Assessment and thus the varied protocols lead to a lack in clarity regarding whether or not the testing conducted is scientifically valid and reproducible.” In response, the EPA cites multiple references and further explanation for how this endpoint was used in the analyses. The response is thorough and precise.

8) A comment was made questioning the clarity regarding selection of the critical effect for derivation of the reference values for the TMB isomers. Again, the EPA provided a detailed clarification of how reference values were determined. The response is thorough and clear.

9) Another comment focuses on large interindividual differences in some of the neurotoxicology responses in one of the studies and the procedures that were used to discount outliers. The comment cites this practice, which is quite standard and statistically valid, as further evidence to bring the choice of critical effect into question. The EPA again clarified how data were presented and acknowledged the concern by adding additional comments in the evidence tables about how the data were analyzed.

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10) A comment was made questioning how the RfC for 1,3,5-TMB was calculated. In response, the EPA summarized the very detailed and clear points that were made in the IRIS document about the toxicokinetic and toxicological similarities among the three TMB isomers. Again, this is a thoughtful and precise response.

11) A comment was made questioning the appropriateness of application of an uncertainty factor (UF) of 10 to account for extrapolation from subchronic exposure to chronic exposure. The commenter suggests that a UF of 3 or less is more appropriate. The EPA responded by stating: "After careful consideration, EPA agrees that a full 10-fold UF_s is not supported by the available data. Given the observation of reversibility in neurotoxicity endpoints reported in subchronic inhalation studies, an uncertainty factor of 3 has been applied in the Draft Assessment. Lowering the UFS to 1 was not supported..." This is a very reasonable and thoughtful response to the comment.

12) A comment was made about the appropriateness of the uncertainty factor for database deficiencies (UF_D). The comment goes on to suggest that use of studies with C9 fraction exposures would lower the uncertainty by providing additional data. As explained in an earlier response, the EPA clearly explains the problems with using C9 fraction exposures and that this would be inappropriate.

13) A comment questioned the appropriateness of conclusions regarding the mutagenicity of 1,2,3-TMB, noting that the study cited found it to be mutagenic in the absence of metabolic activation. Rather than conclude that insufficient data are available to make a conclusion about TMB mutagenicity, the commenter recommends that negative data from C9 fraction exposures be used to make the conclusion that TMB isomers are not mutagenic. In its response, the EPA reiterates how it came to the conclusion of insufficient data, namely that only one study is available that directly addressed the issue.

14) In a further comment on the choice of study to calculate RfC values, a recommendation is made that the EPA should select a study that involved exposure to C9 aromatics. In response, the EPA reiterated the logic for excluding such studies and noted that "sections outlining the derivation of the RfC for each individual TMB isomer have been thoroughly edited to more clearly delineate the process by which the values were derived." Again, this is a thorough, straightforward, and appropriate response.

15) A further comment is made regarding the method used to calculate the RfD and the study used: "For the RfD determination the 90 day oral study with 1,3,5 TMB (Koch Industries, 1995b) is preferable to extensive extrapolation from inhalation data. Results have been accepted by EPA to characterize the hazards of 1,3,5 TMB. Reliance on this study would obviate the need for pharmacokinetic analysis and route-to-route extrapolation." Again the reviewer is trying to

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advocate for use of the C9 fraction exposure study. The EPA provides a thoughtful and thorough explanation for not using the Koch Industries study as the principal study. Further, they explain that of the three reviewers who initially critiqued the IRIS document, two of the three specifically noted that this study was not appropriate to derive reference values. Issues were raised with route of exposure, dosing regimen, and appropriateness of endpoints to human health. However, the EPA in its response notes that "the sections outlining the derivation of the RfD for each individual TMB isomer have been edited to more clearly delineate the process by which the values were derived." Thus, a detailed, thoughtful, and appropriate response was provided.

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Comments from Dr. Miller

General Charge Questions:

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

Response – Overall, the new Preamble achieves the goal of providing a clear and concise description of the guidance and methods the Agency uses when developing IRIS assessments. However, there are a couple of statements made in the Preamble to which this reviewer takes exception. For example, on line 78 of page xxiii, the statement is made “It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 2008)”. What is important is for a study to control the Type I and Type II error rates and the sample size to yield adequate statistical power. If this is achieved, this reviewer submits that it does not matter who funds a study. Case in point – would the IRIS program discount the NHERL Clinical Branch ozone research over a period of years that underpins the NAAQS for this pollutant because they have conducted almost all of the definitive low level exposure studies for this pollutant? Moreover, the studies have been conducted with adequate statistical power and control of the experimental error rates.

On line 53 of page xxv, the statement is made “Studies with adequate power to detect effects at lower exposure levels *are preferred*, to minimize the extent of extrapolation to levels found in the environment” (italics added here for emphasis). The goal should be to *require adequate power* in the conduct of any clinical, animal toxicological, or epidemiological study as this increases the likelihood of obtaining information most useful for the protection of public health.

In Section 1 of the Preamble relative to the scope of the IRIS program, the point is made that exposure assessments are not made in the IRIS documents. While such is understandable, this reviewer appreciates that the Agency included some information on what workplace and residential inhalation exposures can be for the TMBs. This allows the reader to put into perspective the RfC relative to what margin of exposure may be present between the RfC and real world exposures.

In the section describing **Subchronic-to-chronic exposure** (line 87, page xxix), the Preamble states that a UF of 10 is used if the point of departure is based on subchronic studies. An exception to this is a UF of 3 is justified if the subchronic exposure results in steady state levels of the dose metric of interest and if there is evidence that continued exposure is not likely to result in the worsening of the “critical effect” being used to develop the RfC.

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

Response – The new IRIS document structure provides a straightforward layout of the important hazard assessment studies, the dose-response analyses for critical endpoints, and the calculation of RfCs and RfDs for non-cancer endpoints in specific organs, and the identification of overall RfCs and RfDs. While cancer risk estimates are not calculated for the TMB isomers, one can assume that in instances where such calculations would be needed that the necessary information would be presented in a manner similar to what was done for non-cancer endpoints.

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

Response – The Agency has done an excellent job of addressing and incorporating a large number of recommendations contained in the 2011 NRC report. Appendix D in the Supplemental Document specific to the TMBs provides a clear update of what suggested improvements have been incorporated into the IRIS process and which ones remain to be addressed.

4. EPA solicited public comments on the draft IRIS assessment of trimethylbenzenes and has revised the assessment to respond to the scientific issues raised in the comments. A summary of the public comments and EPA's responses are provided in Appendix F of the Supplemental Information to the Toxicological Review of Trimethylbenzenes. Has EPA adequately addressed the scientific issues?

Response – Relative to the RfC, the Agency adequately addressed most of the scientific issues raised in the comments made by the public. However, the Agency's argument for not using the reproductive/developmental study on a C9 mixture that is dominated by TMBs to reduce the data set UF to 1 seems not very scientifically defensible (see this reviewer's comments to Charge Question E-4).

Chemical-Specific Charge Questions

A. Executive Summary

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

Response – For the most part, the conclusions are well described. There are several

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areas where more clarity would be helpful. For example, the paragraph beginning on line 17 of page xxxvi discusses values used of the various UFs. If one multiplies the listed values, the number 270 is obtained but the document states an overall UF value of 300 was used.

Stated on line 16 of page xxxvii to be a source of uncertainty is the fact that Korsak and Rydzynski (1996) did not state if their error terms for exposure levels were standard deviations or standard errors. However, the document authors indicate they deduced that they were standard deviations. This is not really a source of uncertainty because the standard practice in inhalation toxicology is to report variability of measured exposure levels in standard deviations (this arises because computer control systems and other aspects of exposure monitoring result in large numbers of measurements and reporting standard errors is misleading since the variability becomes independent of N by the time 30 or more observations are obtained).

This reviewer does not agree with the statement that the confidence in a database is lower if studies supporting the critical effect predominately come from the same research institute (line 27, p. xxxvii). As noted earlier in this reviewer's comments, the important criteria are that the Type I and Type II experimental error rates are controlled and that the sample size is sufficient to have good statistical power and not whether a particular institute has done most of the research in a given area.

The assumption invoked on line 6 of page xli that oral exposure results in 100 % absorption of 1,2,4-TMB is not scientifically defensible. It is unrealistic to assume that poorly water soluble compounds such as the TMBs are 100 % absorbed if introduced into the gastrointestinal tract. Moreover, this assumption clearly affects the magnitude of the RfD for the TMB isomers. The Agency should conduct a sensitivity analysis relative to the value for absorption.

B. Literature Search Strategy/Study Selection

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

Response – No comments.

C. Hazard Identification

Synthesis of Evidence

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

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weight of evidence for hazard identification has been clearly described and scientifically supported.

Response – No comments.

Summary and Evaluation

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

Response – No comments.

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

Response – No comments.

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?

Response – The PBPK model of Hissink et al. (2007) with some modifications by EPA scientists does a good job of describing the toxicokinetics of 1,2,4-TMB as evidenced by the myriad of graphical results of the BMD modeling that appear in Appendix C. Most of the physiologic parameters of the model are in reasonable agreement with those given by Brown et al. (1997), and the Agency ended up using the data of Brown et al. (1997) in their computer simulations. The chemical specific parameter data given in Table B-5 do not agree with literature values for a number of parameters. The document authors did a good job of describing the uncertainties in the model

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structure and provided an excellent analysis of coding errors in the Hissink et al. (2007) model, the corrections that they made, and a summary of the optimization and validation steps that they took.

The model assumption that the lungs are a well-mixed compartment, while not scientifically correct, does not negatively impact the resulting analyses because the model does not attempt to split the tracheobronchial region from the alveolar region. There is always a gradient within the lower respiratory tract, which would be important to account for if the critical effect used for the RfC was a pulmonary endpoint, particularly since the dose metric would not be based on the average venous blood concentration, but rather on a mass per unit surface area dose metric.

In Section B.2.3, the document overstates the degree of similarity between humans and rats in the metabolic profile of the various TMB isomers. In humans, the patterns for all 3 isomers are quite similar. However, with oral exposure in rats, the profiles appear to be quite different with 73 % being recovered as glycine conjugates in the urine for 1,3,5-TMB as opposed to 33 to 37 % being recovered for the other isomers as well as being recovered as dimethylhippuric acids.

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?

Response – Given that the critical effects upon which the RfC is being determined are neurological and, therefore, are extrapulmonary effects due to inhalation of the TMBs, the selection of venous blood concentration as the internal dose metric is reasonable. Adequate discussion of why this dose metric was selected is provided in Section 2.1.2.

However, there is a discrepancy in the statement of the above charge question relative to what is stated on line 11 of page 2-7. The charge question refers to the steady state weekly average venous blood concentration as the dose metric being used while the document states “Weekly average venous blood 1,2,4-TMB concentration was chosen as the internal dose metric on which to base the POD as it is assumed that the parent compound is the toxic moiety of interest and that average blood concentration of 1,2,4-TMB is assumed to adequately represent the target tissue dose across the multiple tissues of interest”. The text makes no claim of achieving steady state levels on a weekly basis. In fact, the experimental data for both rats and humans show that steady state is not achieved within a weekly exposure period. The half life of the 4th phase clearance of the 3 isomers in humans varies from 78 to 120 hours. Since 5 half lives are required for steady state, steady state cannot be achieved in a week in humans. In rats, the data in Table B-12 show that venous blood first achieves steady state after about 10 days with high levels of exposures for 12 hrs per day for 14

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consecutive days.

Using the PBPK model-estimated internal dose metrics as the dose inputs for BMD modeling required the Agency to drop the high dose exposures from all modeling efforts because the venous blood dose metrics consistently over predicted experimental results for high exposures. This reviewer is of the opinion that the over prediction is due to the fact that the PBPK model does not decompose minute ventilation into its two parts – tidal volume and breathing frequency. Well known is that fact that animals and humans try to maintain a constant minute ventilation level at a given workload by lowering tidal volume and increasing breathing frequency if the material being inhaled is a respiratory irritant. This yields less overall uptake of the irritant material and a shifting upward in the respiratory tract of absorption in the case of a gas or in deposition in the case of a particulate aerosol, thereby protecting the more sensitive alveolar tissue.

Clarification is needed on how the average weekly venous concentration used in the BMD modeling was determined for the analyses of the 90-day exposure studies. Over the course of the exposure period, the animals would add about 200 g of body weight, double their tidal volume and at least double the inhaled dose of TMBs. Were 13 weekly average venous concentrations determined and then the average of these determinations used? Was only the weekly average for the last week of exposure used? Or What? The footnote to Table 2-1 states that for the critical effect study (Korsak & Rydzyski, 1996), the average of the group specific body weights reported in other papers by Korsak were used in internal dose calculations. This statement implies that weekly venous blood levels for body weights of a given week were not computed and then the 13 resulting values averaged, which is what should have been done. The authors of the documents should clarify this topic at the review meeting.

If data were experimentally available on the arterial blood concentration to the brain, this dose metric would be preferable to a mixed venous blood dose metric. However, such data are not available. The uncertainties in the selected dose metric based on mixed venous blood are 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.

Response – The selection of the Korsak & Rydzynski (1996) study as the basis for the derivation of the RfC is adequately described and supported in Section 2.1.5 of the main document. In addition, the Hazard and Dose-Response material in other sections provide good background information that supports the Agency's selection.

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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.

Response – The selection of decreased pain sensitivity as measured by an increased latency to pawlick response after a hot plate test as the critical effect for deriving the RfC for 1,2,4-TMB is adequately supported by current science regarding the interpretation of neurotoxicity tests. The reasons for the selection of this endpoint are clearly described.

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?

Response – The steps the Agency took in the BMD modeling were clearly described in the document. Reference to the Agency's Benchmark Dose Technical Guidance document is frequently invoked. However, it would be useful if the authors referred to specific pages or sections within U.S. EPA (2012a) to help the reader find the supporting material in that document.

This reviewer strongly disagrees with the advice in U.S. EPA (2012a) to use a $p > 0.1$ level for the test of homogeneity of the variance for treatment groups. It has been well established that the analysis of variance is not very sensitive to departures from homogeneity until the rejection level is around 0.01. The Agency's use of $p > 0.1$ to not reject homogeneity causes the high dose to be dropped from BMD modeling. To this reviewer, the justification stated in the Benchmark Dose Technical Guidance document is weak. That justification is listed below

“....Since BMD modeling is usually a curve-fitting exercise involving a suite of models and since it is important that the data be adequately modeled for BMD calculation, it is recommended that $\alpha = 0.1$ be used to compute the critical value¹² for goodness-of-fit, instead of the more conventional values of 0.05 or 0.01.¹³ An exception to this recommendation is when there is an a priori reason to prefer a specific model(s), in which case the more conventional values of $\alpha = 0.05$ or $\alpha = 0.01$ may be considered. *P*-values cannot be compared

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from one model to another since they are estimated under the assumption that the different models are correct; they can only identify those models that are consistent with the experimental results.....

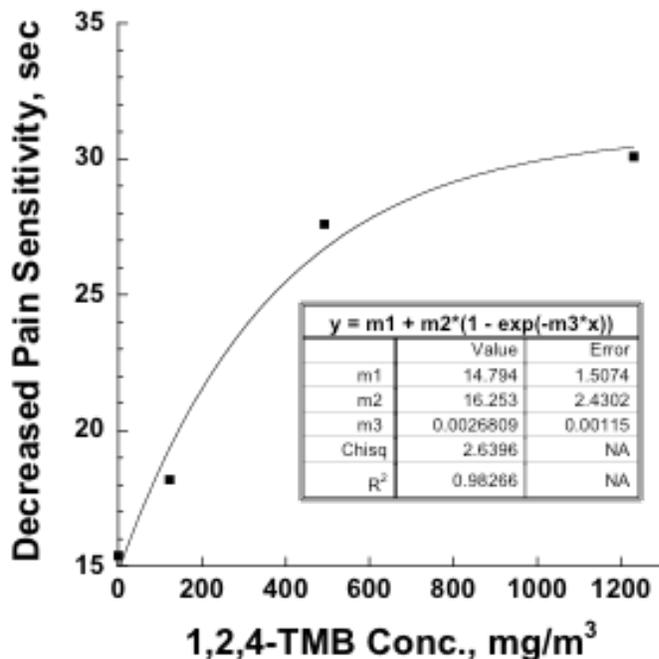
¹² For the χ^2 goodness-of-fit test, the critical value is the $1-\alpha$ percentile of the χ^2 distribution at the appropriate degrees of freedom. We reject for large values of χ^2 , corresponding to p -values less than α , the limiting probability of a Type I error (false positive) selected for this purpose.

¹³ Note that in some cases most of the available model fits may not appear to be adequate on the basis of goodness-of-fit p -values alone, i.e., p -values are less than 0.1. Some of these less adequate fits may be satisfactory when other criteria are taken into account (including the nature of the variability of the endpoint, visual fit, and residuals in the most relevant region of the data range.); expert judgment is useful in these cases.”

Part of the reason this reviewer considers the justification weak is that the sample sizes per group from a regression or curve-fitting viewpoint are more than adequate for the endpoints the Agency chose to model to be able to establish that the response means are appropriately identifying the trend in the concentration-response curve. In addition, use of footnote 13 seems to have been avoided as the BMD modeling documentation in the supplemental document is never invoked or referenced.

The results of the BMD modeling are not clearly described in Appendix C. As an example of what is lacking, consider Table C-2 and the output summary given on pages C-6 and C-7. The functional form should be given for all of the models. Model 2's form is given but the selected best fitting model is exponential Model 4 for which the functional form is not given. For the likelihoods of interest starting on line 11 of page C-7, what are models A1 to A3, model R, etc.? The highest dose used in the Korsak & Rydzyski (1996) study is dropped for this analysis due to the homogeneity of variance test results. However, if all the group means are used and an exponential rising model is fit, the result below is obtained for pain sensitivity versus the concentration of 1,2,4-TMB. The Agency would need to convert the concentrations to internal mixed venous blood concentrations and potentially weight the model by the variance if they chose to include all treatment group data.

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

Response – The rationale for the selection of most of the values of various UFs is well documented in Section 2.1.1 beginning on page 2-12. The only UF that this reviewer questions is the selection of a UF of 3 for database uncertainty because of the lack of a multi-generation reproductive and a developmental neurotoxicity study. The Agency has elected to dismiss the C9 fraction reproductive and developmental studies involving the 3 TMB isomers as part of a broader mixture even though the isomers comprised 55.05 % of the mixture by weight.

Beginning at the bottom of page E-8, the Agency states “Therefore, although there are available peer reviewed studies investigating the toxicity of the C9 fraction, the uncertainty regarding any interactive effects other C9 constituents may have on the ADME of TMB isomers and the general lack of reported effects limit their utility for the assessment of the human

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health risk of individual TMB isomers. For these reasons, these studies were not included in the Toxicological Review". If competitive binding exists between the TMB isomers and the other C9 constituents of the mixture exposures, a conservative approach would be to assume all of the effects are due to the TMB isomers as opposed to a percentage of binding as a function of the percentage of the compound in the mixture. In so doing, one only has to solve for the ppm level of the isomers in the mixture exposure.

The exposure concentration in ppm of each of the organic compounds in the reproductive and developmental study by McKee et al. (1990) can be calculated using the following steps:

- 1: Convert weight percent to number of molecules
- 2: Calculate the total number of molecules in the mixture
- 3: Of the total number of molecules in the mixture, calculate the fraction that is associated with each organic compound.
- 4: Use this fraction (based on number of molecules in the mixture) and the ppm of the mixture in the exposure atmosphere to calculate the exposure concentration in ppm of each organic compound

The results obtained by doing the above yield the table below.

Assigning the entire effects of the C9 mixture studies to the concentration of TMB isomers present in the mixture is a conservative approach. The Agency should re-examine its decision regarding the exclusion of the C9 study results at least as far as their evaluation of the need for a UF of 3 for the database. This reviewer is of the opinion that a UF of 1 for the database is warranted. At any rate, the Agency would be well advised to further examine the database UF. *NOTE: The HERO database only has the abstract for the McKee et al. (1990) paper. Having the full papers in HERO would facilitate review of EPA documents like the one being currently reviewed for TMBs.*

Compound	MW ^a	WT%	WT%/MW	Fraction of Mixture	Mixture Exposure Concentration, ppm		
					100	500	1500
					Compound Concentration, ppm		
o-xylene	116.16	3.2	0.02755	0.0336	3.36	16.81	50.44
cumene	120.19	2.74	0.02280	0.0278	2.78	13.91	41.74
n-propylbenzene	120.19	3.97	0.03303	0.0403	4.03	20.16	60.48
4-ethyltoluene	120.19	7.05	0.05866	0.0716	7.16	35.80	107.40
3-ethyltoluene	120.19	15.1	0.12563	0.1534	15.34	76.68	230.03
2-ethyltoluene	120.19	5.44	0.04526	0.0552	5.52	27.62	82.87
1,3,5-trimethylbenzene	120.19	8.37	0.06964	0.0850	8.50	42.50	127.51
1,2,4-trimethylbenzene	120.19	40.5	0.33697	0.4113	41.13	205.66	616.97
1,2,3-trimethylbenzene	120.19	6.18	0.05142	0.0628	6.28	31.38	94.14
≥ C10's	128.17	6.19	0.04830	0.0590	5.90	29.48	88.43
Totals		98.74	0.81925	1.0000	100.00	500.00	1500.00
Concentration of all TMB isomers, ppm					55.91	279.54	838.62

F.

^a Molecular weight of C10s assigned that for C10H8. Other values of C10s could also be used.

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

1. A 90-day inhalation toxicity study of 1,2,3-TMB in male rats ([Korsak and Rydzyński, 1996](#)) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.

Response – The selection of the Korsak & Rydzyński (1996) study as the basis for the derivation of the RfC is adequately described and supported in Section 2.2.5 of the main document. In addition, the Hazard and Dose-Response material in other sections provide good background information that supports the Agency's selection.

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.

Response – The selection of decreased pain sensitivity as measured by an increased latency to pawlick response after a hot plate test as the critical effect for deriving the RfC for 1,2,4-TMB is adequately supported by current science regarding the interpretation of neurotoxicity tests. The reasons for the selection of this endpoint are clearly described.

3. In order to characterize the observed dose-response relationship comprehensively, benchmark dose (BMD) modeling was used in conjunction with default dosimetric adjustments ([U.S. EPA, 1994b](#)) for calculating the human equivalent concentration (HEC) to identify the point of departure (POD) for derivation of the RfC. Please comment on whether this approach is scientifically supported for the available data, and clearly described.
 - a. Has the modeling been appropriately conducted and clearly described, based on EPA's *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#))?
 - b. Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR equal to a 1 standard deviation change in the control mean for the latency to pawlick response) been supported and clearly described?

Response – The statement on p. 2-24 that the standard deviation (SD) of the highest exposure group caused the variance power law model to not fit the data accurately is puzzling. The SD of 3.4 is essentially the same as the SD of the 123 mg/m³ group and is well within what one would consider as reasonable variation among groups. Similarly to the case for 1,2,4-TMB, this reviewer was able to fit an exponential rising model to the group 1,2,3-TMB mean responses when all groups were included. The deletion by the Agency of the highest exposure group led to a linear model with a BMR involving 1 SD change in the control mean being used.

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

Response – Selection of the value for most of the UFs was adequately defended in Section 2.3.3 for 1,2,3-TMB. However, this section did not discuss why the UF for subchronic to chronic exposure was assigned a value of 1 for decreased body weight in male and in female rats in the developmental study of Saillenfait et al. (2005). In comparison, the maternal decrease in body weight for this UF was given a value of 3.

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

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

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

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

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

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

Response – Since a route-to route extrapolation from inhalation to oral was used for 1,2,4-TMB, the document correctly notes that the UF values for the oral RfD should be the same as they are for the RfC. The document notes that the model used to conduct the route-to-route extrapolation is well characterized and appropriate to use. The scenario for this isomer fits quite well with the decision tree for when an inhalation to oral extrapolation is appropriate, as discussed in Principles of Route-to-Route Extrapolation for Risk Assessment (Eds. T. R. Gerrity and C. J. Henry, Elsevier, pp. 322, 1990).

The authors correctly note in Section 2.4.4 that the assumption of 100 % absorption of 1,2,3-TMB via the oral route is a source of uncertainty. This reviewer does not agree with this assumption, particularly since all TMB isomers are relatively insoluble in water and the mucous secretions in the gastrointestinal tract contain large amounts of water. A percentage less than 100 for oral absorption is warranted, with the net result being that a higher RfD would result. This reviewer recommends that the Agency conduct a sensitivity analysis to the effect of lowering the percentage absorbed to values other than 100 %.

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

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

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

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

Response – Since there are insufficient data available to set an RfD for 1,2,3-TMB, the Agency elected to use the RfD for 1,2,4-TMB as the RfD for 1,2,3-TMB. Given the similarity in structure and ability to affect the same biological endpoints, the approach by the Agency appears reasonable. The rationale is clearly described and the concomitant uncertainties are articulated in the document.

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

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

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

Response – The only available study for potential use in developing an RfD for 1,3,5-TMB was a study by Koch Industries (1995b) that had been submitted to EPA as part of TSCA requirements. An external peer review of this study led the Agency to conclude the study was not suitable to serve as the primary study for calculating a RfD for 1,3,5-TMB, primarily because neurotoxicological endpoints were not studied. Thus, the Agency's invoking of similarities among the 3 TMB isomers relative to their chemistry, toxicology, and toxicokinetics was reasonable for assigning the same RfD value to 1,3,5-TMB as was derived for 1,2,4-TMB. The document clearly described the Agency's logic and position on this matter.

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

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

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Comments from Dr. Rhomberg

General Charge Question 1

Does the new Preamble provide a clear and concise description of the guidance and methods that EPA uses in developing IRIS assessments?

To begin, it must be acknowledged that it is a substantial challenge to make a specific and concise description of a process that at its core depends on coming to reasoned findings about complex matters. IRIS assessments require integration of considerations across diverse data, with different cases hinging on different scientific issues, having varying abundance or quality of data, having different degrees of agreement or disagreement among those data, and having varying levels of understanding of the fundamental underlying biological processes that appear to operate. The challenge for guidance is to be sufficiently specific and operational (in the sense of specifying steps and purely objective criteria for choices) that it can support consistency from case to case and transparency about how and on what basis choices are made, and on the other had to be sufficiently flexible and integrative across the whole body of pertinent evidence to make sound and defensible case-specific judgments. It is easier to explain the application of a formulaic process of steps and rules, but it would be unwise to diminish scientific thoughtfulness and case-by-case judgment only to make the process more transparent. Producing a good Preamble is doubling challenging because many of the parts of the process it describes are in the middle of being reconsidered and reformulated.

General Charge Question 1 asks about the clarity and concision of the Preamble as an explanation, not about the soundness of the process itself or of the guidance it implements. This distinction needs to be borne in mind in formulating a response to the question -- it is quite possible for Committee members to find the Preamble clear and concise but nonetheless take significant issue with the wisdom of parts of what it is describing.

To a substantial degree, the Preamble as currently written succeeds in providing 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 that can be taken in as a whole. It is best to discuss the Preamble as 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 3-7).

The description of "Scope of the IRIS Program" (Section 1) is brief and clear as to what IRIS is, 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. Is the aim to produce comprehensive evaluations fit for any purpose and user or just ones that suffice to meet specific regulatory needs? Is the aim pure objectivity or is there a role for precaution and conservatism? What does it mean for an agent to be taken up by the IRIS process and what does IRIS's failure to take up an assessment task mean? Are the choices of what chemicals to do driven by immediacy of regularly needs

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or by levels of concern for toxicity or by widespread exposure? Is the nomination process (described in lines 46-58 of p.xxii), and the resulting scheduling, one of prioritization or of meeting a threshold for need of IRIS treatment? And whose choice is it ultimately to undertake an assessment? Does EPA see only IRIS assessments as legitimate for regulatory support (except for the legislatively mandated exceptions, as noted) or could non-IRIS assessments serve in some cases? Does the process aim to stand in for the collective judgment of the field, including the assent of peer review, or must it only satisfy the judgments of those in the IRIS program, with peer review being only advisory? It is not that these questions have no answers, but rather that the Preamble -- and specifically Section 1 on Scope -- should set out the objectives and hoped for standards that IRIS assessments will be built to meet.

Both because of the need to make hard-to-explain judgments and because of the transitions underway in the IRIS process, it is important for there to be statements about what the processes and entailed judgments intend to accomplish. This can help explain what goes into -- and is taken to justify -- choices that are hard to explain as mere rule-following. In view of the partial implementation of reforms to the overall process, if the issues the changes are intended to address are discussed, and the objectives of any new methods acknowledged and addressed in good faith to the extent possible even before new processes are implemented, the overall revision of the IRIS process will be smoothed and assessments done before the process is complete will gain credibility and longevity.

Section 2 on the IRIS Process is clear and concise about the steps and what happens at each one. It is rather vague, however, on the nature of the problem formulation step. It is to be hoped that this becomes more than simply a chance to air thoughts and nominate data for consideration to include some genuine discussion (without seeking to constrain the agency's further actions) about the issues needing to be addressed, the prospects for addressing them with available data, and the uncertainties and plausible alternative interpretations that would need to be worked through. Similarly, Section 2 is not very specific about how the input and commentary from Program Offices, from other agencies, from the public, and from the peer reviewers is to be considered. It is clear that a public record of disposition of comments will be made, but there is no discussion as to whom the decision-making is ultimately accountable or whether the peer review process will be consulted about the disposition of its findings. The discussion of Step 5 notes that newly published studies that are critical to conclusions can be brought into consideration, but a more explicit reference to the stopping rule policy (and where its details can be found) would be appropriate.

Sections 3-7 lay out the more particular steps of actually conducting an assessment. A good deal of it is summary of long-extant guidance, and so will be familiar to most readers. The Preamble purpose does require setting out the processes and analyses, but perhaps this could be done somewhat more briefly. On the other hand, the aspects that are under revision need to be either flagged or expressed in more general terms. In particular, the Preface notes that Phase 3 of the IRIS revision process is yet to happen, and this will include review of current methods for weight-of-evidence analysis. The methods for abstracting data, systematically considering study quality and interpretation issues, and ranking relative study impact are also in transition. It needs to be clear what guidance still applies, what is being revised, and (when guidance applies for now but may not in the future) what may change.

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Section 5.5 names the carcinogen classification scheme of the 2005 Carcinogenicity Assessment guidelines, which presumable are part of what is being reconsidered. The same section then cites the ISA criteria for causality (applied in the evaluation of criteria pollutants) as "another example" and then even further on raises the issue that the agency is investigating what descriptors to use and may use these or others. This is confusing as to what applies at present, and is an instance in which discussing the intent of descriptors is probably more useful than recounting definitions that may or may not be used and may or may not be seen as in keeping with the spirit of the overall revision process.

The Preamble itself is not guidance; it only describes, summarizes, and explains guidance. This is not always clear, and an unambiguous statement to this effect should be added. This is especially critical because -- being only summaries and explanations -- the treatment in the Preamble is less developed and unaccompanied by the fuller discussion about motivation, meaning, interpretation, and scientific justification of the briefly described analyses, presumptions, standards, or judgments, and without reference to the fuller treatment, there is danger that an oversimplified version may be latched onto by readers and mistaken for policy. In some places where existing guidance is described and explained, there is a citation to the full guidance document, but on many spots, there is no such citation. Citations are necessary if the Preamble is to adhere to the distinction between established policy and explanation, noted above. They also give readers an indication that there is a fuller description of the issue to be found and where to find it. Uncited assertions about methods, interpretations, or standards of evidence are in danger of being taken (and perhaps mistaken) for established policy, and the Preamble could be inappropriately cited to support claims of such status.

Among these latter uncited assertions are several that are not familiar from existing guidance or announced policy. They seem to have crept into the Preamble's description of methodology, and so the concern is beyond merely editorial and includes the question of whether statements represent real policy changes and whether such changes have been appropriately vetted, discussed, reviewed, and implemented. Different Committee members may cite different instances as of most concern, but among those to be mentioned are the statement on p.xxii (line 67) that negative genetic toxicity studies carry less weight than positive ones, the statement on 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. All of these can be -- and indeed should be -- debated, but in any case, it is necessary to support such statements with citation to existing policy or guidance. If these are not existing policy, then they should be flagged as matters under discussion.

General Charge Question 2 --

Are the steps and structure clear and do they contribute to the ability to make the assessment concise and easy to follow?

It should be the objective to make it possible to read the document in three different modes: (1) quickly to

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

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

The second way of reading -- for the choices and reasoning -- has also markedly improved, though there are some suggestions that can be made, as is returned to in discussion further below.

The third way of reading -- the examination particulars and the ability efficiently to find them documented with sufficient detail, is much improved from the former state of IRIS documents. The relegation of a lot of the details to well structured appendices is helpful. The set of focused appendices helps a reader to find the place where particular study aspects or analyses of issues is to be found. The organization of the appendices -- and the consistency of presentation across IRIS documents -- are important in making the place to find details clear. Although the general structure of the appendix entries can be discerned, the plans for the structure and consistency they are to adhere to has not been provided, so it will take some time and examination of other documents following the same plan for readers and reviewers to develop a good sense of the consistent structure that will aid in making particulars easily found. The use of appendices simultaneously allows presentation of more detail than may have been captured in earlier generations of IRIS documents and also avoids cluttering the main body of the IRIS document -- where interpretation and evaluation are considered. The appendix approach also frees the main document from seeming to need to present all the details before drawing any interpretation. The typical model for a scientific paper on a particular investigation uses the data-then-interpretation structure, but a complex assessment document makes this approach difficult to follow. Once it is clear that the detailed data were examined first, despite their relegation to appendices that appear at the end of the document, the main text is freed to make for a more readable documentation of evaluation and interpretation.

For each documented study, it might be good to have a consistently formatted table that presents the study-specific considerations that bear on evaluation of study quality and pertinence, including shortcomings and assumptions that are needed in interpretation of the study's outcomes.

It may also be useful to have for each study a short text section that briefly provides an overview (not repeating tabulated details) of the nature of the study, its examined endpoints, and main findings of apparent relevance. The aim would not be to draw interpretations but rather to provide context for the tabulated details, so that the details need not be read in full to gain an idea of the general nature of the study and its importance to the assessment as a whole.

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It is clear that the intent of this structure is to free the main document text to focus on the second way of reading -- to see the choices that were made in the analysis (among possible endpoints, among studies to represent and characterize those endpoints, and analyses and interpretations of their bearing on human risk estimation). The challenge is to bring the appropriate data and level of detail from the appendices into the main body, so that the interpretations and choices can be justified and documented, without overwhelming the interpretation discussion or leaving potentially relevant parts of findings out. Sorting this out is the essence of the systematic review process, and though there have been clear strides ahead, it is also evident that more work is left to be done. The key to this process is to be forthright about not just what is chosen for inclusion but also what is left out; not just what supports an interpretation, but what seems unexplained or even inconsistent; not just what interpretations for bearing on human risk can be proposed, but what alternative interpretations could also be plausible.

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

The Literature Search Strategy section is brief and focuses only on identification of pertinent studies from the literature. It should be clear that this is only the first step of systematic review, which needs to be followed by evaluation of each study in terms of design, quality, shortcomings, main findings (including both positive and negative findings) and evaluation of the reliability of individual study results. There is a further process of comparing results across studies when they address phenomena that ought to have commonality if there is a true underlying effect, and also an evaluation of how any effects seen in particular studies are concluded to generalize to other settings, such that their bearing on the larger assessment can be judged. The individual-endpoint sections of the Hazard Identification have some discussion about interpretation across studies and evaluations of bearing and relevance, though somewhat more extensive discussion of interpretation rationales and consideration of alternatives would be beneficial. But it is this middle section of systematic review -- after the studies are chosen but before the interpretation of their overall bearing gets considered -- that does not have a clear home in the current document structure. As the agency develops its approach to systematic review, including defined ways for abstracting data, judging study quality, documenting factors bearing on interpretation and its limits, and considering the bearing for suites of studies, it will be important to develop the document structure that presents, documents, and explains these considerations. It is noteworthy that the Preamble has a section (Section 5) on evaluation of causality, the execution of which depends on the existence of such a documented review and evaluation process, but the present document has no particular place where the Preamble's named considerations -- strength, consistency, specificity, temporal relationship, biologic plausibility, coherence, natural experiments, and analogy -- are systematically considered or documented.

It may be useful to consider adding a brief section on pharmacokinetics and metabolism before the section on Hazard Identification. The aim would be only to set the context for the interpretation of studies bearing on hazard, and the main presentation of pharmacokinetic details should continue to reside in the appendix. The main text's section would note such things as extent of absorption, rapidity of elimination, main metabolic processes, main means of clearance (and what part of that is by metabolism), indications whether metabolic saturation or enzyme induction might play a relevant role in toxicity studies, and any notable unusual differences between experimental animals and humans. Again, the point would not be

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just to list specifics (which can remain in an appendix) but to provide the basic insights that might bear on how toxicity data are interpreted or on the limits to such interpretation.

Within the Hazard Identification section, the separation of endpoints is useful (though potential ties between endpoints and consistency of their behavior in light of pharmacokinetic and mode-of-action considerations should not be overlooked). The tabulation of studies is useful, and the preservation of the particular dose levels and dose-specific responses are important details to include. The links to the detailed description of studies in the appendices helps to make those appendices directly supportive and makes finding of relevant particulars more efficient. The exposure-response arrays are useful summary devices, though they should not be read as meta-analysis forest plots or otherwise be used as the justification of conclusions. Nonetheless, they provide a valuable means to see an overview of the data. It is perhaps unfortunate that it is difficult to preserve the distinction between several studies on a given effect (especially if the studies appear to disagree) and also that the particular dose-levels shown are only the extremes, the NOAEL, and the LOAEL. It would be useful, for instance, to see how many dose levels without effect exist below the NOAEL. There is also no ready way to visualize dose-response patterns, and one must rely on the data in the study tables.

In the dose-response section, the tabulation of PoDs, HECs, and applied UFs is useful, allowing endpoints to be compared and the distinction between a low PoD with few UFs and a high PoD with many UFs to be seen.

It represents an important advance that the Hazard Identification sections for each endpoint have specific places where there is discussion of consistencies and inconsistencies among data, on the bearing of studies for the task of human risk evaluation, on the knowledge of mode of action (even if it to say that little is known), and on alternative interpretations that could be hypothesized for sets of results across studies and for the bearing of the available data on causal interpretations. The structure to the text that makes a point of visiting each of these issues in an orderly way for each endpoint is important to advancing the explanation of the basis for conclusions and enhancing transparency. This said, some of the interpretation passages suffer from trying to be too concise, so as not to interrupt the larger exposition. When there is more to be said on some of these issues, it would be good to find a structural way (perhaps more appendices) to document the working through of the arguments without unduly bogging down the main discussion.

As it stands, both the Hazard Identification and Dose-Response sections simply dive in to the first endpoint or analysis to be considered, and then have separate sections on each. There is little overview to prepare a reader for what is coming or to point to the parts that are critical versus those that are there for completeness. In general, in aid of enabling a reader to grasp the main lines of argument and only go into detail when needed, it might be good for both the Hazard Identification and the Dose-Response sections to have an initial paragraph setting out the main things that will be considered and indicating which considerations (to be developed in the subsequent text) are the most notable for the larger assessment process. A parallel paragraph at the end of each of these chapters could summarize what its contents mainly bring to the larger assessment process, and name the choices of things to focus on and things that have been deemed ancillary. The aim of these paragraphs would be to make it possible to read the

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document in more detail than provided in the Executive Summary (which largely documents findings) but still as a quick run through to see the deeper structure of the report and where to focus search for more information on particular aspects. That is, the initial and last paragraphs as proposed would not be justifications of choices, but only a guide to the more detailed discussion in each section.

General Charge Question 3 --

How is EPA's progress?

In general, there has been a lot of progress in restructuring the document to make the main body focus on documenting and explaining the interpretations, choices, and analyses, and relegating the listing of the contributing information to appendices. The use of links to the appendices aids in using them as support, without encumbering the flow of the main arguments. At the same time, the details and particulars matter as they are used in constructing arguments, and it will be an ongoing challenge to bring enough into the main text to document the reasoning, avoid leaving important aspects hidden in the appendices, and still have an easily read document that fully explains the choices and conclusions made.

The process of systematic review still needs development. There still needs to be further development and documentation of the process of identifying literature has progressed, but the further process of establishing standard practices for abstracting relevant data, for evaluating study quality, strengths and shortcomings, and raising considerations for how the data are to be brought to bear. This includes the phase of evaluating individual studies, that of comparing the results of studies of similar objective into characterizations of their joint bearing in a way that addresses discordant results, and the overall integration across lines of evidence into overall judgments about causality and about appropriate dose-response analyses.

In this development, it should be borne in mind that the process of systematic review is not solely one of identifying the "right" or the "best" data, with the interpretation and bearing on risk evaluation becoming clear once the right choices are made. The integration and weight-of-evidence evaluation process requires a good deal of consideration of interpretations of the data – even the best or most appropriate data. This process needs to consider how results of particular studies are to be generalized so as to apply to other situations (and especially to the expected action of the agent in humans at the exposures they experience), it needs to provide some account of why other study results might disagree, and it needs to consider not just possible interpretations or the ones generating particular risk concern, but also others that may explain the array of results differently, with different consequences for risk estimation.

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

The recommendations for revision of the IRIS process that come from the NAS "Roadmap" (Chapter 7 of the Formaldehyde review) and other sources. A good principle to follow in conducting assessments during the process of revision is to consider the reasons behind the recommendations, and to make good faith efforts to address the issues forthrightly, even if in doing so the methods may not yet be fully developed and agreed upon. That is, in the absence of fully developed new methods, trying to address as well as one can the issues behind the recommended methodological and procedural changes is a good way

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to make assessments as reformed as they can be, and apt to find better acceptance for a longer time as the overall IRIS process continues to advance.

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Comments from Dr. Taioli

A. Executive Summary

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

The "confidence" section of the summary needs some more thought in my view. While it is clear what the consequences of the "confidence" score are (for example, as stated on page XXX, low confidence indicates that the reference value is especially vulnerable to change with further testing), it is not clear what are the quantitative elements considered to calculate the actual score. The description of how a score is derived seems very qualitative and variable according to the individual paragraphs. In general, it should be quantitative, defined a priori, and standardized across the sections of the executive summary.

B. Literature Search Strategy/Study Selection

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

The description of the selection process should separate the reasons and number of articles "considered" but not included for human and animal studies. Right now it is difficult to assess the goodness of the exclusion criteria without knowing the reasons for exclusion for human studies, for example. While it is very useful that section B4 of the supplement reports all the details on each human study, it would be very important to have a summary table of the studies related to each health effect. For example, a table with the 9 studies on neurotoxicity in humans, reporting study design, inclusion, exclusion criteria, number of subjects, main results. This is common practice in epidemiologic reviews and meta-analyses. The current way of presenting the study is very analytical, but hard to summarize.

Kinetic studies in supplement table B 13: not sure why Janasik 2008 and Jones 2006 are not included.

Summary and Evaluation

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

Yes this section is written very clearly and concisely. I would include something more about dose-response effects, even though most of the studies are complex mixtures. I would also compare the magnitude of doses used in animal studies with the magnitudes of doses humans are currently exposed to, even if the study looks at the chemical within a mixture.

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

I am not sure that we have everything that has been published on this topic. I found these articles on Medline, for example:

Silva CL, Passos M, Câmara JS. Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of potential urinary cancer biomarkers--a powerful strategy for breast cancer diagnosis.

Talanta. 2012 Jan 30;89:360-8. doi: 10.1016/j.talanta.2011.12.041. Epub 2011 Dec 22.

Silva CL, Passos M, Câmara JS. Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry. Br J Cancer. 2011 Dec 6; 105(12):1894-904. Epub 2011 Nov 15.

Gaschen A, Lang D, Kalberer M, Savi M, Geiser T, Gazdhar A, Lehr CM, Bur M, Dommen J, Baltensperger U, Geiser M. Cellular responses after exposure of lung cell cultures to secondary organic aerosol particles. Environ Sci Technol. 2010 Feb 15;44(4):1424-30. doi: 10.1021/es902261m.

I suspect that if we look into "biomarkers" and "cancer" and TMB, we can find more of these articles. We need to make sure that we read these articles and comment them. Section 1.2.3 appropriately comments on the metabolic steps that TMB undergoes in the body after inhalation or oral exposure, and how this affects different population ages in different ways; this could be nicely connected to carcinogenicity and biomarkers of TMB metabolism.

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

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

I am ok with this, although I reserve my last comments after I see a review of the biomarkers papers

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Comments from Dr. York

TMB Panel Revision for General Charge Question 4.

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

Public comments on the draft IRIS assessment of trimethylbenzene (Appendix F) focused on the standards and transparency of the draft document and several scientific areas: 1) why EPA did not use the available data on C9 mixtures (mostly TMB isomers and ethyltoluene isomers, which according to the comments, have similar toxicological profiles) for the IRIS evaluation; 2) why EPA identified the critical endpoint as pain sensitivity based on a transient latency in paw lick to a hot plate stimulus following subchronic exposure, that was not evident after chronic exposure and was a reversible response after two-weeks post-exposure; and 3) why the 1,3,5-TMB oral gavage toxicity study (Koch Industries, 1995), the results of which were already accepted by EPA, was not used to reduce the identified uncertainties

EPA has reasonably addressed the standards and transparency comments in Appendix F.

The omission of data from the two TSCA (4a) Test Rules (U.S. EPA. 1985 and 1993) need to be included in the Review Document. The Test Rule for 1,3,5-TMB (Fed. Reg. vol. 58 no. 216, pp. 59681-82) and the protocols that were developed, are critical in understanding why: multiple species were not used; exposures were only on weekdays; group sizes were 10/sex; certain animal housing was used; blind observations were not used; and no attempt was made to link the results to an extensive literature review of other organic solvents. It needs to be clearly stated that endpoints involving TMB blood concentrations, behavior, neurotoxicity, electrophysiology and respiratory function were not in the Test Rule nor in the 1979 EPA Toxic Substances Control Act (TSCA) testing guidelines 40 CFR 798.2650, and therefore not required for the Koch Industries (1995) study.

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

One developmental toxicity study (Saillenfait et al., 2005) following inhalation exposure to 1,3,5-TMB was identified in the literature and was considered as a potential principal study for the derivation of the RfC for 1,3,5-TMB. However, the candidate RfC derived for 1,3,5-TMB based on this study (and the critical effect of decreased maternal weight gain) was 20-fold higher than the RfC derived for 1,2,4-TMB (based on decreased pain sensitivity). Given the available toxicological database for 1,2,4-TMB and 1,3,5-TMB, there are several important similarities in the two isomers' neurotoxicity that support an RfC for 1,3,5-TMB that is not substantially

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different than the RfC derived for 1,2,4-TMB. Additionally, the available toxicokinetic database for the two chemicals indicates that internal dose metrics would be comparable. Thus, EPA concluded that deriving such disparate RfCs for these two isomers was not scientifically supported. Rather, EPA concluded that given the similarities in toxicokinetics and toxicity between the two isomers, there was sufficient evidence to support adopting the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB.

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

A developmental inhalation toxicity study by Saillenfait et al. (2005) was considered as a potential study to identify a critical effect for 1,3,5-TMB RfC derivation because there existed no chronic or subchronic studies to support RfC derivation (unlike the other two isomers where at least subchronic toxicity studies had been conducted). Anne-Marie Saillenfait is a well-published developmental toxicologist in France and the Institut National de Recherche et de Sécurité (full name is National Institute for Research and Security for the Prevention of Occupational Accidents and Diseases) works under the auspices of the National Health Insurance Fund composed of equal numbers of representatives of employers and representatives of the unions. That said, the Saillenfait study is well-conducted and follows EU guidelines for that time. They used an appropriate animal model and strain, exposure chamber generation, five concentration groups, atmosphere sampling and analysis, group size, maternal and fetal evaluations, and statistical data analyses. Therefore, I consider it a robust developmental toxicity study. The one short coming is the manuscript lacks sufficient reporting for some observations (clinical observations, body weights, uterine weights) for a clearer picture of maternal and fetal toxicity. Examination of the actual study report with the individual dam and fetal data would circumvent this short coming.

The authors of the Saillenfait paper selected the 100 ppm (492 mg/m³) for the maternal NOAEL for mesitylene (1,3,5-TMB isomer) and 300 ppm (1476 mg/m³) exposure as the maternal LOAEL based on decreased maternal weight gain and food intake. I agree with this assessment. The decreased corrected average maternal weight gain over the treatment period (GDs 6-21 minus the gravid uterine weight) for the 300 ppm (1476 mg/m³) group was 20g. While the 20g decrease over the 15-day treatment was not statistically significantly different from the average 29g decrease in corrected maternal body weight for the 300 ppm (1476 mg/m³), it was 31.0% below the control group value. This percentage decrease (-31%) in corrected pregnant dam average weights is more than a minimal response and is biologically significant.

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Saillenfait et al. selected 100 ppm (492 mg/m³) as the maternal NOAEL and 300 ppm (1476 mg/m³) as the maternal LOAEL based on decreased mean body weight gain and food consumption values; the developmental NOAEL was 300 ppm (1476 mg/m³) and the developmental LOAEL was 600 ppm (2952 mg/m³) based on decreased mean male fetal body weights.

In the Toxicology Review of Trimethylbenzene (August 2013), EPA appears to set the maternal NOAEL at 300 ppm (1476 mg/m³) and the maternal LOAEL at 600 ppm (2952 mg/m³) based on decreased corrected body weight gain, higher exposure levels than Saillenfait et al. EPA set the developmental NOAEL at 300 ppm (1476 mg/m³) and the developmental LOAEL at 600 ppm (2952 mg/m³) based on decreased male fetal body weights, the same exposure levels as Saillenfait et al. (see Tables 2-12 and 2-13). Why was this the case?

I disagree with Saillenfait's developmental NOAEL and LOAEL assessments. There is clearly a decrease in mean male fetal body weight for the 300 ppm (1476 mg/m³) group (5.50 ± 0.31g versus the control group of 5.80 ± 0.41g, which is a 5.2% decrease). Albeit, there is no statistical significance indicated on Table 3 of the Saillenfait paper. Using the BMD approach, EPA states a 5% decrease in fetal body weight relative to control values was determined to be a minimal, biologically significant response (see p.2-8, in the Toxicology Review of Trimethylbenzene (August 2013).

I would have set the 100 ppm (492 mg/m³) as both the maternal and developmental NOAEL and 300 ppm (1476 mg/m³) as the maternal and developmental LOAELs based on statistically significant decreased maternal body weights (GDs 13-21, 6-21, and corrected weight gain), statistically significantly decreased food consumption values (GDs 2-13, 13-21, and 6-21) and biologically significantly decreased fetal body weights (male, female and both sexes combined).

I also disagree with the selection of BMR exposure levels in the Toxicological Review of Trimethylbenzenes (August, 2013). In Section 1.1.3. Reproductive and Developmental Effects (p.1-32), the document states "The decrease in food consumption at 1476 mg/m³ 1,3,5.TMB (92% relative to controls) was not considered to be a marker of adversity given no accompanying decrease in maternal weight gain was observed at that concentration". This is absolutely not the case. In the Saillenfait paper, Table 1, statistically significant decreases were observed GDs 13-21 (-12.6%) and GDs 6-21 (-12.6%) and matched the statistically significant decreases in food consumption values observed over the same periods, GDs 13-21 and GDs 6-21. I assume the document chose to select the Corrected Weight Gain in dams, which was not statistically significant (although reduced by 31%), as the marker. For this endpoint, there is no food

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consumption value for comparison (because one can't distinguish what amount of food the pregnant dam is eating for herself and what she is eating for her litter).

Further down in the same paragraph (p. 1-32), the document states "Fetal body weights were decreased (statistically significantly) by 5-13% at concentrations > 2,952 mg/m³ of 1,2,4-TMB and 1,3,5-TMB". As pointed out above, male fetal body weights were decreased 5.2% at 1,476 mg/m³ of 1,3,5-TMB.

The Toxicological Review of Trimethylbenzenes (August, 2013), in Section 2.3.2. Methods of Analysis for 1,3,5-TMB (p. 2-36), used 2974 mg/m³ as the POD (p. 2-37) for the developmental endpoint (decreased male fetal body weight) which was not the NOAEL listed in Table 2-13 (p.2-38). Shouldn't 1471 mg/m³ have been used for the POD? This will change the whole calculation for POD_{HEC} presented in the table.

In Section 2.3.1. Identification of Studies and Effects Other Than Cancer for 1,3,5-TMB, there were three other errors in Table 2-12 that need to be addressed. The female fetal body weight average for the 100 ppm (492 mg/m³) group should be 5.47± 0.21 and not 5.74± 0.21 (it is correct in other tables of the document). The level of significance for decreased maternal body weight gain for the 600 ppm (2,952 mg/m³) group should have two (***) and not one (*) asterisk to indicate the $p < 0.01$ level. The table also states with a footnote (b) that the numbers of live fetuses is not explicitly reported. However, the authors did report them in the Saillenfait et al. (2005) manuscript in Table 3. The total numbers of fetuses were 297, 314, 282, 217 and 236, for the control and exposure groups, respectively, and should be included in Tables 2-2 and 2-12 of the draft TMB Review document.

The scientific justification for not deriving an RfC based on the available data for 1,3,5-TMB and using the derived RfC for 1,2,4-TMB isomer is supported in the document. An alternative derivation may give a RfC of 1.7×10^{-1} (see below), which may lend additional support to a RfC of 5×10^{-2} based on an isomer.

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

My first concern is the duration adjustment applied to the inhalation developmental toxicity study in Section 2.3.2. Methods of Analysis for 1,3,5-TMB (p. 2-37). In A Review of the Reference Dose and Reference Concentration Process (EPA 2002), Section 4.4.2.2., Duration Adjustment for Inhalation Developmental Toxicity Studies – A Current Exception, the document states "A notable exception to duration adjustment of inhalation exposures is for inhalation developmental toxicity studies in which this practice historically has not been done. The current guidelines for developmental toxicity risk assessment (EPA 1991) recommend against duration adjustment (i.e., from a discontinuous to a continuous exposure) as a default procedure unless toxicokinetic data are available to indicate an accumulation with continuous exposure". I am not sure if this

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is still EPA's position and there appears to be no TK data from pregnant rats available. Not using a duration adjustment for a developmental toxicity study seems reasonable since each of the 15 exposure days is a critical period in organ development and sensitivity, and most developmental toxicants display a threshold internal dose that must be reached to disrupt normal development. Therefore, it seems improper to do a duration adjustment and determine a POD_{ADJ} (mg/m^3) for 1,3,5-TMB based on 6/24 hours.

I agree with the $UF_A = 3$, UF_H of 10, the UF_L of 1. However, I disagree with the UF_S of 3 (maternal) and 1 (developmental) and the UF_D of 3. In the case of UF_S , a subchronic to chronic uncertainty factor of 3 to account for extrapolation from a 15-day repeat exposure (not a typical 90-day repeated exposure) to a chronic exposure which is typically 2 years in a rat does not seem sufficient. The TMB document (Section 2.1.3) states "A full subchronic to chronic uncertainty factor of 10 was not applied in this case as there was evidence of reversibility of...neurotoxic effects". The document goes on to say "in the case of neurotoxicity, chronic exposures may overwhelm the adaptive responses observed after termination of subchronic exposure, potentially resulting in more severe and/or irreversible changes in neurological function". How is the reversibility of effects from a subchronic study relevant here? That is, how can neurotoxic effects be reversible when applied for a life-time continuous exposure (i.e., there is no post-exposure period)? As pointed out in the EPA RfC review document (EPA 2002), "[it] is also important to keep in mind that effects that may initially appear to be reversible may re-appear later or be predictive of later adverse outcomes". The UF_S of 1 for the developmental endpoints should follow the same rationale as the maternal endpoint. The UF_S should be the default of 10 for both maternal and developmental endpoints.

EPA should reconsider the database uncertainty factor (UF_D) of 3 applied to account for database deficiencies as it also seems inadequate. The database is lacking chronic and subchronic toxicity studies in two species, a single species two-generation reproductive toxicity study, a second species (usually rabbit) developmental toxicity study, a developmental neurotoxicity study (since the critical effect is potentially neurotoxicity) and a PBPK model. It seems that the UF_D should be the default of 10.

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Alternative Derivation of Candidate RfC Values for 1,3,5-TMB

Endpoints	NOAEL/LOAEL ppm (mg/m ³)	POD* (mg/m ³)	HEC (mg/m ³)	UF _A	UF _H	UF _L	UF _S	UF _D	Comp. UF	Candidate RfC value (mg/m ³)
Developmental Endpoints										
Decreased fetal bw, males	100/300 (497/1471)	497	497	3	10	1	10	10	3000	1.7 x 10 ⁻¹
Decreased fetal bw, females	100/300 (497/1471)	497	497	3	10	1	10	10	3000	1.7 x 10 ⁻¹
Maternal Endpoints										
Decreased maternal BW gain	100/300 (497/1471)	497	497	3	10	1	10	10	3000	1.7 x 10 ⁻¹

*Value not duration adjusted

The above 1,3,5-TMB candidate RfCs are based on the NOAEL/LOAEL approach. The RfC value of 1.7 x 10⁻¹ is approximately three-fold different than using the 1,2,4-TMB of 5 x 10⁻² based on a neurological endpoint (decreased pain sensitivity).

In the end, I think the RfC for 1,3,5-TMB can be based on the 1,2,4-TMB isomer, but the Saillenfait et al. study is a solid supporting study. The 1,2,4-TMB has three subchronic rat studies, a one species developmental toxicity study and a PBPK model. The 1,2,4-TMB and 1,2,3-TMB isomers have pain sensitivity (hot-plate) thresholds following short-term and subchronic inhalation exposures at similar exposure levels of 100 ppm (492 mg/m³) and both have similar chemical and physical properties as well as comparable physiologically-based parameters for rats and humans. The RfC for all three isomers should be 5 x 10⁻². My one caveat is the 'pain sensitivity' endpoint needs to be tested by a scientifically-validated method and must be reproducible by labs outside the Nofer Institute of Occupational Medicine.

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

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

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

The oral route of exposure was not identified in any chronic or subchronic studies for 1,2,4-TMB, in any species, and lacked oral developmental toxicity studies in a rodent and nonrodent (usually rabbit) species. The route-to-route extrapolation from inhalation to oral exposure using the modified Hissink et al. (2007) PBPK model to derive an oral RfD for 1,2,4-TMB seems scientifically sound.

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

The oral route of exposure was not identified in any chronic or subchronic studies for 1,2,4-TMB; therefore, a route-to-route extrapolation (inhalation to oral) was used with a modified PBPK model (Hissink et al., 2007). Several acute gavage studies using TMB isomers or mixtures were identified in the literature to produce neurotoxicity. The available 1,2,4-TMB inhalation data were demonstrated to have similar qualitative profiles of toxicokinetics (adsorption, distribution, metabolism and elimination) across exposure routes and there was no evidence to suggest different toxicity profiles. There was no portal of entry effects by either route. Observed neurotoxic effects of acute oral exposure of 1,2,4-TMB in rats were similar to effects observed following short-term inhalation exposure to 1,2,4-TMB. The one exposure component added to the PBPK model was to assume continuous oral ingestion and 100% absorption by constant infusion

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into the liver. There were no oral 1,2,4-TMB data available to calibrate the modified model so the assumption that 100% of the dose would enter the liver is untested and therefore uncertain. EPA indicates this is a common assumption but provides no reference. A reference should be added or a more detailed explanation given.

I agree with the $UF_A = 3$, UF_H of 10, the UF_L of 1 and the UF_S of 3. However, I disagree with the UF_D of 3. The reversibility of the pain sensitivity for a lifetime RfD is still a concern (see above). The database uncertainty factor (UF_D) of 3 applied to account for database deficiencies does not seem to afford adequate protection. The database is lacking an oral chronic toxicity study in two species, a single species two-generation reproductive toxicity study, a second species rabbit developmental toxicity study, a developmental neurotoxicity study (since the critical effect is neurotoxicity) and is based on a modified PBPK model. The UF_D should be the default of 10. This would make the composite $UF=1000$.

Other Comments

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

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

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

The Koch Industries, (1995a,b) 14- and 90-day oral studies with 1,3,5-TMB, while not adequate studies for principal studies for the RfD, could be used to support the RfD. The RfD derived from this study could be compared to the RfD from the route-to-route extrapolation with PBPK models for other isomers using available endpoints.

The external peer reviewers commented that the study was not appropriate as the basis of a reference dose because the exposures were 1) oral gavage, a less likely route of human exposure; and 2) a single daily bolus, 5-days/week, is not an optimal for TK studies for materials with rapid elimination (Huo et al. 1989, demonstrated that 99% of TMB and its metabolites are eliminated in 24 hours). Each weekend leaves 48 hours for 1,3,5-TMB and its metabolites to be cleared. It should be noted that 5-days/week gavage exposures were acceptable by EPA and OECD when this study was conducted in 1995. The third criticism was that the study did not investigate endpoints "more pertinent to human health" (e.g., "behavioral, respiratory or electrophysiological" endpoints). To be fair,

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these data were submitted under a TSCA 4(a) Test Rule. Those protocols are strictly hazard identification. The addition of behavioral, respiratory or electrophysiological endpoints would have been unheard of, especially at the time the study was conducted and extremely costly. Another reviewer's criticism was that neurotoxicity, being a critical endpoint, could have been included in the Koch protocol design. This would have been a valid point if all the Nofer Institute studies had been published prior to 1995. (Hindsight is always 20-20).

Positive points for the Koch 90-day study were 1) it was conducted by GLPs and in a different laboratory than the other inhalation subchronic studies for the other isomers; 2) group sizes were 10/sex/middle 3 dose groups and 20/sex/control and high dose groups; 3) body weights, clinical chemistry, hematology, and organ weights data collected were comparable to the 90-day inhalation studies; 4) there were male and female data available; and 5) there was a 28-day recovery group composed of 10/sex/control and high dose groups.