



BEFORE THE
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

COMMENTS OF THE HYDROCARBON SOLVENTS PANEL
OF THE AMERICAN CHEMISTRY COUNCIL
IN RESPONSE TO EPA'S DRAFT IRIS TOXICOLOGICAL REVIEW
OF TRIMETHYLBENZENE ASSESSMENT

Notice of Public Comment Period and Listening Session;)
Draft Toxicological Review of Trimethylbenzene in Support)
of Summary Information on the Integrated Risk) EPA-HQ-ORD-2012-16027
Information System (IRIS))
77 Fed. Reg. 126)

Leslie Berry
Director
American Chemistry Council

Allison Starmann
Assistant General Counsel
American Chemistry Council

August 28, 2012



EXECUTIVE SUMMARY

The American Chemistry Council's Hydrocarbon Solvents¹ (Panel) represents the major US producers of hydrocarbon solvents including trimethylbenzene. The Panel appreciates the opportunity to comment on EPA's Draft Integrated Risk Information System (IRIS) Toxicological Review of Trimethylbenzene Assessment (Draft IRIS Assessment) which is the subject of an external peer-review. We strive to ensure appropriate product stewardship, and, as part of our mission, address important science and public policy issues related to the hydrocarbon solvents industry, including EPA's Draft IRIS Assessment.

EPA has made improvements to the readability of the Draft IRIS Assessment, and the Agency has provided an expanded detail for the literature search strategy, presentation of evidence tables, and discussion of study data and rationale for deriving the toxicity values. The utilization of the Health and Environmental Research Online (HERO) system to access abstracts of interest was also helpful in reviewing the Draft Assessment. EPA, however, has failed to conduct a scientifically sound literature search and has not used a systematic approach to weigh the available evidence. Due to these shortcomings, the selection of the key endpoint and studies for determining the RfC and RfD for trimethylbenzene isomers (TMB) is not scientifically sound.

Key deficiencies with the Draft IRIS Assessment include:

- The NAS recommended that EPA's IRIS Office use a consistent and transparent process for identifying and evaluating studies for inclusion in the IRIS assessments, yet EPA has continued to fail to do so.
- EPA failed to utilize the best available science, as well as a 'weight-of-evidence' approach that considers all relevant information and its quality in the Draft IRIS Assessment.
- The literature search strategy should be revised to ensure that the selection of the key endpoint and the definitive study are determined by evaluating all useful data in a transparent and consistent manner. The literature search should include and clearly note any data submitted to and accepted by EPA under regulatory mandate, and in the public domain;
 - the TMB isomers and mixtures of isomers should be considered equivalent and the data set fully applied accordingly, and
 - the inclusion of the extensive body of published data available on C9 aromatic hydrocarbon solvents tested by inhalation under the TSCA Section 4(a) test rule (FR50 20662, 1985 and FR58 59667, 1993) would greatly enhance the database available on TMB isomers individually and address many of the uncertainties raised in the Draft IRIS Assessment.

¹ Panel Members are Chevron Phillips, CITGO, ExxonMobil Chemical Company, and Sasol North America.



- The selection of the key endpoint and the definitive study for the RfD and RfC calculations were not appropriate;
 - pain sensitivity should not be used as the key endpoint for the RfC as there is no clear dose response or reproducible finding related to the pain sensitivity endpoint alone for the individual isomers. The discussion of pain sensitivity for the RfC determination should be revised to accurately emphasize that decreases in pain sensitivity and increases in pain latency were observed only when animals were tested immediately after 90 days of treatment (Korsak and Ryzdzyński 1996), but not when the animals were held without treatment for any extended period of time indicating the transient nature of the response. The significant persistence in response postulated by EPA was only apparent after foot-shock was introduced which according to EPA itself “can complicate interpretations regarding effects on discrete neurological function.”
- The most useful study for the determination of the RfC is Clark et al., not the study selected in the DRAFT IRIS Assessment. Clark et al. provides a longer duration of exposure and the outcome is consistent with the 90 day inhalation study of 1,2,3 TMB (Korsak et al., 2000), and the 90 day oral toxicity study of 1,3,5-TMB (Koch Industries, 1995).
- The most useful study for the determination of the RfD is Koch Industries, 1995, not the study selected in the Draft IRIS Assessment. Koch Industries, 1995 is preferable to extensive extrapolation from inhalation data.

The Panel urges EPA to substantially revise the Draft IRIS Assessment to accurately incorporate the best available science. As set forth in these comments, the Draft IRIS Assessment does not accurately represent the health effects associated with exposure to TMB, and should be revised before initiating the external review process. The Draft Assessment should utilize a consistent and transparent data evaluation procedure for evaluating and weighing the full body of evidence in compliance with the Information Quality (IQ) Guidelines. In light of the ongoing external peer-review of the Draft IRIS Assessment, ACC reserves the right to supplement its comments.



INTRODUCTION

The Panel appreciates the opportunity to comment on EPA's Draft Integrated Risk Information System (IRIS) Toxicological Review of Trimethylbenzene Assessment (Draft IRIS Assessment) which is the subject of an external peer-review. As set forth in these comments, EPA has failed to comport with the EPA IQ Guidelines and to fully adopt the recommendations of the National Academies of Sciences (NAS) in its Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde, which are also applicable to this review of TMB. Importantly, the Draft IRIS Assessment is "influential" scientific risk assessment information, and therefore, must adhere to a rigorous standard of quality. Throughout the Draft IRIS Assessment, EPA has failed to employ a transparent and consistent approach to data selection and evaluation. Moreover, the Draft IRIS Assessment is not based on the best available science.

The literature search strategy and study selection as presented is significantly flawed. The process by which some studies were either not considered or not used was not transparent, or consistently and reliably applied. The inclusion of the extensive body of published data available on C9 aromatic hydrocarbon solvents tested by inhalation under the TSCA Section 4(a) test rule (FR50 20662, 1985) would greatly enhance the database available on TMB isomers individually and address many of the uncertainties raised in the Draft IRIS Assessment.

The Panel urges the EPA to substantially revise the Draft IRIS Assessment on trimethylbenzene to accurately convey the best available science, and utilize consistent and transparent data evaluation procedures for evaluating and weighing the full body of evidence in compliance with the IQ Guidelines and NAS recommendations. In light of the ongoing external peer-review of the Draft IRIS Assessment, ACC reserves the right to supplement its comments.

I. General Comments

1. The Draft IRIS Assessment is subject to EPA and OMB Information Quality Guidelines.

Congress enacted the Information Quality Act (IQA), Pub. L. No. 106-554, 114 Stat. 2763A-153 to 2763A-154, to "ensur[e,] and maximiz[e,] the quality, objectivity, utility and integrity of information . . . disseminated by Federal agencies" such as EPA. The IQA required the Office of Management and Budget (OMB) to issue government-wide guidance, which each federal agency was to follow in issuing its own guidelines. In February 2002, OMB issued its guidelines. EPA issued its agency-specific guidelines (EPA IQ- Guidelines) later that year.² The purpose of the

² 67 Fed. Reg. 8452 (Feb. 22, 2002); see EPA, *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency*, available at http://www.epa.gov/quality/informationguidelines/documents/EPA_InfoQualityGuidelines.pdf.



EPA IQ Guidelines is to apply the OMB Guidelines to the agency's particular circumstances, and to "establish administrative mechanisms allowing affected persons to seek and obtain correction of information . . . disseminated by the agency that does not comply with the [OMB] guidelines. . . ." EPA's Draft IRIS Assessment must meet OMB's guidelines as well as the EPA IQ guidelines.

2. The Draft IRIS Assessment is influential information and must adhere to a rigorous standard of quality.

The Draft IRIS Assessment is "influential" scientific risk assessment information as set forth in EPA IQ Guidelines³ because it is a "[m]ajor work product[] undergoing peer review," and "will have . . . a clear and substantial impact (i.e., potential change or effect) on important public policies or private sector decisions."⁴ The Draft IRIS Assessment, therefore, must adhere to a rigorous standard of quality.⁵ The substance of the information must be "accurate, reliable, and unbiased."⁶ Additionally, EPA must employ "a higher degree of transparency regarding (1) the source of the data used, (2) the various assumptions employed, (3) the analytic methods applied, and (4) the statistical procedures employed."⁷ EPA must use the best available science and supporting studies, as well as "a 'weight-of-evidence' approach that considers all relevant information and its quality."⁸

As set forth below, EPA has failed to comport with the information quality guidelines. The assessment is not of high quality because it does not present an objective and transparent presentation of the best available scientific information. For example, throughout the Draft IRIS Assessment, EPA has failed to employ a clear and consistent approach to data selection and evaluation. Moreover, the Draft IRIS Assessment is not based on the best available science because it does not consider all the available high quality, highly relevant scientific information. Numerous studies, including EPA issued TSCA Test Rule data, were either not reviewed or only

³ Pub.L.No. 106-554. The Information Quality Act was developed as a supplement to the Paperwork Reduction Act, 44 U.S.C. §3501 et seq., which requires OMB, among other things, to "develop and oversee the implementation of policies, principles, standards, and guidelines to . . . apply to Federal agency dissemination of public information."

⁴ EPA IQ Guidelines at 19 (internal citations omitted); OMB Guidelines For Ensuring and Maximizing the Quality Objectivity Utility and Integrity of Information Disseminated by Government Agencies [Hereinafter OMB Information Quality Guidelines] 67 F.R. 8452, 8455 February 22, 2002.

⁵ Quality includes objectivity, utility, and integrity.

⁶ EPA IQ Guidelines at 22; OMB IQA Guidelines at 8453.

⁷ EPA IQ Guidelines at 21.

⁸ EPA IQ Guidelines at 21. "In this approach, a well-developed, peer-reviewed study would generally be accorded greater weight than information from a less well-developed study that had not been peer-reviewed, but both studies would be considered." *Id.* at 26. The definition of best available science mirrors that articulated in *Chlorine Chemistry Council v. EPA*, 206 F.3d 1286 (D.C. Cir. 2000), referring to "the availability at the time an assessment is made." EPA IQ Guidelines at 23.



superficially considered by EPA. Based on our review of the data, the choice of key endpoint is not consistent or supported when weighing the totality of the evidence, which shows a lack of a dose-response relationship as well as a transitory response that is not sustained. The Draft IRIS Assessment must: (1) rely on the best available scientific information regarding hazard; (2) employ consistent, objective methods and models; and (3) utilize transparent data evaluation procedures for evaluating and weighing the full body of evidence.

3. The Preamble does not satisfy the NAS recommendations

In the Draft IRIS Assessment, EPA has included a section titled “Preamble to the IRIS Toxicological Reviews” that includes a summary discussion of the scope of the IRIS program, process for developing IRIS assessments, study selection, data evaluation and derivation of toxicity values. In 2011 recommendations by the NAS during its review of the EPA’s draft Formaldehyde assessment, stated that.

“Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various non-cancer outcomes. The committee emphasizes that it is 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 the RfCs and unit risk estimates.”

The NAS recommendations are applicable to the Draft IMB IRIA assessment. As currently written, the preamble offers an abbreviated view of EPA policies, guidance and standard practices but fails to include the detail necessary to provide useful information on how the Agency reviews or weighs the scientific information for inclusion in its toxicological review as discussed in the NAS recommendations. Unfortunately, in providing this abbreviated view, critical information has been omitted. In addition, it is not appropriate to use the preamble to an IRIS assessment as a means to communicate new criteria, guidance and approaches, that have not been properly peer reviewed, to the public. The adoption of new approaches should be done through an open and robust process that involves peer review and stakeholder participation before being implemented in an assessment. For further elaboration regarding the full extent of our concerns on the preamble, please see the ACC comments submitted on the Draft IRIS Assessment for Ammonia.⁹ The comments submitted previously are equally applicable to this assessment.

⁹ Comments Submitted by the American Chemistry Council, Center for Advancing Risk Assessment Science and Policy (ARASP) on the Draft Toxicological Review of Ammonia .Docket ID: EPA-HQ-ORD-2012-0399. <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-ORD-2012-0399-0017>.



II. EPA’s utilization of a consistent and transparent procedure for identifying, selecting and evaluating appropriate studies for inclusion in the Draft IRIS Assessment is critical to ensure and maximize the document’s quality.

The Draft IRIS Assessment’s classification as “influential” information requires its content to meet rigorous standards of consistency and transparency. In failing to employ a consistent and transparent procedure for identifying, evaluating and selecting appropriate studies for the Draft IRIS Assessment, EPA has not complied with its IQ guidelines and severely undermined the legitimacy and utility of the document. What follows is a number of suggestions that, if adopted, can bring the Draft IRIS Assessment into accordance with both OMB and EPA IQ guidelines and significantly improve the accuracy and value of the document.

1. EPA IRIS Office should search scientific literature databases for toxicity information for trimethylbenzenes.

Identifying appropriate studies to serve as the basis for the Draft IRIS Assessment is critical to the objectivity and utility of the document. In failing to conduct a thorough literature search, EPA has undermined the integrity of the Draft IRIS Assessment and eroded its utility. EPA identifies “maximiz[ing] the quality, including objectivity, utility and integrity of disseminated information” as the primary purpose of the EPA IQ guidelines.¹⁰ Further, objectivity “focuses on whether the disseminated information is being presented in an accurate, clear, complete and unbiased manner, and ... is accurate, reliable and unbiased.”¹¹ The initial step in ensuring “accurate, clear, complete and unbiased” information is through the identification and appropriate characterization of all relevant studies. This can only be achieved through the identification of the most appropriate databases for finding relevant studies for the particular chemical.

The Draft IRIS Assessment identifies a solid core set of databases [consolidated in the HERO system, p. xxxvii] to serve as a starting point for the research of any chemical substance. For example, in the case of TMB, EPA’s failure to review databases from other EPA Program Offices resulted in the omission of data from two TSCA Section 4(a) test rules (FR50 20662, 1985 and FR58 59667, 1993) and High Production Volume Program data. This omission is just one example of the flawed and incomplete process EPA currently employs in identifying relevant studies and suggests that other studies may have been missed as well.

¹⁰ EPA IQ Guidelines at 15.

¹¹ EPA IQ Guidelines at 15.



2. The literature search data base should be expanded to include all relevant databases for non-cancer endpoints

The decision to consider the TMB isomers as equivalent is appropriate but not fully exploited in the Draft IRIS Assessment. If EPA considers TMB isomers to be equivalent as is appropriate based on the data submitted, and accepted by the EPA under TSCA Section 4(a) test rule (FR50 20662, 1985 and FR58 59667, 1993), then data on any of the isomers, or on mixtures of isomers, predominantly TMBs with other similar hydrocarbons [e.g. C9 aromatic including ethyltoluene] can be used to characterize the hazards of TMBs individually or collectively. Inclusion of the extensive body of published data available on C9 aromatic hydrocarbon mixture tested by inhalation under the TSCA Section 4(a) test rule (FR50 20662, 1985 and FR58 59667, 1993) would greatly enhance the database available on TMB isomers individually, and address many of the uncertainties raised in the Draft IRIS Assessment.

In the 1985 TSCA 4(a) test rule, EPA determined that because C9 aromatics were manufactured as complex substances and exposures are usually to the mixed isomers, testing of a mixture of 55% TMBs and 28% ethyltoluene (ET) isomers would provide reasonable upper bound estimates for the toxicity of TMB and ET isomers. The overall program addressed genetic toxicity, subchronic neurotoxicity, reproductive toxicity in rats (3 generations), and developmental toxicity in the mouse. EPA acknowledged that studies addressing developmental toxicity in the rat and repeated systemic toxicity of 90 day and 12 month durations were already available and did not include these endpoints in the test rule requirements. Since EPA considered the data generated from studies of C9 aromatic mixture representative of the toxicity of the component isomers, these studies provide valuable data for the toxicological assessment of TMB and should be considered in establishing the Inhalation RfC.

It is unclear why these critical studies, conducted pursuant to an EPA test rule, submitted to the EPA in 1993 and evaluated by EPA were excluded from the Draft IRIS Assessment. The IRIS document, did however, provide a vague explanation as to why certain published data were omitted from the assessment

“These reports were not peer-reviewed and they either did not use appropriate durations of exposure that would support derivation of chronic health reference numbers (e.g., 14 days), reported minimal and difficult to interpret toxic effects, or investigated mixtures containing TMB isomers” (IRIS Literature Search Strategy xxxix, lines 16-19).”

Exclusion of studies of mixtures of TMB is not consistent with the principle of similarity used in the Draft IRIS Assessment. If all TMBs are equivalent, any study on a mixture of predominantly TMBs should be as relevant as a study on individual isomers. In general, these C9 studies (55% TMB isomers; 28% ET isomers) resulted in minimal toxicity (Table 1) at doses substantively



higher than the studies on individual TMB isomers selected for key endpoints. EPA considered the data generated from studies of C9 aromatic mixture representative of the toxicity of the component isomers under the TSCA Section 4(a) rule, providing valuable data for the toxicological assessment of TMBs. Studies on the 4-ET isomer alone indicated no genetic toxicity (Janik-Spiechowicz and Wysznska, 1998) and minimal systemic effects at high concentrations (Swiercz et al., 2000). The Swiercz et al, 2000 study reported the concentrations of ethyltoluene which caused respiratory rate decrease [RD50 = 4216mg/m³] in mice were higher than levels of TMB isomers [RD50 = 2553 – 2844mg/m³ range for isomers] that produced similar effects. Ethyltoluene was less irritating than TMB isomers. Inhalation exposure of rats to 4-ET at concentrations of 477 or 2337mg/m³, 6 hours/day, 5 days/week for 4 weeks resulted in respiratory effects at the maximum dose similar to the responses reported for trimethylbenzenes but no adverse effects at 477mg/m³. The highest dose of 2337mg/m³ in the 4-ET 4-week study was similar to the calculated ET isomer percentage of 2100mg/m³ in the highest dose of 7500mg/m³ in the TSCA C9 aromatic studies at which minimal adverse effects were reported, indicating that any toxicity was likely due to TMB concentrations. Overall the available evidence indicates that ethyl toluene isomers, like TMB isomers, pose few toxicological hazards.

Table 1. Results of 1985 Test Rule C9 Aromatic Fraction Testing

Test	Assay or Doses	Results	Reference
Genetic Toxicity	Ames <i>Salmonella</i> assay CHO HGPRT forward mutation CHO chromosome aberration CHO -SCE Rat chromosome aberration	All studies negative results for gene mutation (<i>Salmonella</i> , CHO/HGPRT mutation) or cytogenetic effects. C9 aromatics unlikely to be genotoxic carcinogen	Schreiner et al., 1989
Subchronic Neurotoxicity - Rats	100, 500, 1500ppm (500, 2500, 7500mg/m ³) 6hr/day, 5 days/wk for 90 days	No adverse effects for motor activity, functional observation battery or neuropathology	Douglas et al., 1993
Developmental toxicity – Mice Female ^a	100, 500, 1500ppm (500, 2500, 7500mg/m ³) 6hr/day Gest day 6 through Gest day 15	1500ppm – 50% mortality 500ppm - maternal and fetal body weights reduced 100ppm – no effects	McKee et al., 1990
Reproductive Toxicity Rats 30M,30F/group parental	100, 500, 1500ppm (500, 2500, 7500mg/m ³) 6hr/day, 7 days/wk 10 wks pre-	No adverse effects on reproductive parameters. Maternal and offspring	McKee et al., 1990



	mating, 2 wks mating (both sexes) females GD0 to GD20 Females not exposed to postnatal day 4 to weaning at LD21. Offspring began exposure after weaning.	body weight effects at 1500ppm	
Repeated dose toxicity Rats [Earlier study to C9 mixture product] ^b	450, 900, 1800 mg/m ³ 5d/week for 12 months	Primary effect liver weight increase with no adverse pathologic correlate at 1800 mg/m ³	Clark et al., 1989

a- Other developmental toxicity publications in rats for mixed C9 (Lehotsky et al., 1985 and Ungary et al., 1983) and individual isomers (Saillenfait et al., 2005)

b- EPA considered this study sufficient to fulfill the repeat dose requirement and did not require an additional study in the C9 test rule program

In the establishment of the Oral RfD, EPA appears to justify exclusion of the 14 day and 90 day Koch Industries (1995a,b) studies using 1,3,5 TMB because they would "*not qualitatively enhance hazard identification, quantitatively enhance dose-response analysis or substantially decrease uncertainty in the assessment*" (IRIS Literature Search Strategy xxxix, lines 14 through 21). This justification is simply unfounded, and the Panel strongly disagrees with this decision.

The Draft IRIS Assessment states that “no chronic, subchronic, or short-term oral exposure studies were found in the literature¹²” for 1,3,5-TMB. This is incorrect; there is an oral toxicity study performed by the request of EPA Office of Water Chemicals Final Test Rule (FR58 59667, 1993). The data from Koch Industries (1995a,b) was accepted by the EPA, and later relied upon by the EPA’s Office of Pollution Prevention and Toxics (OPPT) for the Organization for Economic Cooperation and Development’s (OECD) High Production Volume (HPV) Screening Information Data Set (SIDS) program in the C9 aromatic hydrocarbon solvents dossier for 1,3,5 TMB. This study is therefore, “known to the public” and peer evaluated under the OECD’s HPV. The study has also been summarized in a publication by Firth, 2008 in which an RfC of 0.4mg/kg-d was proposed.

There is no sound justification as to why the results of the 90 day Koch Industries (1995) study was considered not relevant to and should not be used for regulatory purposes. In the absence of a peer reviewed oral study for any trimethylbenzene, the Koch study provides direct results for oral exposure to 1,3,5-TMB in rats and does, in fact, enhance both the hazard identification and dose response analysis. Since it was conducted by the most relevant route of exposure (which EPA’s Draft IRIS Assessment agrees is the oral route), it also decreases uncertainty. Dose levels were 0,

¹² Page xxxiv and in Sect 2.4 p. 2-46 of the Draft IRIS Assessment



200 and 600mg/kg/day 5 days/week for 13 weeks (a total of 65-66 doses). The study found no overt expressions of behavioral neurotoxicity at any dose however, increased liver and kidney weights and an increase in serum phosphorous levels were seen at 600mg/kg resulting in a LOAEL = 600mg/kg and a NOAEL = 200mg/kg. Using a conservative NOAEL = 200mg/kg/day would likely result in an oral RfD in the range of 0.2mg/kg with an uncertainty factor of 1000, significantly higher than the RfD = 0.006mg/kg-day calculated by IRIS by extrapolation from inhalation values, at least in part by eliminating the route-to-route uncertainty factor. Furthermore, since IRIS acknowledges the similarity in toxicological responses among the TMB isomers, an RfD based on animal data for 1,3,5 TMB could reasonably be extrapolated to the other 2 isomers.

III. The selected key endpoint and study is not appropriate.

EPA has selected decreased pain sensitivity expressed as increased latency to response as the critical effect for TMB toxicity. The basic study design involves exposing animals to individual isomers and then measuring latency to paw-lick in response to hot plate exposure. Korsak and Rydzynski (1996) was the principal study for the derivation of the RfC for 1,2,4 TMB (Draft IRIS Assessment xxxiv, lines 9-11). Authors provided data on both acute and repeated exposure (3 months) and reported exposure to TMB resulted in an increased latency in response when measured immediately after treatment but found no effects 2 weeks post-exposure for animals in the repeat dose study. The most likely explanation is that exposure to TMB isomers results in acute, reversible responses. Acute effects are related to the most recent exposures, and are not the consequence of repeated exposures. Furthermore, results for the pain sensitivity endpoint in the neurotoxicity study with C9 aromatics (Douglas et al, 1993) found no adverse effects in animals examined at 5, 9 and 13 weeks during and after exposure to higher levels than employed by Korsak and Rydzynski. Although Korsak and Rydzynski (1996) was identified as the key study, significant emphasis was placed on subsequent studies in which animals were exposed for only 4 weeks duration and held for longer periods and foot shock was introduced (Wiaderna et al., 1998; Wiaderna et al, 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al, 1997) to support a position that the observed pain sensitivity was not an acute response but that exposure to TMB isomers results in persistent impairment as long as 50-51 days post exposure, long after TMB had been eliminated from the body. However, the studies actually demonstrated that pain sensitivity per se was not persistent. Moreover, these studies show some inconsistencies in their findings.

- Korsak and Rydzynski (1996) exposed rats to 1,2,3- and 1,2,4-TMB isomers and tested them for pain sensitivity after 90 days exposure using the hot plate assay. There were significant increased latency effects at all 3 exposure levels (25, 100, 250 ppm) when the testing was done immediately after termination of exposure. They tested the rats 2 weeks post exposure and there were no differences between exposure levels.



- Gralewicz et al. (1997) exposed animals to 1,2,4 TMB for 4 weeks, held them for 35 days post-exposure and tested at days 50-51 and then tested them using the hot plate assay and found no effects. They then shocked the animals and re-tested them, finding no effects. They then tested the rats 24 hours after foot shock, finding a significant increased time to response in the 100 and 250 ppm groups.
- Wiaderna et al. (1998) exposed rats to 1,2,3 TMB for 4 weeks, tested them at 50 and 51 days after exposure using a hot plate assay only and no effects were seen, confirming any acute response had disappeared. After foot shock was administered, latency to foot lick in the hot plate assay was increased at all doses including controls to a similar degree. When tested 24 hours after foot shock a significant increase in latency time to response was found at 100ppm but not 25 or 250ppm.
- Gralewicz and Wiaderna (2001) exposed rats to the 1,2,3-, 1,2,4-, and 1,3,5- isomers of TMB for 4 weeks at 100 ppm. They held the animals for 39 days post exposure and then tested them on days 50-51 for pain response, finding no effects. Then they shocked the animals and tested for pain sensitivity immediately after foot-shock and 24, 72 and 120 hours post-shock. Increased latency time was observed at 24 hours for 1,2,4 TMB and 1,3,5 TMB but at 72 hours post-shock significant reductions in latency time to response were found in the experiments with 1,3,5 TMB at 72 hours post-shock and 1,2,4- and 1,3,5- at 120 hours. No effects were seen with 1,2,3-TMB isomers.
- Wiaderna et al. (2002). exposed rats to 1,3,5-TMB at levels of 25, 100 and 250 ppm for 4 weeks, held them for 35 days post exposure., tested on days 50-51 and found no effects in the hot plate test and no effects immediately after foot shock or at any intermediate point before the 240 hours post-shock assessment at which point a significant reduction in latency time was found at all exposure levels. However, as the reduction in latency was similar across the dose groups, no dose response was apparent. Results did not replicate significant differences reported by Gralewicz and Wiaderna (2001) for 1,3,5 TMB at 72 and 120 hours post-shock.

The studies cited above as the key study (Korsak and Rydzynski 1996) and supporting studies were performed in the same laboratory with the same group of investigators and yet presented variable results. Effects were not consistent across isomers (i.e. Wiaderna et al (1998) saw effects with 1,2,3 TMB but no effects were reported with 1,2,3 TMB by Gralewicz and Wiaderna, (2001)). Dose responses were not demonstrated as effects when present were more pronounced at 100ppm than 250ppm. Effects were not directionally consistent, latency increased in some studies, decreased in others. Statistically significant findings in non-acute studies suggestive of persistence in response were found only after foot shock administration. No agreed guidelines for study conduct and rationale for administering foot shock were cited and thus the varied protocols



lead to a lack in clarity regarding whether or not the testing conducted is scientifically valid (representing an endpoint of concern) and reproducible. Indeed incorporation of foot shock complicates the interpretation of the studies as acknowledged by EPA (page. 1-21, lines 8-10) “*Most of the neurotoxicity tests in animals incorporated the application of footshock which, depending on the procedure, can involve multiple contributing factors and can complicate interpretations regarding effects on discrete neurological function.*” Finally, looking at the weight of the evidence, there is no clear dose response or reproducible finding related to the pain sensitivity endpoint for the individual isomers. See Appendix A for a summary of pain sensitivity studies with trimethylbenzene isomers measuring latency in response to hot plate without or with foot shock.

IV. The Summary and Evaluation Section Need Significant Revisions

The Panel recommends significant revision of this section to clearly explain the variability of results in pain sensitivity studies and how these differences affect their value for identifying the RfC. The Panel is particularly concerned about the selection of the pain sensitivity endpoint and Korsak and Rydzynski (1996) as the principal study because EPA is applying the RfC for 1,2,4 TMB calculated from this dataset to the other isomers when the studies conducted more recently that the 1996 study clearly show that not all isomers responded similarly. In addition, extrapolating from this uncertain endpoint to very low values for the RfD, is simply not necessary when there is an available 90 day oral study with 1,3,5 TMB.

1. Pain Sensitivity Endpoint

The discussion of pain sensitivity should be revised to accurately emphasize that decreases in pain sensitivity and increases in response latency were observed only when animals were tested immediately after 90 days of treatment (Korsak and Rydzynski 1996), but not when the animals were held without treatment for any extended period of time indicating the transient nature of the response, and that significant persistent effects were only reported after foot-shock was introduced. On page 1-3 lines 2 and 3 “*In these studies, treatment-related, statistically significant changes in pain sensitivity at $\geq 492 \text{ mg/m}^3$ 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB were observed 24 hours after rats were given a footshock; no statistically significant effects at any concentration were observed prior to or immediately following footshock*” should be expanded to qualify results of relevant studies. Significant effects were seen 24 hours after foot shock with 2 of 3 TMB isomers [1,2,3 TMB showed no effect] according to Gralewicz and Wiaderna (2001) and in 1,2,3-TMB treated rats in a separate study (Wiaderna et al, 1998) but in the other studies significant differences were reported in assessments conducted 3-10 days after foot shock was administered but not after 24 hours.

The Draft IRIS Assessment page 1-3, lines 16-20 state that “*The decreases in pain sensitivity measured in the subchronic and acute studies were observed immediately after exposure with no*



significant effects persisting 2 weeks after exposures were terminated (Korsak and Rydzynski 1996). In contrast, performance in the hot plate test was significantly impaired following short-term exposure to the TMB isomers when tested 50-51 days after exposure...”, but this is not a correct statement. In fact none of these studies reported any significant differences in persistence until the foot shock step was introduced.

The determination of RfC for 1,2,3 TMB using the Korsak and Rydzynski (1996) pain sensitivity endpoint resulted in a candidate RfC of 1.63×10^{-2} similar to that calculated for 1,2,4 TMB which rounds off to 0.02mg/m^3 .

In discussion at the Listening Session [August 1st, 2012] it was stated that IRIS used the “step down” technique to develop the assessment. This appears to be incorrect as the document itself indicates pain sensitivity is the key endpoint. The “step-down” method is considered in the section on cognitive function (pages 1-6 and 1-7), in Table 1-1. Wiaderna et al. 1998 employed comparisons of step down latencies for pain sensitivity in successive trials. Gralewicz and Widerna (2001) reported large individual differences in each group in step down latency for pain sensitivity and foot shock. *“In order to reduce the with-in group variability, data from two rats with the lowest and highest mean step-down latency in the first post shock trial were excluded from data sets for each group of rats”*. This suggests it was necessary to adjust the data to get significance in the Gralewicz and Wiaderna (2001) study raising further questions about the suitability of these data for risk assessment purposes. Finally, if the “step down” data are key, the EPA should consider revising the Draft IRIS Assessment as this distinction is not clear from the document. For example Table ES-1 (page xxvii) identifies decreased pain sensitivity as the basis for the reference calculations, and Table 1-1 (page 1-9) identifies paw-lick latency as the indicator of pain sensitivity. Thus, after further review of the IRIS documentation, it seems to us that pain sensitivity rather than changes in step-down performance defines the critical endpoints.

In developing the RfC for 1,3,5 TMB IRIS chose to discount the developmental toxicity study performed by Saillenfait, 2005 as the key study even in the absence of adequate neurotoxicity data for this isomer. The apparent reason (page xxxi) is that the no effect level differed from neurotoxicity studies. The relevant question here is which studies available on a given material provide the most robust response on which to base the assessment. The significance of the no effect level for the developmental toxicity may become more apparent when a broader database is considered in evaluating toxicity of TMBs employing studies which show minimal inhalation induced toxicity at much higher doses.

2. Uncertainty Factors

In developing the RfC for 1,2,4 TMB and the other isomers EPA attributes an uncertainty factor of 10 for extrapolation from subchronic exposure to chronic exposure (page 2.11, lines 21-27). *“The*



10-fold uncertainty factor is applied to the POD identified from the subchronic study on the assumption that effects observed in a similar chronic study would be observed at lower concentrations for a number of possible reasons, including potential cumulative damage occurring over the duration of the chronic study or an increase in the magnitude or severity of effect with increasing duration of exposure.” However, the Korsak and Rydzynski (1996) study does not demonstrate any cumulative damage from exposure to TMB as effects are not seen two weeks after exposure is terminated. Indeed how can this study be used as the principal study for derivation of the RfC if evidence of persistent effects are not observed in this study? Transient [acute] effects as reported by Korsak and Rydzynski (1996) are dependent on the most recent exposure and are reversible when exposures are terminated and the test material is cleared from the central nervous system. Pain sensitivity was observed but in the absence of footshock no cumulative damage was observed and in other studies where cumulative effects were reported following footshock, latency results were variable, again questioning the reliability of conclusions based on persistence of effects for this endpoint. So it does not seem justified to add extensive uncertainty factors to account for differences in study duration based on results from Korsak and Rydzynski (1996). If pain sensitivity is indeed an acute effect, which the Panel discusses above, a UF of 3 or less is more appropriate.

3. Developmental and Multigeneration Reproductive/Developmental toxicity

In determining uncertainty factor for database deficiencies (UF_D), EPA cites the absence of multigeneration and developmental neurotoxicity studies for all three isomers as contributing to the rationale for a UF = 3. The results of the 3-generation study for High Flash Aromatic Naphtha [C9 aromatics] (McKee et al., 1990) in which reduced litter size, lower birth weight and poor survival in the rat F3 generation at doses lower than those which caused similar effects in the F1 and F2 generations was used by EPA to suggest a lower point of departure (POD) could results from a multigeneration study with an individual isomer. However, if this 3 generation C9 aromatic study is included in the data set to evaluate TMB toxicity the need for data base deficiency UF will be virtually eliminated. The increased toxic effects in the third generation of this study related more to direct toxicity resulting from exposure of very young animals with small body size to high levels of solvent beginning at weaning than to effects on reproductive or developmental parameters. The outcome was a toxic response, not a reproductive effect. In fact the study data show this effect was not observed in the second generation when the pups were older and larger at the start of the exposure period. It is unlikely that another multigeneration study will result in a LOAEL lower than that resulting from a developmental study. In addition there are behavioral developmental studies performed with Aromatol, a blended C9 aromatic hydrocarbon mixture (Lehotzky et al., 1985a,b) which evaluated the effects on the nervous system of offspring from rats exposed to 600, 1000, and 2000mg/kg/day 24hours/day from day 7-15 of gestation with no adverse nervous system effects in offspring reported. Prenatal Aromatol inhalation had no adverse effects on maturation of gait, motor coordination, and activity avoidance



response on offspring at days 21, 36 or 90 day after delivery. This study was considered reliable by EPA in the TSCA Sect 4 C9 aromatic test rule. Incorporating the results of these studies into the IRIS Assessment in combination with the Saillenfait et al 2005 should provide sufficient data to overcome any deficiencies in the developmental/reproductive area and eliminate the need for any additional uncertainty factors to account for database deficiencies, reducing the uncertainty factor to 1.

4. Weight of Evidence for Carcinogenicity

The Panel agrees that the database for TMBs provides “inadequate information to assess carcinogenic potential” of these isomers. The database for TMBs, however, supports the likelihood that TMBs are not mutagens and are unlikely to be genotoxic carcinogens. The only animal carcinogenicity study identified, exposure to 1, 2, 4 TMB by oral gavage (Maltoni et al., 1997), was poorly presented with no statistical analysis. Follow-up analysis by EPA yielded no statistically significant results.

Of the genetic toxicity study available, Janik-Spiechowicz et al. (1998) did not find positive results in the *Salmonella* bacteria assay for 1, 2, 4 or 1, 3, 5 TMB but did report positive results in all *Salmonella* strains with 1,2,3 TMB in the absence of metabolic activation. This positive result seems unusual as aromatic hydrocarbons when mutagenic tend to require metabolic activation to express gene mutation (Hermann et al., 1980). Furthermore although numerical data were presented for 1,2,4 TMB and 1,3,5 TMB, for 1,2,3 TMB only fold increases at 5ul in TA97a and TA98, at 10ul for TA102 and at 20ul for TA100 were reported with no numerical data over dose ranges to support the 1,2,3 TMB conclusions. This presentation makes the conclusion of positive gene mutation results for 1, 2, 3 TMB open to question. In a subsequent study with a mixed aromatic solvent [Farbasol] containing 46% TMBs and 40% ethyl toluene isomers and 4-ET alone, no *Salmonella* mutagenicity was observed for the solvent or 4-ET tested alone (Janik-Spiechowicz and Wyszynska, .1998). The increased incidence of sister chromatid exchanges [SCE] in mice with each isomer suggests the ability of the TMBs to induce DNA perturbation. SCE studies were performed with only 5 male mice per group, only 50 cells/animal were examined and data were only presented graphically with sporadic significant results over the dose groups for each isomer. In contrast, the micronucleus assays performed like the SCE assay at doses equivalent to percentages of the LD50 for each isomer and comprised of sufficient number of animals and adequate reporting of data, did not demonstrate positive results for any isomer. This absence of positive results in the micronucleus assay, a definitive endpoint for cytogenetic damage, indicates that clastogenicity is not expressed. Similar cytogenetic results were also reported in the Janik-Spiechowicz and Wyszynska (1998) study with Farbasol a mixed C9 aromatic solvent.



Gene mutation or clastogenic activity was not seen with the C9 aromatic hydrocarbons tested in the *Salmonella* assay or the forward mutation HGPRT assay in Chinese hamster ovary cells. Negative results for cytogenetic damage were also reported in an *in vitro* chromosome assay, and *in vitro* SCE with Chinese hamster ovary cells, and in an inhalation chromosome aberration assay in rats (Schreiner et al., 1989). These results support the likelihood that trimethylbenzenes are not mutagens and are unlikely to be genotoxic carcinogens.

V. Recommendations and Calculations for RfD and RfC values for TMBs

The most useful study for the determination of the RfC is Clark et al (1989, see Table 1), a one year inhalation study in rats at doses of 450, 900 and 1800mg/m³. This study provides a longer duration of exposure and the outcome is consistent with the 90 day inhalation study of 1,2,3 TMB (Korsak et al., 2000), and the 90 day oral toxicity study of 1,3,5-TMB (Koch Industries, 1995).

The 90 day neurotoxicity study with C9 aromatics (Douglas et al., 1993, see Table 1) which was performed at higher doses than Clark et al, (1989) and evaluated standard neurotoxicity endpoints; motor activity, functional observation battery including the hot plate latency response [without foot shock] at 5, 9 and 13 weeks of exposure is also useful as supporting information as no adverse effects were identified.

For the RfD determination the 90 day oral study with 1,3,5 TMB (Koch Industries, 1995) performed at doses of 50, 200, 600mg/kg/day 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 these studies obviates the need for pharmacokinetic analysis and route to route extrapolation. The more extensive data base accompanying these studies reduces the uncertainties identified with the current investigation and avoids reliance on studies with interpretational difficulties.

It is essential that the correct data sets be evaluated in the toxicity assessment process. Studies should be designed and performed to accordance with established criteria and produce consistent and reproducible results. When the most robust studies and most relevant endpoints are identified, EPA recommended methods using mode of action determinations, weight of evidence parameters and appropriate uncertainty factors dependent on quality of the studies, extent of the database and scientific judgment can be applied to calculate regulatory values. An example of RfC and RfD calculations was reported by Firth, 2008 for the studies cited above. RfC calculation for 3 inhalation studies resulted in an overall recommended conservative value of 3 mg/m³ based on Clark et al (1989) 1 year toxicity study = 3 mg/m³; Douglas et al (1993) 90 day neurotoxicity study = 4 mg/m³ and McKee et al (1990) developmental toxicity = 4 mg/m³. An RfD based on the Koch Industries (1995) 90 day oral 1,3,5 TMB study was 0.4mg/kg/day [The author considered effects at the highest oral dose of 600mg/kg to be reversible and considered this the NOAEL adjusted to



429mg/kg/day] . ACC encourages EPA to review all available data on TMBs and C9 mixtures reevaluate to calculations for RfC and RfD.

Conclusion

The American Chemistry Council's Hydrocarbon Solvents Panel appreciates the opportunity to comment on EPA's Draft IRIS Toxicological Review of TMB. For all the reasons discussed herein, the Panel urges EPA to substantially revise the Draft IRIS Assessment consistent with the comments herein to accurately identify and convey the best available science and weight-of-evidence in compliance with EPA's IQ Guidelines and NAS recommendations.



References

- Clark, D.G., Butterworth, S.T, Roderick, and Bird, M.G. 1989. The inhalation toxicity of high flash aromatic naphtha. *Tox Indust Health* 5: 415-428
- Douglas, J.F., McKee, R.H., Cagen, S.Z. et al. 1993. A neurotoxicity assessment of high flash aromatic naphtha. *Tox Indust Health* 9: 1047-1058
- Firth, M.J. 2008. Derivation of a chronic reference dose and reference concentration for trimethylbenzenes and C9 aromatic hydrocarbon solvents. *Reg Toxicol and Pharmacol* 52: 248-256.
- Gralewicz, S., Wiaderna, D., Tomas, T., and Rydzynski, K., 1997. Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4 trimethylbenzene) in the rat. *Neurotox Teratol* 19: 327-333.
- Gralewicz, S; and Wiaderna, D. 2001. Behavioral effects following subacute inhalation exposure to m-xylene or trimethylbenzene in the rat: A comparative study. *Neurotoxicology* 22: 79-89.
- Hermann M, Chaudi, O., Weill, N. et al. 1980. Adaptation of the Salmonella/mammalian microsome test to the determination of the mutagenic properties of mineral oils. *Mut Res* 77: 327-339.
- Janik-Spiechowicz, E., Wyszynska, K., Dziubaltowska, E. 1998. Genotoxicity evaluation of trimethylbenzene. *Mut Res.* 412: 299-305
- Janik-Spiechowicz, E., and Wyszynska, K. 1998. Genotoxicity of Farbasol and its component: 4-ethyltoluene. *Mutat Res* 427: 95-100
- Koch Industries, Incorporated. 1995. 90-day oral gavage toxicity study of 1,3,5 trimethylbenzene in rats with a recovery group. (44618). Wichita, KS: Koch Industries, Inc.
- Korsak, Z., and Rydzynski, K. 1996. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. *Int J Occup Med Environ Health* 9: 341-349.
- Korsak, Z; Stetkiewicz, J; Majcherek, W; et al., 2000. Subchronic inhalation toxicity of 1,2,3-trimethylbenzene (hemimellitene) in rats. *Int J Occup Med Environ Health* 13: 223-232
- Lehotzky K., Szeberenyi, J, Ungvary, G and Kiss, A. 1985a. The effect of prenatal Aromatol exposure on the nervous systems of offspring among rats. *Egeszsegtudomany* 29: 389-397
- Lehotzky K., Szeberenyi, J, Ungvary, G and Kiss, A. 1985b. Behavioural effects of prenatal exposure to carbon disulphide and to Aromatol in rats. *ARCH Toxicol suppl* 8: 442-446



- Maltoni, C; Ciliberti, A; Pinto, C; et al. 1997. . Results of long-term experimental carcinogenicity studies of the effects of gasoline, correlated fuels, and major gasoline aromatics on rats. *Ann N Y Acad Sci* 837: 15-52.
- McKee, R.H., Wong, Z.A., Schmitt, S. et al. 1990. The reproductive and developmental toxicity of high flash aromatic naphtha. *Tox Indust Health* 6: 441-460.
- Saillenfait, A.M., Gallissot, Sabate, J.P., and Morel, G. 2005. Developmental toxicity of two trimethylbenzene isomers, mesitylene and pseudocumene in rats following inhalation exposure. *Food Chem Tox* 43: 1055-1063.
- Schreiner, C.A., Edwards, D.A., McKee, R.H. et al. 1989. The mutagenic potential of high flash aromatic naphtha. *Cell Biol Tox* 5: 169-188.
- Swiercz, R., Rydzynski, K., Jajte, J. et al. 2000. Studies on dermal, ocular and respiratory effects of 4-ethyltoluene in experimental animals. *Int J Occup Med Environ Health* 13: 307-15.
- Ungary, G., Tatrai, E., Lorinz, M. et al. 1983. Study of the embryotoxic effect of Aromatol, a new aromatic C9 mixture. *Egeszsegtudomány* 27: 138-148.
- US EPA TSCA 4(a) 1985 Identification of Specific Chemical Substances and Mixture Testing Requirements: Ethyltoluenes, trimethylbenzenes, and the C9 aromatic hydrocarbon fraction. 40CFR 50 (96) 20662-20677 (May 17, 1985)
- US EPA 1993. Office of Water Chemicals: Final Test Rule. Oral 14 day repeat dose and oral 90 day subchronic toxicity studies: 1,3,5 Trimethylbenzene (CAS No. 108-67-8). 40CFR 58(216) 59667-59682 (Nov 10, 1993).
- Wiaderna, D; Gralewicz, S; Tomas, T. 1998. Behavioral changes following a four-week inhalation exposure to hemimellitene (1,2,3-trimethylbenzene) in rats. *Int J Occup Med Environ Health* 11: 319-334.
- Wiaderna, D; Gralewicz, S; Tomas, T. 2002. Assessment of long-term neurotoxic effects of exposure to mesitylene (1,3,5-trimethylbenzene) based on the analysis of selected behavioral responses. *Int J Occup Med Environ Health* 15: 385-392.



Appendix A: Summary of Pain Sensitivity Studies with Trimethylbenzene isomers measuring latency in response to hot plate without or with foot shock

Reference	TMB Isomers	Doses [ppm]	Study Duration	Results	
Korsak and Rydzynski 1996	1,2,3 TMB 1,2,4 TMB	25, 100, 250	90 days no footshock	Immediate post exposure: <u>Increased latency</u> – 25, 100, 250ppm	2 weeks postexposure – no effects at any doses
Gralewicz et al, 1997	1,2,4 TMB	25, 100, 250	4 weeks, tested 50-51 days after end of exposure Before/after footshock	At 50 days No effects on latency; before footshock; No effects on latency immediately following 2 min footshock	At 51 days 24 hours post footshock. <u>Increased latency</u> at 100 and 250ppm,
Wiaderna et al., 1998	1,2,3 TMB	25, 100, 250	4 weeks tested at 50-51 days after end of exposure Before/after footshock	At 50 days No effects on latency before footshock; After footshock: No treatment related increase in latency	At 51 days 24 hours post footshock. <u>Increased latency</u> at 100ppm but not at 250ppm,
Gralewicz and Wiaderna, 2001	1,2,3 TMB 1,2,4 TMB, 1,3,5 TMB	100	4 weeks tested 50-51 days after end of exposure Before/after footshock	At 50-51 days post exposure : No effects on latency without footshock With footshock: no effects immediately after, increased latency at 24 hours with 1,2,4 TMB and 1,3,5TMB	At 72 and 120 hrs post foot shock <u>Decreased latency</u> at 72 and 120 hrs with 1,2,4 TMB and at 120hrs with 1,3,5 TMB No effects with 1,2,3 TMB
Wiaderna et al, 2002	1,3,5 TMB	25, 100, 250	4 weeks tested at 50-51 days after end of exposure Before/after footshock	At 50-51 days post exposure : No effects on latency without footshock With footshock: no effects immediately after or at 24, 48, 72, 96 or 120hrs post footshock	At 240 hours post footshock, <u>decreased latency</u> at 25, 100, 250ppm similar across all dose groups, no dose response.

