



Overview of the Draft IRIS Assessment of Trimethylbenzenes

**Presentation for the
TMBs Augmented Chemical Assessment Advisory Committee of the Science
Advisory Board
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Outline of Presentation

- **Briefly, this presentation will discuss**
 - How the Toxicological Review implements the 2011 NRC recommendations
 - The key aspects of the Toxicological Review, especially how toxicological similarities between TMB isomers and toxicokinetic modeling were used to fill in data gaps in isomer-specific databases
 - Major public comments received and EPA's responses to those comments



Implementation of 2011 NRC Recommendations

- **The new document structure enhances clarity, reduces volume, addresses redundancies and inconsistencies and includes:**
 - A Preamble that describes the assessment methods
 - An executive summary that concisely summarizes major conclusions
 - A detailed literature search strategy and study selection section
 - Use of the HERO database
 - Distinct sections on hazard identification and dose-response assessment
 - Standardized evidence tables in place of long text descriptions
 - Standardized study evaluation (describing strengths and weaknesses) by including more systematic synthesis and integration of information by health outcome
 - A dose-response section with detailed analyses and candidate reference values for multiple endpoints
 - Clear description of decision points



General Information

- **Uses of Trimethylbenzenes (TMBs)**
 - Blending agent in gasoline formulations (as part of the C9 fraction)
 - Solvent in research and industry
 - Dyestuff intermediate
 - UV oxidation stabilizer for plastics
 - Paint thinner
- **Exposures**
 - Primarily via inhalation
 - General population exposures associated with combustion and refining activities
 - Occupational exposures occur in the oil/gas extraction and printing industries
 - Oral ingestion possible through contaminated food or drinking water
 - 6.1 million pounds of 1,2,4-TMB released to the environment in 2012
 - 5.5 million pounds as point or fugitive air emissions
 - 456,000 pounds disposed of in on- and off-site injection wells or landfills

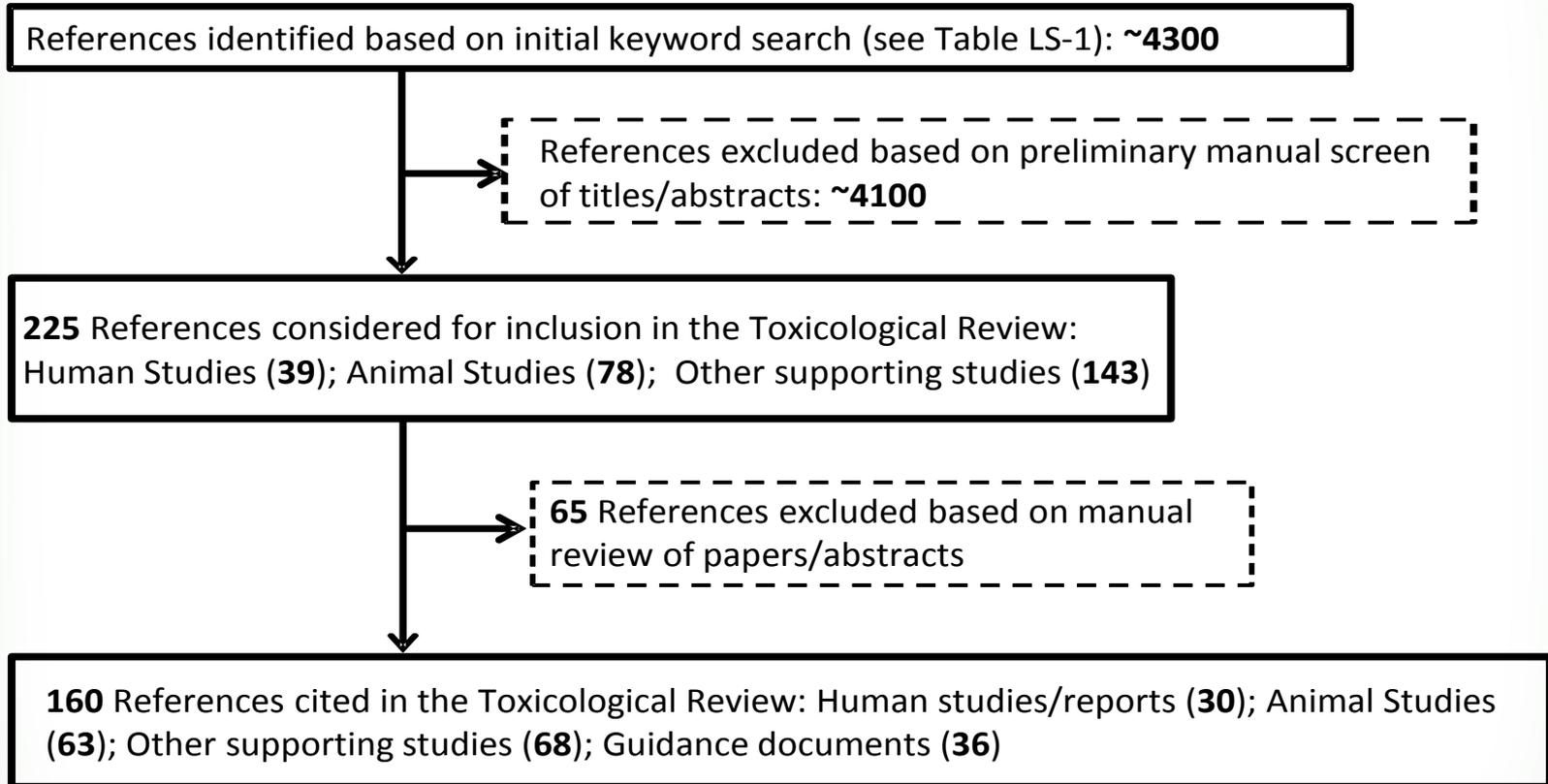


Scope of Toxicological Review

- **Critical review of publicly available literature on individual isomers of trimethylbenzene (1,2,3-, 1,2,4-, or 1,3,5-TMB)**
 - Does not include quantitative analysis of complex mixtures that contain TMB isomers
 - Human studies involving exposures to mixtures containing TMB isomers considered qualitatively in hazard identification
 - Animal studies investigating exposure to C9 fraction not used in toxicity value derivations (Appendices E & F)
- **TMBs nominated to IRIS Program due to presence at 41 Superfund sites:**
 - 1,2,4-TMB: 35 sites (83%)
 - 1,3,5-TMB: 38 sites (93%)
 - 1,2,3-TMB: 5 sites (12%)
 - Unspecified isomers: 7 sites (17%)



Literature Search Strategy



Some references provide information on more than one topic, and therefore, may be included in more than one study type. Accordingly, the sum of the references in subcategories of studies will not match the number of references for the larger category.



TMBs Database

	Human	Experimental						
		Chronic	Sub-chronic	Short-term	Acute	Develop- mental	Toxico- kinetics	Mechanistic Information*
Inhalation								
1,2,3-TMB	✓		✓	✓	✓		✓	
1,2,4-TMB	✓		✓	✓	✓	✓	✓	
1,3,5-TMB	✓			✓	✓	✓	✓	
Oral								
1,2,3-TMB					✓		✓	
1,2,4-TMB		✓			✓		✓	
1,3,5-TMB			✓		✓		✓	

* MOA information is either inferred from observed toxicological effects (i.e., inflammatory responses in the respiratory system following inhalation exposure), in vitro studies, or from compounds with similar structures (i.e., toluene or xylene)



Hazard Identification - Inhalation

Health Outcome Measure	Exposure Duration	1,2,3-TMB	1,2,4-TMB	1,3,5-TMB
Nervous System Effects	acute	✓	✓	✓
	Pain Sensitivity	subchronic	✓	✓
Pain Sensitivity following foot shock challenge	short-term	✓	✓	✓
Neuromuscular Function	acute	✓	✓	✓
	subchronic	✓	✓	
Motor Function / Anxiety	short-term	✓	✓	✓
Sensitization	short-term	✓	✓	
Cognitive Function	short-term	✓	✓	✓
Electrocortical activity	acute	✓	✓	✓
Respiratory Effects	acute	✓	✓	✓
	Subchronic	✓	✓	
Developmental Effects	gestational		✓	✓
Hematological Effects	subchronic	✓	✓	
Carcinogenicity	chronic			

The table denotes the presence or absence of data and does not distinguish between outcomes measures with larger vs. smaller databases



Study Selection for RfC Derivation

- **Four sub-chronic studies identified as adequate for dose-response analysis –**
 - Korsak and Rydzyński (1996) – 1,2,3-TMB and 1,2,4-TMB
 - Korsak et al. (2000a) – 1,2,4-TMB
 - Korsak et al. (2000b) – 1,2,3-TMB
 - Saillenfait et al. (2005) – 1,2,4-TMB and 1,3,5-TMB
- **All studies used appropriate laboratory animal models (rats), reasonable ranges of exposure levels, adequate numbers of animals per exposure group and statistical tests**
- **Three studies (Korsak et al., 2000a,b; Saillenfait et al., 2005) reported actual exposure concentrations to be within 10% of target concentrations**



RfC Derivation – 1,2,4-TMB

- **Decreased pain sensitivity observed in acute, short-term, and subchronic studies and was selected as the critical effect**
- **Available rat PBPK model (Hissink et al., 2007) was used to calculate internal blood dose metrics for use with BMD modeling; the human PBPK model was then used to calculate the human equivalent concentration (HEC)**

Critical effect	Point of departure	Uncertainty factor	Chronic RfC (mg/m ³)
Decreased pain sensitivity Korsak and Rydzyński (1996)	POD _{HEC} (mg/m ³) = 15.8	Total UF = 300 UF _A = 3 UF _H = 10 UF _S = 3 UF _D = 3	5 × 10 ⁻²



RfC Derivation – 1,2,3-TMB

- **Decreased pain sensitivity observed in acute, short-term, and subchronic studies and was selected as the critical effect**
- **No PPBK model available for 1,2,3-TMB, all BMD modeling conducted using external exposure concentrations; HEC calculated using default dosimetric adjustments**

Critical effect	Point of departure	Uncertainty factor	Chronic RfC (mg/m ³)
Decreased pain sensitivity Korsak and Rydzyński (1996)	POD _{HEC} (mg/m ³) = 16.3	Total UF = 300 UF _A = 3 UF _H = 10 UF _S = 3 UF _D = 3	5 × 10 ⁻²



Key Scientific Issue Addressed in Toxicological Review

- **Some TMB isomer toxicity databases are inadequate for derivation of reference values**
 - How to leverage toxicity and toxicokinetic data available for other TMB isomers in order to derive reference values for another isomer?

Isomer	Reference Concentration	Reference Dose
1,2,4-TMB	Suitable subchronic toxicity study to support RfC derivation (5×10^{-2} mg/m ³)	-
1,2,3-TMB	Suitable subchronic toxicity study to support RfC derivation (5×10^{-2} mg/m ³)	-
1,3,5-TMB	No available chronic or subchronic study to support RfC derivation	-

- Developmental toxicity study (Saillenfait et al., 2005) was considered**
 - Use of decreased maternal weight as critical effect would result in an RfC = 1 mg/m³
 - This RfC would be 20-fold greater than that derived for 1,2,4-TMB (5 × 10⁻² mg/m³)
- This difference is not consistent with the toxicological and toxicokinetic database that demonstrates the two isomers are very similar to one another and suggests that reliance on this study would underestimate the toxicity of 1,3,5-TMB.**

Health Outcome Measure	Exposure Duration	TMB Isomer Potency
Pain Sensitivity	acute	1,2,4-TMB ≈ 1,3,5-TMB
Pain Sensitivity following foot shock challenge	short-term	1,2,4-TMB ≈ 1,3,5-TMB
Neuromuscular Function	acute	1,2,4-TMB ≈ 1,3,5-TMB
Motor Function / Anxiety	short-term	1,2,4-TMB ≈ 1,3,5-TMB
Cognitive Function	short-term	1,3,5-TMB > 1,2,4-TMB
Electrocortical activity	acute	1,3,5-TMB > 1,2,4-TMB
Respiratory Effects	acute	1,2,4-TMB ≈ 1,3,5-TMB
Developmental Effects	gestational	1,2,4-TMB = 1,3,5-TMB



Key Scientific Issue Addressed in Toxicological Review

- **1,2,4-TMB and 1,3,5-TMB are similar in their physiochemical, toxicokinetic, and toxicological properties**
- **Given the similarities between the isomers and the likely underestimation of toxicity associated with a RfC derived from Saillenfait et al., 2005, the RfC for 1,3,5-TMB was adopted from 1,2,4-TMB**

Isomer	Reference Concentration	Reference Dose
1,2,4-TMB	Suitable subchronic toxicity study to support RfC derivation ($5 \times 10^{-2} \text{ mg/m}^3$)	-
1,2,3-TMB	Suitable subchronic toxicity study to support RfC derivation ($5 \times 10^{-2} \text{ mg/m}^3$)	-
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers ($5 \times 10^{-2} \text{ mg/m}^3$)	-



Hazard Identification - Oral

- **No chronic or subchronic studies were identified that investigated the non-cancer toxicity of 1,2,4-TMB or 1,2,3-TMB following oral exposures.**
- **Only one subchronic study was identified that examined the effects of oral exposure to 1,3,5-TMB – Koch Industries (1995b).**
 - Minor changes in hematological parameters (increased monocytes) and clinical chemistry parameters (altered sodium, chloride, potassium levels) were noted.
 - Study was unpublished industry report; EPA sought a external peer review of the study which highlighted several deficiencies in the study that precluded using it for RfD derivation.



Key Scientific Issue Addressed in Toxicological Review

- **Some TMB isomer toxicity databases are inadequate for derivation of reference values**
 - How to leverage toxicity and toxicokinetic data for other TMB isomers in order to derive reference values for another isomer?

Isomer	Reference Concentration	Reference Dose
1,2,4-TMB	Suitable subchronic toxicity study to support RfC derivation ($5 \times 10^{-2} \text{ mg/m}^3$)	No available chronic or subchronic study to support RfD derivation
1,2,3-TMB	Suitable subchronic toxicity study to support RfC derivation ($5 \times 10^{-2} \text{ mg/m}^3$)	No available chronic or subchronic study to support RfD derivation
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers ($5 \times 10^{-2} \text{ mg/m}^3$)	No available chronic or suitable subchronic study to support RfD derivation



RfD Derivation – 1,2,4-TMB

- **In the absence of route-specific data to derive an RfD for 1,2,4-TMB**
 - A route-to-route extrapolation was performed with the available human PBPK model (Hissink et al., 2007)
 - Assumptions: constant oral ingestion and 100% absorption via constant infusion into the liver
 - Available toxicity and toxicokinetic database supports the use of a route-to-route extrapolation

Critical effect	Point of departure	Uncertainty factor	Chronic RfD (mg/kg-day)
Decreased pain sensitivity Korsak and Rydzyński (1996)	POD_{HEC} (mg/kg-day) = 6.3	Total UF = 300 $UF_A = 3$ $UF_H = 10$ $UF_S = 3$ $UF_D = 3$	2×10^{-2}



Key Scientific Issue Addressed in Toxicological Review

- **Some TMB isomer toxicity databases are inadequate for derivation of reference values**
 - How to leverage toxicity and toxicokinetic data for other TMB isomers in order to derive reference values for another isomer

Isomer	Reference Concentration	Reference Dose
1,2,4-TMB	Suitable subchronic toxicity study to support RfC derivation	Route-to-route extrapolation from RfC for 1,2,4-TMB using PBPK model
1,2,3-TMB	Suitable subchronic toxicity study to support RfC derivation	No available chronic or subchronic study to support RfD derivation
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers	No available chronic or suitable subchronic study to support RfD derivation



RfD Derivation – 1,2,3-TMB and 1,3,5-TMB

- **The physiochemical, toxicological and toxicokinetic properties demonstrates the 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB are very similar to one another**
- **1,2,3-TMB**
 - Qualitative patterns of neurotoxicity similar between 1,2,4-TMB and 1,2,3-TMB
 - Equal RfC values derived independently for both isomers
 - Similarities in blood:air partition coefficients and bloodstream absorption data indicate blood levels would be similar for the two isomers
 - Qualitative metabolic profiles are similar between the two isomers
- **1,3,5-TMB**
 - 1,3,5-TMB and 1,2,4-TMB elicit similar neurotoxic effects following acute and short-term studies
 - No evidence exists to suggest toxicity profiles would differ for 1,3,5-TMB following oral or inhalation exposures
 - Similarities in blood:air partition coefficients and bloodstream absorption data indicate blood levels would be similar for the two isomers
 - Qualitative metabolic profiles are similar between the two isomers



Key Scientific Issue Addressed in Toxicological Review

- Given the similarities the similarities in the physiochemical, toxicological and toxicokinetic properties of the three isomers, the RfD for 1,2,4-TMB was adopted as the RfD for both 1,2,3-TMB and 1,3,5-TMB.

Isomer	Reference Concentration	Reference Dose
1,2,4-TMB	Suitable subchronic toxicity study to support RfC derivation	Route-to-route extrapolation from RfC for 1,2,4-TMB using PBPK model
1,2,3-TMB	Suitable subchronic toxicity study to support RfC derivation	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers



Major Public Comments Received and EPA's Response

- **Comment - EPA should include multiple C9 fraction inhalation studies in the assessment, and should base RfC derivations on these studies (pages F-2, F-12)**
- **EPA's Response:**
 - The C9 fraction is a complex mixture containing at most only 55% TMB isomers

Constituent	Clark et al. (1990)	Douglas et al. (1993)	Schreiner et al. (1989)	McKee et al. (1990)
TMBs	44.81	55.05	55.05	55.05
ethyltoluenes	30.96	27.59	27.59	27.59
<i>o</i> -xylene	2.27	3.20	3.20	3.20
<i>n</i> -propylbenzene	4.05	3.97	3.97	3.97
Non-aromatics	0.46	n/r	n/r	n/r
cumene	n/r	2.74	2.74	2.74
Benzene	n/a	n/r	n/r	n/r
C10s	8.31*	6.19**	6.19**	6.19**
Unknown	9.15	1.26	1.26	1.26

n/r = not reported; n/a = reported to not be present; * = 1-methyl-3-n-propylbenzene, 1,2-diethylbenzene, and 1-ethyl-3,5-dimethylbenzene; ** specific C10 compounds not reported



Major Public Comments Received and EPA's Response

- **Comment - EPA should include multiple C9 fraction inhalation studies in the assessment, and should base RfC derivations on these studies (pages F-2, F-12)**
- **EPA's Response (continued):**
 - In the Federal Register Notice announcing the C9 fraction testing requirements, EPA agreed that “assessing the toxicity of the C9 mixture as a complete entity should provide a reasonable upper bound for the toxicity of the individual ethyltoluene and TMB [isomers] in the C9 mixture”
 - However, this assumption has been shown to be inaccurate given the results of multiple peer-reviewed studies that have demonstrated that individual TMB isomers elicit clearly adverse toxicological effects.
 - EPA also agreed that testing the C9 fraction was appropriate on the assumption that TMBs were not released to the environment in substantial quantities nor expected to persist once released
 - This also has been shown to be incorrect as substantial quantities of TMB isomers are released to the environment (2012 TRI), they persist (detected at Superfund sites) and human exposures do occur



Major Public Comments Received and EPA's Response

- **Comment - Decreased pain sensitivity is not an appropriate endpoint for derivation of the 1,2,3-TMB and 1,2,4-TMB RfCs due to issues surrounding recovery, latency, and application of external stimuli (page F-4)**
- **EPA's Response**
 - EPA has more thoroughly cited the relevant guidance documents in support of selecting decreased pain sensitivity as the critical effect
 - Neurotoxicity guidelines state that reversible effects and latent effects are of high concern
 - The *Review of the Reference Dose and Reference Concentration Process* states that “effects that may initially appear to be reversible may re-appear later or be predictive of later adverse outcomes”



Major Public Comments Received and EPA's Response

- **Comment - EPA should not discount the available 1,3,5-TMB developmental toxicity study for RfC derivation (page F-10)**
- **EPA's Response:**
 - The RfC derivation section for 1,3,5-TMB contains an extensive discussion of derivation of RfC based on maternal and fetal effects observed in Saillenfait et al. (2005)
 - The draft assessment has been revised to include these RfCs as candidate values
 - The original determination remains that use of an RfC for 1,3,5-TMB that is 20-fold greater than that derived for 1,2,4-TMB is inconsistent with evidence indicating that the isomers are similar regarding their physiochemical, toxicokinetic, and toxicological properties
 - Therefore, use of an RfC derived from endpoints observed in Saillenfait et al. (2005) likely underestimates the toxicity associated with 1,3,5-TMB (i.e., would not be protective of the assumed neurotoxicity)



Major Public Comments Received and EPA's Response

- **Comment - EPA should include 1,3,5-TMB subchronic oral study in the Toxicological Review and should base the RfD derivation on this study (page F13)**
- **EPA's Response:**
 - This study was submitted to EPA under a TSCA 4(a) test rule and had not previously been peer-reviewed. EPA sought an independent peer review in order to consider this study in the assessment (Versar, 2013)
 - A majority of the reviewers concluded that the study was not appropriate for derivation of an RfD for the following reasons:
 - The NOAEL is likely an artifact of the study investigating insensitive endpoints (body weight, gross pathology)
 - Study is not reliable as it does not investigate the endpoint of concern for TMBs (i.e., neurotoxicity)
 - Although some clinical signs were observed, these were too general to be predictive of neurotoxicity

- **The TMBs assessment:**
 - Derives new reference values for individual TMB isomers
 - Uses physiochemical, toxicokinetic, and toxicological similarities and toxicokinetic modeling to fill in data gaps in isomer-specific databases in order to derive toxicity values
 - Addresses public comments
 - Implements many of the 2011 NRC recommendations and represents a significant advance for the IRIS Program



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