EPA/635/R-16/161Fc
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Toxicological Review of Trimethylbenzenes: Executive Summary

[CASRNs 25551-13-7, 95-63-6, 526-73-8, and 108-67-8]

September 2016

Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

EXECUTIVE SUMMARY

Occurrence and Health Effects

Trimethylbenzenes (TMBs) are a commercially available mixture of three individual isomers: 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB. TMB isomers are produced during petroleum refining and production of aromatic hydrocarbons with nine carbons (i.e., C9 aromatic fraction). As the vast majority of the C9 fraction is used as a component of gasoline, vehicle emissions are expected to be the major anthropogenic source of TMBs. TMBs are volatile hydrocarbons, and humans are thus exposed to these isomers primarily through breathing air containing TMB vapors, although ingestion through food or drinking water is also possible.

Effects on the nervous, respiratory, and hematological (i.e., blood) systems have been reported in occupationally- and residentially-exposed humans, but these effects were observed following exposure to complex mixtures containing TMB isomers, thus making it difficult to determine the contribution of each TMB isomer to the observed health effects. Health effects that are roughly analogous to those seen in humans have been observed in animals exposed to the individual isomers. Effects on the nervous system, including cognitive effects and decreased pain sensitivity, are the most widely observed effects in animals. Effects on other systems, including the respiratory and hematological systems, have also been observed in animals. Both 1,2,4-TMB and 1,3,5-TMB have been observed to elicit effects on pregnant animals and developing fetuses, but at exposure levels greater than those that cause effects on the nervous system. There is inadequate information to evaluate the carcinogenicity of TMBs.

Effects Other Than Cancer Following Inhalation Exposure

The relationship between exposure to 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB and health effects has been evaluated in studies of (1) exposed human adults, (2) animals exposed via inhalation for acute, short-term, and subchronic durations, and (3) animals exposed gestationally via inhalation.

Human studies included occupational exposure to various solvent mixtures containing TMBs. Health effects noted in these studies were eye irritation, neurological effects (hand tremble, abnormal fatigue, lack of coordination), and hematological effects. Residential exposure to mixtures containing 1,2,4-TMB were observed to be associated with asthma. However, as these studies involved exposures to mixtures containing multiple TMB isomers and other volatile organic

compounds (VOCs), it is difficult to ascertain the specific contribution of each TMB isomer to the specific health effects reported. Studies involving controlled exposures of healthy adult volunteers to individual isomers also exist, although these studies generally reported little or no sensory irritation or effects on the respiratory system. One controlled human exposure study reported some deficits in attention following exposure to white spirit, a complex mixture containing 1,2,4-TMB.

Animal inhalation studies included acute and short-term studies of TMBs that reported respiratory irritation (decreased respiration rates) and neurological effects (decreased pain sensitivity, altered cognitive function, and decreased anxiety and/or increased motor function) that are consistent with effects seen in human studies. Four subchronic inhalation studies for 1,2,3-TMB and 1,2,4-TMB observed exposure-response effects in multiple systems, including the nervous, hematological, and respiratory systems. In these studies, disturbances in central nervous system (CNS) function, including decreased pain sensitivity and decreased neuromuscular function and coordination, appear to be the most sensitive endpoints following exposure to 1,2,3-TMB or 1,2,4-TMB. No subchronic studies were found that investigated exposure to 1,3,5-TMB. One developmental toxicity study observed maternal and fetal toxicity (i.e., decreased maternal weight gain and fetal weight) following exposure to either 1,2,4-TMB or 1,3,5-TMB; other indices of fetal toxicity (i.e., fetal death and malformations) were not affected by exposure.

Inhalation Reference Concentration (RfC) for TMBs for Effects Other Than Cancer

The RfC for TMBs was derived using benchmark dose (BMD) modeling coupled with physiologically-based pharmacokinetic (PBPK) modeling or default dosimetric methods. BMD modeling was conducted using external exposure concentrations as the dose inputs and either a benchmark response (BMR) level of 5% change (fetal weight) or 1 standard deviation (SD) of the control mean (all other endpoints). Once a lower confidence limit on the benchmark dose (BMDL) (or a no-observed-adverse-effect level [NOAEL] or lowest-observed-adverse-effect level [LOAEL] in cases where no models fit the data) was identified as the point of departure (POD), a human equivalent concentration (HEC) was calculated for each endpoint using either a PBPK model (1,2,4-TMB) or default dosimetric adjustments (1,2,3-TMB and 1,3,5-TMB).

To each HEC, a composite uncertainty factor (UF) was applied to account for uncertainties in the TMB database:

- 3 to account for uncertainty in extrapolating from laboratory animals to humans (interspecies variability),
- 10 to account for variation in susceptibility among members of the human population (interindividual variability),
- 3 to account for subchronic-to-chronic extrapolation due to the use of a subchronic study, and

- 3 to account for deficiencies in the database (no TMB-specific developmental neurotoxicity studies were available).

Full details of the selection and application of the UFs are available in Section 2.1.3. Dividing the candidate HECs by this composite UF of 300 yielded the organ/system-specific RfCs presented in Table ES-1.

Table ES-1. Organ/system-specific chronic RfCs for individual TMB isomers

Effect	Isomer	Basis	RfC (mg/m^3)	Composite UF	Study Exposure Duration	Confidence
Neurological Korsak and Rydzyński (1996)	1,2,4-TMB	Decreased pain sensitivity	6×10^{-2}	300	Subchronic	Low to medium
	1,2,3-TMB		5×10^{-2}	300	Subchronic	Low to medium
Hematological Korsak et al. (2000a), Korsak et al. (2000b)	1,2,4-TMB	Decreased clotting time	8×10^{-2}	300	Subchronic	Low to medium
	1,2,3-TMB	Decreased segmented neutrophils	6×10^{-2}	300	Subchronic	Low to medium
Respiratory Korsak et al. (2000a), Korsak et al. (2000b)	1,2,4-TMB	Inflammatory lung lesions	2×10^{-1}	300	Subchronic	Low to medium
	1,2,3-TMB		2×10^{-1}	300	Subchronic	Low to medium
Developmental[a] Saillenfait et al. (2005)	1,2,4-TMB	Decreased fetal weight	4	100	Gestational	Low to medium
	1,3,5-TMB		4	100	Gestational	Low to medium
Maternal[a] Saillenfait et al. (2005)	1,2,4-TMB	Decreased maternal weight	3	300	Subchronic	Low to medium
	1,3,5-TMB		4×10^{-1}	300	Subchronic	Low to medium
Chronic Overall RfC (Neurological)	**All TMB isomers**	**Decreased pain sensitivity**	**6×10^{-2}**	**300**	**Subchronic**	**Low to medium**

[a] Intended to be used for gestational exposures

Neurotoxicity is the most consistently observed endpoint in the toxicological database for TMBs, and decreased pain sensitivity was observed in multiple studies following exposures to 1,2,3- or 1,2,4-TMB for short-term or subchronic durations. Given the consistency of this effect and the determination that decreased pain sensitivity is an appropriate adverse effect with which to derive reference values (see Section 2.1.5), in accordance with the EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), decreased pain sensitivity was selected as the critical effect and Korsak and Rydzyński (1996) was selected as the principal study for derivation of the RfC for TMBs. No subchronic study was available that investigated neurotoxicity endpoints following exposure to

1,3,5-TMB, resulting in the lack of an isomer-specific neurotoxicity RfC for this isomer. However, as discussed in Section 1.2.7, the available toxicological database for all three isomers, across all exposure durations, indicates there are important similarities in the isomers' neurotoxicity that are supportive of an RfC for 1,3,5-TMB that is not substantially different than the RfC derived for other TMB isomers. Also supporting this conclusion is the observation that TMB isomers display important similarities with regard to chemical properties and toxicokinetics, including similarities in blood:air partition coefficients, respiratory uptake, and absorption into the bloodstream (see Section 1.2.7 and Appendices C.1 and C.2). These similarities support the conclusion that an RfC for 1,3,5-TMB would be similar to those calculated for 1,2,3- or 1,2,4-TMB. The RfC for 1,2,4-TMB was selected over the RfC for 1,2,3-TMB as the RfC for the entire TMB database due to increased confidence in that it was calculated via the application of a validated PBPK model, whereas the 1,2,3-TMB value was estimated using default dosimetric methods. **Therefore, the chronic RfC for all TMBs was set at 6×10^{-2} mg/m^3 based on neurological effects following exposure to 1,2,4-TMB.** However, this overall RfC for TMBs can be used for any TMB isomer alone, or in situations when individuals are exposed to a mixture of TMB isomers. The individual organ- or system-specific RfCs may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

In addition to providing an RfC for chronic exposures in multiple systems, this document also provides an RfC for subchronic-duration exposures. In the case of TMBs, all of the studies used to calculate the chronic RfCs were subchronic or gestational in duration. Therefore, the methods to calculate subchronic RfCs are identical to those used for calculation of chronic RfCs, minus the application of a subchronic-to-chronic UF (UF_S) (see Table ES-1). It should be noted that the subchronic RfC values for the developing fetus are identical to the chronic RfC values for the developing fetus as gestation represents a critical window of susceptibility and no UF_S was applied to account for less-than-chronic exposure in either case. The subchronic inhalation RfC is intended for use with exposures for more than 30 days, up to approximately 10% of the lifespan in humans. **The subchronic RfC for TMBs was set to 2×10^{-1} mg/m^3 based on neurological effects following exposure to 1,2,4-TMB.**

Table ES-2. Organ/system-specific subchronic RfCs for individual TMB isomers

Effect	Isomer	Basis	RfC (mg/m^3)	Composite UF	Study Exposure Duration	Confidence
Neurological Korsak and Rydzyński (1996)	1,2,4-TMB	Decreased pain sensitivity	2×10^{-1}	100	Subchronic	Low to medium
	1,2,3-TMB		2×10^{-1}	100	Subchronic	Low to medium
Hematological Korsak et al. (2000a), Korsak et al. (2000b)	1,2,4-TMB	Decreased clotting time	2×10^{-1}	100	Subchronic	Low to medium
	1,2,3-TMB	Decreased segmented neutrophils	2×10^{-1}	100	Subchronic	Low to medium
Respiratory Korsak et al. (2000a), Korsak et al. (2000b)	1,2,4-TMB	Inflammatory lung lesions	6×10^{-1}	100	Subchronic	Low to medium
	1,2,3-TMB		6×10^{-1}	100	Subchronic	Low to medium
Developmental [a] Saillenfait et al. (2005)	1,2,4-TMB	Fetal weight	4	100	Gestational	Low to medium
	1,3,5-TMB		4	100	Gestational	Low to medium
Maternal [a] Saillenfait et al. (2005)	1,2,4-TMB	Decreased maternal weight	8	100	Subchronic	Low to medium
	1,3,5-TMB		1	100	Subchronic	Low to medium
Subchronic Overall RfC (Neurological)	**All TMB isomers**	**Decreased pain sensitivity**	**2×10^{-1}**	**100**	**Subchronic**	**Low to medium**

[a] Intended to be used for gestational exposures

Confidence in the Chronic Inhalation RfC for 1,2,4-TMB

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994)*.

Confidence in the study from which the critical effect was identified is low to medium. The study is a peer-reviewed study that utilized three dose groups plus untreated controls, employed an appropriate number of animals per dose group, and performed appropriate statistical analyses. However, sources of uncertainty exist that reduce confidence in this study.

One area of uncertainty regarding this study is the lack of reported actual concentrations. However, as the methods by which the test atmosphere was generated and analyzed were reported in sufficient detail, and given the fact that this laboratory has used this methodology in subsequent studies and achieved appropriate actual concentrations (i.e., within 10% of target concentrations),

the concern regarding the lack of reported actual concentrations is reduced. Another source of uncertainty is the fact that the principal study does not explicitly state that the reported measures of variance in Table 1 of that reference are SDs. However, careful analysis of the reported levels of variance and magnitude of statistical significance indicate that the measures of variance are SDs. Supporting this conclusion is the observation that all other papers from this laboratory report variance as SDs. The critical effect on which the RfC is based is well-supported as the weight of evidence for TMB-induced neurotoxicity is coherent across species (i.e., human, mouse, and rat), coherent across isomers, and consistent across multiple exposure durations (i.e., acute, short-term, and subchronic).

The database for TMBs includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. However, confidence in the overall database is low to medium because it lacks chronic and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. The overall confidence in the RfC for TMBs is low to medium.

Effects Other Than Cancer Observed Following Oral Exposure

Only one subchronic study was identified that examined the effects of oral exposure to 1,3,5-TMB. Effects in the hematological system, including changes in clinical chemistry parameters and differential white blood cell (WBC) numbers, were observed following exposure to 1,3,5-TMB via gavage in rats. Altered organ weights were also observed in multiple systems (kidneys, liver). The alterations to clinical chemistry parameters and organ weights were observed in the absence of histopathological changes in relevant systems, and were thus considered to be compensatory in nature. Discounting effects that could be non-adverse or compensatory in nature left an observed increase in monocytes in male rats as the only statistically significant effect on which to base the reference dose (RfD) derivation. While a slight increase in monocytes may be of questionable adversity if taken with no context of the larger TMB database, a number of endpoints involving the alteration of WBC counts have been observed in the inhalation toxicity database. It was therefore deemed that the observed increase in monocytes following oral exposures was possibly indicative of an underlying toxicity to the hematological system also evident following inhalation exposure.

Oral Reference Dose (RfD) for TMBs for Effects Other Than Cancer

The RfD for TMBs was derived using BMD modeling coupled with default dosimetric methods. BMD modeling was conducted using external exposure concentrations as the dose inputs and a BMR level of 1 SD of the control mean. Once a BMDL was identified as the POD, a human equivalent dose (HED) of 3.0 mg/kg-day was calculated for increased monocytes using default dosimetric adjustments (i.e., body weight to the ¾ power).

To the estimated HED, a composite UF was applied to account for uncertainties in the TMB database: 3 to account for uncertainty in extrapolating from laboratory animals to humans (interspecies variability), 10 to account for variation in susceptibility among members of the

human population (interindividual variability), 3 to account for subchronic-to-chronic extrapolation due to the use of a subchronic study, and 3 to account for deficiencies in the database (no TMB-specific developmental neurotoxicity studies were available). Full details of the selection and application of the UFs are available in Section 2.2.3. **Dividing the HED by this composite UF of 300 yielded an RfD of 1×10^{-2} mg/kg-day that can be applied to any TMB isomer individually or to mixtures of TMB isomers.**

In addition to the RfD calculated for TMBs from oral data, an RfD was calculated from inhalation data using a route-to-route extrapolation to address the lack of suitable neurotoxicity data in the oral TMB database. It is clear from the inhalation database for TMB that neurotoxicity is an important endpoint for derivation of reference values, especially given the consistency with which neurotoxicity is observed in the TMB database, across all isomers following acute oral and acute, short-term, and subchronic inhalation exposures. Ultimately, the fact that oral and inhalation neurotoxic endpoints are comparable, and that neurotoxic endpoints resulted in the most strongly supported RfCs in the inhalation database, it is reasonable to expect that neurotoxicity-based PODs would be critical for deriving RfDs. The available database for 1,2,4-TMB supports the use of route-to-route extrapolation; sufficient evidence exists that demonstrates similar qualitative profiles of metabolism (i.e., observation of dimethylbenzoic and hippuric acid metabolites) and patterns of parent compound distribution across exposure routes (Section C.2, Appendix C).

Therefore, assuming that oral exposure would result in the same systemic effect as inhalation exposure (i.e., altered CNS function, measured as decreased pain sensitivity), an oral exposure component was added to the PBPK model by EPA (Section C.3.3.5, Appendix C), assuming 100% absorption of the ingested 1,2,4-TMB by constant infusion of the oral dose into the liver and an idealized pattern of six ingestion events (see Section 2.2.3). **Using the modified PBPK model resulted in an HED of 3.5 mg/kg-day, which was divided by the composite UF of 300 to estimate an RfD of 1×10^{-2} mg/kg-day.** Although identical to the RfD calculated from the oral 1,3,5-TMB data for increased monocytes, this value of 1×10^{-2} mg/kg-day was ultimately selected as the RfD for TMB isomers based on multiple lines of evidence in the oral and inhalation database, including commonalities in the pattern of neurotoxic effects observed following oral and inhalation exposures, similarities in blood:air and tissue:air partition coefficients and absorption into the bloodstream between TMB isomers, and qualitative metabolic profiles that suggest that first-pass metabolism through the liver is not expected to differ greatly between the three isomers.

In addition to providing RfDs for effects in the hematological and nervous systems, this document also provides values for subchronic RfD values for exposures that may be of concern in a less-than-lifetime context. In the case of TMBs, the oral 1,3,5-TMB study and the inhalation 1,2,4-TMB study used for the route-to-route extrapolation for the calculation of the chronic RfDs were both of subchronic duration. Therefore, the methods used to calculate subchronic RfDs is identical to that used for calculation of chronic RfCs, minus the application of a UF_S. This results in a composite UF of 100 (interspecies UF [UF_A] of 3, intraspecies UF [UF_H] of 10, UF_S of 1, and database

UF [UF_D] of 3). Dividing the POD for hematological effects (3.01 mg/kg-day) and neurotoxicity effects (3.5 mg/kg-day) by the composite UF of 100 results in RfDs of 3×10^{-2} and 4×10^{-2} mg/kg-day for decreased monocytes and decreased pain sensitivity, respectively. **The subchronic RfD was set to 4×10^{-2} mg/kg-day based on neurological effects following exposure to 1,2,4-TMB**. The subchronic oral RfD is intended for use with exposures for more than 30 days, up to approximately 10% of the lifespan in humans.

Confidence in the Chronic Oral RfD for 1,2,4-TMB

The confidence in the oral database for TMB is low because it only contains acute oral studies investigating neurotoxicity endpoints for multiple isomers, and one subchronic study investigating general toxicity endpoints for one isomer (1,3,5-TMB). This database was used to derive an RfD, but given the concern over the lack of a suitable neurotoxicity study, the confidence in this RfD is low. A PBPK model was utilized to perform a route-to-route extrapolation to determine a POD for the derivation of the RfD from inhalation data. The confidence in the study from which the critical effect was identified is low to medium (see Section 2.1.7). The inhalation database for 1,2,4-TMB includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. However, confidence in the overall database for TMB is low to medium because it lacks chronic and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the application of the available PBPK model for the purposes of a route-to-route extrapolation, the overall confidence in the RfD for TMB is low.

Evidence of Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is "inadequate information to assess carcinogenic potential" of TMBs. No chronic inhalation studies that investigated cancer outcomes were identified in the literature for 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. One cancer study in which rats were exposed to 1,2,4-TMB via gavage at one experimental dose of 800 mg/kg-day reported marginal increases in total malignant tumors and head tumors (e.g., neuroesthesioepitheliomas), but provided no statistical analyses of the results. A number of methodological issues limit the utility of this study (e.g., only one dose group and no discussion of histopathological analyses). Therefore, **a quantitative cancer assessment for TMBs was not conducted.**

Susceptible Populations and Lifestages

No TMB-specific data that would allow for the identification of populations or lifestages with increased susceptibility to TMB exposure exist. However, some data gleaned from the related compound, toluene, do provide some suggestive evidence that periods in early life represent periods of susceptibility to solvent exposure. Therefore, it can be reasonably assumed that exposures in early life to individual TMB isomers are of particular concern.

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