1. DOSE-RESPONSE ANALYSIS

1.1. Inhalation Reference Concentration for Effects other than Cancer for 1,2,3-TMB

1.1.1. Identification of Candidate Principal Studies and Critical Effects for 1,2,3-TMB

The nervous, hematological, and respiratory systems are the primary targets of inhaled 1,2,3-TMB in humans and experimental animals, and effects in these systems have been identified as hazards following inhalation exposure to 1,2,3-TMB. Human data are preferred over animal data for deriving reference values when possible because the use of human data is more relevant in the assessment of human health and avoids the uncertainty associated with interspecies extrapolation introduced when animal data serve as the basis for the RfC. In this case, literature exists on the noncancer effects of 1,2,3-TMB exposure in humans, including neurological, hematological, and respiratory toxicities; however, no human studies are available that would allow for dose-response analysis. The human studies evaluated TMB exposures occurring as a complex solvent or VOC mixtures, and this consideration along with other uncertainties including high imprecision in effect measures due to low statistical power, lack of quantitative exposure assessment, and lack of control for co-exposures, limits their utility in derivation of quantitative human health toxicity values. However, these studies provide supportive evidence for the neurological, hematological, and respiratory toxicity of TMB isomers in humans.

Several studies investigating 1,2,3-TMB noncancer effects in experimental animal models were identified in the literature. No chronic studies were available, although several acute, short-term, and subchronic studies were identified. 1,2,3-TMB-induced toxicity was observed across several organ systems in two subchronic studies by Korsak et al. (2000b) and Korsak and Rydzyński (1996). These were the only subchronic studies identified in the peer-reviewed literature. Data from these studies pertaining to the primary hazards observed in humans and animals identified in Chapter 1, neurological, hematological, and respiratory toxicity, were considered as candidate critical effects for the purpose of determining the point of departure (POD) for derivation of the inhalation RfC for 1,2,3-TMB. Neurotoxicity was also observed in both acute and short-term inhalation studies and respiratory toxicity was also observed in acute studies. However, the high exposure concentrations used in acute studies and the short exposure durations of both acute and short-term studies limit their applicability for quantitative assessment of chronic human health effects. Nevertheless, as with the human mixture studies, these studies provide

qualitative information regarding the consistency and coherency of these effects across the 1,2,3-TMB database.

The two subchronic studies by Korsak et al. (2000b) and Korsak and Rydzyński (1996) are considered to be adequate for dose-response analysis. Both studies used rats, an appropriate laboratory animal species, and utilized appropriate sham-exposed controls. Animals were exposed to 1,2,3-TMB reported as > 97% pure (impurities not reported). The studies utilized an appropriate route (inhaled air) and duration (subchronic) of exposure. The studies used a reasonable range of appropriately-spaced exposure levels to facilitate dose-response analysis. An appropriate latency between exposure and development of toxicological outcomes was used, and the persistence of some outcomes after termination of exposure was investigated. Adequate numbers of animals per exposure group were used, and appropriate statistical tests including pair-wise and trend analyses were performed.

With regard to reporting of exposure methodologies, Korsak et al. (2000b) reported actual exposure concentrations, as measured by gas chromatography, to be within 10% of target exposure concentrations. This increases the confidence in the overall evaluation and adequacy of this study. Although Korsak and Rydzyński (1996) do not report actual, measured exposure concentrations, this study uses the same exposure methodology as Korsak et al. (2000b); suggesting that it is likely that the actual exposure concentrations in this study are within 10% of target exposure concentrations. Target and actual concentrations for these studies are listed in Table 1-1.

Table 1-1. Target and actual exposure concentrations used in BMD modeling of 1,2,3-TMB endpoints considered for the derivation of the RfC

Reference	Species/ Sex	Target Exposure Concentration (mg/m^3)	Actual Exposure Concentration (mg/m^3)
Korsak and Rydzyński (1996)	Rat, male	123	n/a
		492	n/a
		1230	n/a
Korsak et al. (2000b)	Rat, male	123	128
		492	523
		1230	1269
	Rat, female	123	128
		492	523
		1230	1269

n/a: not applicable

These subchronic studies examined 1,2,3-TMB-induced toxicity in multiple organ systems. Neurological, hematological, and respiratory endpoints that demonstrated statistically

significant pair-wise increases or decreases relative to control were considered for the derivation of the RfC for 1,2,3-TMB (Table 1-2). These endpoints included decreased pain sensitivity in male rats (Korsak and Rydzyński, 1996), and decreased RBCs and increased reticulocytes in male rats, decreased segmented neutrophils and increased lymphocytes in male and female rats, and increased inflammatory lung lesions in female rats (Korsak et al., 2000b). Changes in liver organ weights and clinical chemistry parameters from Korsak et al. (2000b) were not further considered due to the lack of accompanying hepatocellular histopathological alterations in exposed animals. Changes in splenic organ weights were similarly not considered further due to a lack of any observed histopathological changes in that organ. Increases in reticulocytes in females were not further considered due to non-monotonicity in response (i.e., non-significant increases in high exposure concentration animals).

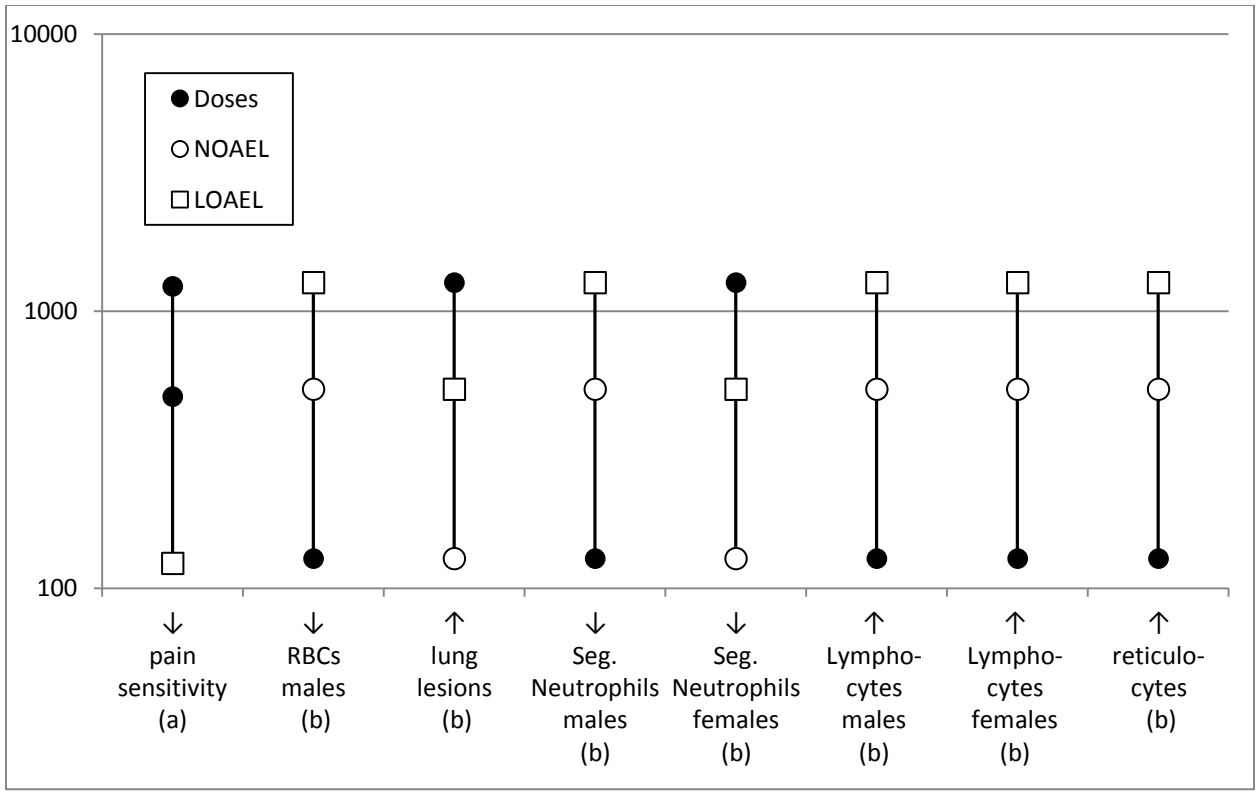
Table 1-2. Non-cancer endpoints resulting from subchronic inhalation exposure to 1,2,3-TMB considered for the derivation of the RfC

Endpoint	Species/ Sex	Exposure Concentration (mg/m^3)			
		0	123	492	1,230
Neurological Endpoints					
Decreased pain sensitivity (measured as latency to paw-lick in seconds)	Rat, male	9.7 ± 2.1[a] (n = 30)	11.8 ± 3.8[d] (n = 20)	16.3 ± 6.3[e] (n = 10)	17.3 ± 3.4[c] (n = 10)
Hematological Endpoints					
Decreased RBCs (10^6/cm^3)	Rat, male	9.49 ± 2.03 (n = 10)	10.2 ± 1.29 (n = 10)	10.11 ± 1.27 (n = 10)	8.05 ± 1.38[b] (n = 10)
Decreased segmented neutrophils (%)	Rat, male	24.8 ± 4.5 (n = 10)	25.4 ± 5.8 (n = 10)	20.7 ± 5.8 (n = 10)	17.7 ± 8.3[b] (n = 10)
	Rat, female	23.1 ± 6.1 (n = 10)	19.7 ± 3.4 (n = 10)	16.4 ± 4.2[b] (n = 10)	11.9 ± 7.1[c] (n = 10)
Increased lymphocytes (%)	Rat, male	71.2 ± 5.0 (n = 10)	71.6 ± 6.8 (n = 10)	75.4 ± 4.7 (n = 10)	79.3 ± 78.0[c] (n = 10)
	Rat, female	73.2 ± 7.9 (n = 10)	77.5 ± 4.9 (n = 10)	80.4 ± 5.1 (n = 10)	84.0 ± 78.0[c] (n = 10)
Increased reticulocytes (%)	Rat, male	2.8 ± 1.3 (n = 10)	2.1 ± 1.7 (n = 10)	3.8 ± 2.1 (n = 10)	4.5 ± 1.8[b] (n = 10)
Respiratory Endpoints					
Increased inflammatory lung lesions	Rat, female	[e] (n = 10)	[e] (n =10)	[e] (n = 10)	[e] (n = 10)

[a] Values are expressed as mean ± one standard deviation
[b] $p < 0.05$
[c] $p < 0.01$
[d] Level of significance not reported in Table 1 from Korsak and Rydzyński (1996), however the results of an ad-hoc t-test (performed by EPA) indicated significance at $p < 0.01$
[e] Incidences for exposure groups not reported; however, based on qualitative information reported in the study, i.e., that female rats exhibited a statistically significant increase in inflammatory lung lesions at 492 mg/m^3.
Adapted from Korsak et al., (2000b) and Korsak and Rydzyński (1996)

Impaired neuromuscular function and coordination, measured as performance on rotarod apparatus, was also observed in rats exposed to 1,2,3-TMB. The use of rotarod data from Korsak and Rydzyński (1996) was initially considered for quantification of candidate RfC values for 1,2,3-TMB. However, upon critical evaluation of the exposure-response information in the study it was determined that the endpoint was reported in a manner that reduced the confidence in the observed effect levels. The critical limitation noted for these data related to the presentation of rotarod performance, which is best represented as a continuous variable, as opposed to a quantal variable such as that presented by Korsak and Rydzyński (1996). In contrast to the percent failures reported by the study authors, the most widely used and accepted measurement for rotarod performance in rodents is latency to fall from the rotating rod (Brooks and Dunnett, 2009; Kaspar et al., 2003; Bogo et al., 1981), typically with an arbitrary upper limit on the maximum latency allowed to prevent confounding by fatigue. Although the quantal percent failures data can provide useful information, these measures require an arbitrary selection of the length of time required for successful performance; there is no scientific consensus on an optimal time for this parameter. In addition, when identifying effect levels based on the data presented by Korsak and Rydzyński (1996), latencies on the rod of 1 and 119 seconds would be treated identically as failures when, in fact, they indicate very different levels of neurological dysfunction (Bogo et al., 1981). This adds uncertainty when trying to extrapolate to a concentration associated with a minimally adverse effect. Finally, quantal presentation of data does not allow for interpretations related to intra-rat and intra-group variability in performance. Due to these reporting limitations, impaired neuromuscular function and coordination, measured as performance on the rotarod apparatus, was excluded from consideration for derivation of the RfC for 1,2,3-TMB.

Endpoints carried forward for derivation of an RfC for 1,2,3-TMB, along with their exposure ranges and NOAEL and LOAEL values (identified by EPA), are graphically represented in Figure 1-1.



Solid lines represent range of exposure concentrations. (a) Korsak and Rydzyński (1996); (b) Korsak et al. (2000b)

Figure 1-1. Exposure response array for endpoints resulting from inhalation exposure to 1,2,3-TMB considered for the derivation of the RfC

1.1.2. Methods of Analysis for Reference Concentration Derivation for 1,2,3-TMB

As discussed above, endpoints observed in Korsak et al., (2000b) and Korsak and Rydzyński (1996) that demonstrated statistically significant (at least at the $p < 0.05$ level) pair-wise increases or decreases relative to control for at least one exposure group were considered for the derivation of the RfC for 1,2,3-TMB; these effects are listed in Table 1-2 above. This assessment used the BMD approach, when possible, to estimate a POD for the derivation of an RfC for 1,2,3-TMB (see the *Supplemental Information* for detailed methodology). The BMD approach involves fitting a suite of mathematical models to the observed dose-response data using EPA's BMDS (version 2.2). Each fitted model estimates a BMD and its associated BMDL corresponding to a selected BMR. For continuous data (i.e., decreased pain sensitivity, decreased RBCs, decreased segmented neutrophils, increased lymphocytes, increased reticulocytes) from the Korsak and Rydzyński (1996) and Korsak et al. (2000b) studies, no information is available regarding the change in these responses that would be considered biologically significant, and thus a BMR equal to a change in the

mean equal to 1 standard deviation of the model estimated control mean was used in modeling the endpoints, consistent with the *Benchmark Dose Technical Guidance* (U.S. EPA, 2000). The estimated BMDL is then used as the POD for deriving the RfC.

The suitability of the above methods to determine a POD is dependent on the nature of the toxicity database for a specific chemical. Some endpoints for 1,2,3-TMB were not amenable to BMD modeling for a variety of reasons, including responses only in the high exposure group with no significant changes in responses in lower exposure groups (e.g., decreased RBCs) and absence of incidence data (e.g., increased inflammatory lung lesions). Increased lymphocytes were excluded from analysis by BMD modeling due to the unusually high standard deviations reported in the high exposure group. In cases where BMD modeling was not feasible, the NOAEL/LOAEL approach was used to identify a POD. Additionally, for decreased pain sensitivity, the reported SD of 3.4 in the high exposure group resulted in an inability of the variance power model to fit the data adequately. For this reason, the high dose was dropped in order to facilitate model fitting. Detailed modeling results are provided in the *Supplemental Information*.

Because an RfC is a toxicity value that assumes continuous human inhalation exposure over a lifetime, data derived from inhalation studies in animals need to be adjusted to account for the noncontinuous exposures used in these studies. In the Korsak et al., (2000b) and Korsak and Rydzyński (1996) studies, rats were exposed to 1,2,3-TMB for 6 hours/day, 5 days/week for 3 months. Because no PBPK model exists for 1,2,3-TMB, the duration-adjusted PODs for neurological effects in rats were calculated as follows:

POD_{ADJ} (mg/m^3) = POD (mg/m^3) × hours exposed per day/24 hours × days exposed per week/7 days

Therefore, for decreased pain sensitivity from Korsak and Rydzyński (1996), the POD_{ADJ} would be calculated as follows:

POD_{ADJ} (mg/m^3) = 97.19 mg/m^3× 6 hours/24 hours × 5 days/7 days

POD_{ADJ} (mg/m^3) = 17.36 mg/m^3

The calculated POD_{ADJ} (mg/m^3) values for all neurological, hematological, and respiratory endpoints considered for RfC derivation are presented in Table 1-3.

Table 1-3. Summary of dose-response analysis and point of departure estimation for endpoints resulting from subchronic inhalation exposure to 1,2,3-TMB

Reference	Endpoint	Species/ Sex	POD Basis	Best-fit Model; BMR	Candidate POD (mg/m^3)	Candidate POD_{ADJ} [a] (mg/m^3)
Neurological Endpoints						
Korsak and Rydzyński (1996)	Decreased pain sensitivity	Rat, male	BMDL	Linear; 1 SD	97.19	17.36
Hematological Endpoints						
Korsak et al. (2000b)	Decreased RBCs	Rat, male	NOAEL	n/a[b]	523	93.39
	Decreased segmented neutrophils	Rat, male	BMDL	Exponential 2; 1 SD	534.81	95.50
		Rat, female	BMDL	Hill; 1 SD	99.21	17.72
	Increased lymphocytes	Rat, male	NOAEL	n/a	523	93.39
		Rat, female	NOAEL	n/a	523	93.39
	Increased reticulocytes	Rat, male	BMDL	Linear; 1 SD	652.90	116.58
Respiratory Endpoints						
Korsak et al. (2000b)	Increased inflammatory lung lesions	Rat, female	NOAEL	n/a	128	22.86

[a] Duration adjusted POD_{ADJ} (mg/m^3) = POD × (6 hours/24 hours) × (5 days/week) in accordance with EPA guidance (U.S. EPA, 2002).
[b] No model was able to fit data adequately, or data were deemed insufficient for BMD modeling

1.1.3. Derivation of Reference Concentrations for 1,2,3-TMB

Because the majority of selected endpoints for consideration as the critical effect (decreased pain sensitivity, decreased RBCs, decreased segmented neutrophils, increased lymphocytes, increased reticulocytes) result primarily from systemic distribution of 1,2,3-TMB, and no available PBPK model exists for 1,2,3-TMB, the human equivalent concentration (HEC) for 1,2,3-TMB was calculated by the application of the dosimetric adjustment factor (DAF) for systemically acting gases (i.e., Category 3 gases), in accordance with the U.S. EPA RfC Methodology (U.S. EPA, 1994b). Additionally, although the observation of lung lesions would normally indicate portal-of-entry effects, various factors support the conclusion that 1,2,3-TMB is a systemically-acting toxicant, including the isomer's relatively low water-solubility and non-reactivity as well as the observation that the majority of 1,2,3-TMB-induced effects are systemic in nature. Gases with these properties are expected to preferentially distribute to the lower regions of the respiratory tract where larger surface areas and thin alveolar-capillary boundaries facilitate uptake. Respiratory absorption of 1,2,3-TMB into the bloodstream has been observed to be

relatively high (~60% dose) following inhalation exposures to humans (Järnberg et al., 1996). Therefore, increased inflammatory lung lesions are assumed to result from systemic distribution of 1,2,3-TMB in the bloodstream of exposed animals. DAFs are ratios of animal and human physiologic parameters, and are dependent on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal-of-entry [i.e., systemic]) (U.S. EPA, 1994b). For gases with systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

$$\mathbf{DAF = (Hb/g)_A/(Hb/b)_H}$$

$$\mathbf{DAF = 62.6/66.5}$$

$$\mathbf{DAF = 0.94}$$

where:

$\mathbf{(H_{b/}g)_A}$ **= the animal blood:air partition coefficient**

$\mathbf{(H_{b/}g)_H}$ **= the human blood:air partition coefficient**

In cases where the animal blood:air partition coefficient is lower than the human value (Meulenberg and Vijverberg, 2000; Järnberg and Johanson, 1995), resulting in a DAF < 1, the calculated value is used for dosimetric adjustments (U.S. EPA, 1994b). For example, the HEC for decreased pain sensitivity reported in Korsak and Rydzyński (1996) is calculated as follows:

$$\mathbf{POD_{HEC} = POD_{ADJ}\ (mg/m^3) \times DAF}$$

$$\mathbf{POD_{HEC} = POD_{ADJ}\ (mg/m^3) \times 0.94}$$

$$\mathbf{POD_{HEC} = 17.36\ mg/m^3 \times 0.94}$$

$$\mathbf{POD_{HEC} = 16.32\ mg/m^3}$$

Table 1-4 presents the calculated HECs for the candidate critical effects, selected uncertainty factors (UFs) and the resulting derivation of candidate RfCs from the two subchronic toxicity studies (Korsak et al., 2000b; Korsak and Rydzyński, 1996).

Table 1-4. Candidate POD_{ADJ} values, human equivalent concentrations (HECs), and applied uncertainty factors used in the derivation of Candidate RfCs for 1,2,3-TMB

Reference	Endpoint	POD_{ADJ} (mg/L)	HEC (mg/m^3)[a]	Uncertainty Factors (UF)						Candidate RfC (mg/m^3)[b]
				UF_A	UF_H	UF_L	UF_S	UF_D	UF_{TOTAL}	
Neurological Endpoints										
Korsak and Rydzyński (1996)	Decreased pain sensitivity	17.36	16.32	3	10	1	10	3	1,000	1.63×10^{-2}
Hematological Effects										
Korsak et al. (2000b)	Decreased RBCs, males	93.39	87.79	3	10	1	10	3	1,000	8.78×10^{-2}
	Decreased segmented neutrophils, males	95.50	89.77	3	10	1	10	3	1,000	8.98×10^{-2}
	Decreased segmented neutrophils, females	17.72	16.66	3	10	1	10	3	1,000	1.67×10^{-2}
	Increased lymphocytes, males	93.39	87.79	3	10	1	10	3	1,000	8.78×10^{-2}
	Increased lymphocytes, females	93.39	87.79	3	10	1	10	3	1,000	8.78×10^{-2}
	Increased reticulocytes, males	116.58	109.58	3	10	1	10	3	1,000	1.10×10^{-1}
Respiratory Effects										
Korsak et al. (2000b)	Increased inflammatory lung lesions, females	22.86	21.49	3	10	1	10	3	1,000	2.15×10^{-2}

[a] Human equivalent concentration
[b] As calculated by application of uncertainty factors, not rounded.

Neurotoxicity is the most consistently observed endpoint in the toxicological database for 1,2,3-TMB. According to EPA's *Guidelines for Neurotoxicity Risk Assessment* (1998), many neurobehavioral changes are regarded as adverse, and the observation of correlated and replicated measures of neurotoxicity strengthen the evidence for a hazard. Decreased pain sensitivity, as measured as latency to paw-lick, is a measure of nociception (i.e., decreased pain sensitivity) and therefore this endpoint represents an alteration in neurobehavioral function (U.S. EPA, 1998). Decreased pain sensitivity was observed in two studies investigating short-term and subchronic exposure durations (Wiaderna et al., 1998; Korsak and Rydzyński, 1996), and in the presence of other metrics of altered neurobehavior, including impaired neuromuscular function and coordination and altered cognitive

function. Additionally, neurotoxicological endpoints (e.g., hand tremble, weakness) were observed in human worker populations exposed to complex VOC mixtures containing 1,2,3-TMB; indicating consistency and coherency of effects in humans and animals following exposure to 1,2,3-TMB.

The U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) note that effects that are reversible in minutes, hours, or days after the end of exposure and appear to be associated with the pharmacokinetics of the agent and its presence in the body may be of less concern than effects that persist for longer periods of time after the end of exposure. Pain sensitivity was observed to return to control levels two weeks after termination of subchronic exposure (Korsak and Rydzyński, 1996). However, in short-term studies of TMBs, there is evidence indicating that decreased pain sensitivity associated with exposure to TMBs is not rapidly reversible and not associated with clearance of the chemical from the body. TMB isomers have been observed to clear rapidly from blood and nervous tissues (Section B.2, Appendix B), and decreased pain sensitivity persisted for up to 50-51 days after termination of short-term exposures (Wiaderna et al., 1998). Short-term neurotoxicity studies of the related 1,2,4-TMB isomer also observed a persistence of decreased pain sensitivity after termination of exposure (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997). Taken as a whole, the database does not support the characterization of decreased pain sensitivity associated with exposure to 1,2,3-TMB as rapidly reversible upon clearance from the body. Given the consistency of decreased pain sensitivity across independent studies and multiple durations of exposure in animal studies, and the consistency of observed neurotoxicity in animals and humans, EPA concluded that there is strong evidence that neurotoxicity is a toxicity hazard associated with exposure to 1,2,3-TMB. Further, EPA concluded that decreased pain sensitivity is an adverse effect and is the most appropriate effect on which to base the RfC. **Therefore, the candidate RfC for neurotoxicity based on decreased pain sensitivity was selected as the RfC for 1,2,3-TMB.**

A POD_{HEC} of 16.3 mg/m^3 for decreased pain sensitivity (Korsak and Rydzyński, 1996) was used as the POD to derive the chronic RfC for 1,2,3-TMB. The uncertainty factors (UFs), selected and applied in accordance with the procedures described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (2002) (Section 4.4.5 of the report), address five areas of uncertainty resulting in a composite UF of 1,000. This composite UF was applied to the selected POD to derive an RfC.

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences

between rats and humans following inhalation exposure to 1,2,3-TMB. In this assessment, the use of a DAF to extrapolate external exposure concentrations from rats to humans reduces toxicokinetic uncertainty in extrapolating from the rat data, but does not account for the possibility that humans may be more sensitive to 1,2,3-TMB than rats due to toxicodynamic differences. A default UF_A of 3 was thus applied to account for this remaining toxicodynamic and residual toxicokinetic uncertainty.

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response in the human population following inhalation of 1,2,3-TMB. No information is currently available to predict potential variability in human susceptibility, including variability in the expression of enzymes involved in 1,2,3-TMB metabolism.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR equal to a change in the mean equal to 1 standard deviation of the model estimated control mean for decreased pain sensitivity was selected under the assumption that this BMR represents a minimal, biologically significant change for this endpoint.

A subchronic to chronic uncertainty factor, UF_S, of 10 was applied to account for extrapolation from a subchronic exposure duration study to derive a chronic RfC. 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 exposure 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.

A database uncertainty factor, UF_D, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for database deficiencies. Strengths of the database include the two well-designed subchronic studies that observe exposure-related effects in multiple organ systems (nervous, hematological, and respiratory systems) in Wistar rats exposed to 1,2,3-TMB via inhalation. However, the database lacks a multi-generational reproductive toxicity study and a developmental toxicity study investigating effects due to 1,2,3-TMB exposure. Normally, the lack of both of these types of studies in a toxicity database would warrant the application of a 10-fold UF_D in accordance with EPA's *Review of the Reference Dose and Reference Concentration Processes* (2002). Although there is no developmental toxicity study for 1,2,3-TMB, Saillenfait et al. (2005) investigated the developmental toxicity of the

other two TMB isomers (1,2,4-TMB and 1,3,5-TMB) and observed developmental toxicity at levels much higher than those eliciting neurotoxicity, hematotoxicity, and respiratory toxicity in adult animals (Korsak studies). Given that toxic effects were observed at lower concentrations in adult rats exposed to 1,2,4-TMB and 1,3,5-TMB compared with rats exposed in utero and the similarities in toxicity profiles amongst the three isomers, it is unlikely that the inclusion of a developmental toxicity study for 1,2,3-TMB would result in a POD that is lower than the POD associated with neurotoxicity for this isomer. Thus, the application of an UF to account for the lack of a development toxicity study is not warranted.

Although, a multi-generation reproductive study does not exist for 1,2,3-TMB, there is a multi-generation developmental/reproductive study for high flash naphtha, of which 1,2,3-TMB is a constituent. This study demonstrates effects on postnatal growth at lower exposures in the F_3 generation (2,460 mg/m^3) compared to the F_2 or F_1 generation (7,380 mg/m^3) (McKee et al., 1990), but did not observe a consistent effect on reproductive parameters. This raises some concern that addition of a multi-generational reproductive study for 1,2,3-TMB might result in the identification of a lower POD.

EPA's *Review of the Reference Dose and Reference Concentration Processes* (2002) also recommends that the database uncertainty factor take into consideration whether there is concern from the available toxicity database that the developing organism may be particularly susceptible to effects in specific organ systems. TMBs (unspecified isomer) are able to cross the placenta (Cooper et al., 2001; Dowty et al., 1976); therefore, as neurotoxicity is observed in adult animals, there is concern that exposure to 1,2,3-TMB may result in neurotoxicity in the developing organism. EPA's *Guidelines for Neurotoxicity Risk Assessment* (1998) identify specific effects observed in adult animals (e.g., cognitive and motor function) as effects that can also affect the developing organism exposed in utero. EPA's *Guidelines for Neurotoxicity Risk Assessment* (1998) also indicate that neurotoxicants may have greater access to the nervous system in developing organisms due to an incomplete blood-brain barrier and immature metabolic detoxifying pathways. Therefore, there is some concern that the lack of a developmental neurotoxicity study is a deficiency in the database and that the inclusion of such a study would potentially result in a lower POD than the POD for neurotoxicity identified from the available 1,2,3-TMB toxicity database. In summary, a 3-fold database UF was applied to account for the lack of both a multi-generation reproductive study and a developmental neurotoxicity study in the available database for 1,2,3-TMB.

Application of this **1000-fold composite UF** to the POD_{HEC} yields the following chronic RfC for 1,2,3-TMB:

RfC = POD_{HEC} ÷ UF

RfC = 16.3 mg/m^3 ÷ 1,000

RfC = 0.02 mg/m^3 = 2 × 10^{-2} mg/m^3 (rounded to one significant digit)

1.1.4. Uncertainties in the Derivation of the Reference Concentration for 1,2,3-TMB

As presented above, the UF approach following EPA practices and RfC guidance (U.S. EPA, 2002, 1994b), was applied to the POD_{HEC} in order to derive the chronic RfC for 1,2,3-TMB. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolation from animals to humans, a diverse human population of varying susceptibilities, POD determination methodologies (NOAEL, LOAEL, or BMDL), and database deficiencies.

The critical effect selected, decreased pain sensitivity, does not introduce substantial variability into the RfC calculation as selection of alternative hematological or respiratory effects would result in similar RfCs that would be equivalent when rounding to one significant digit (i.e., 2 × 10^{-2} mg/m^3 , see Figure 1-2). Some uncertainty exists regarding the selection of the BMRs for use in BMD modeling due to the absence of information to determine the biologically significant level of response associated with the endpoints. However in cases such as this, the selection of a BMR of 1 standard deviation for continuous endpoints is supported by EPA guidance (U.S. EPA, 2000). Uncertainty regarding the selection of particular models for individual endpoints exists as selection of alternative models could decrease or increase the estimated POD and consequently, the RfC. The selection criteria for model selection was based on a practical approach as described in EPA's BMD Technical Guidance (U.S. EPA, 2000). Uncertainty may exist in the default dosimetry methods used to calculate HEC estimates, but such uncertainties would apply equally to all endpoints.

1.1.5. Confidence Statement for the Reference Concentration for 1,2,3-TMB

Confidence levels of high, medium, or low are 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, 1994b). **Confidence in the principal study from which the critical effect was identified, Korsak and Rydzyński (1996) is medium.** The study is a well-conducted peer-reviewed study that utilized three dose groups plus untreated controls, and an

appropriate number of animals per dose group, and performed statistical analyses. One area of uncertainty regarding this study is the lack of reported actual exposure 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 (Korsak et al., 2000b) and achieved appropriate actual concentrations (i.e., within 10% of target concentrations), the concern regarding the lack of reported actual concentrations is minimal. The critical effect on which the RfC is based is well-supported as the evidence for 1,2,3-TMB-induced neurotoxicity is consistent and coherent across multiple animals species (i.e., human and rat) and across multiple exposure durations (i.e., acute, short-term, and subchronic) (Lutz et al., 2010; Wiaderna et al., 1998; Korsak and Rydzyński, 1996). **Confidence in the database for 1,2,3-TMB is low to medium** as the database includes acute, short-term, and subchronic toxicity studies in rats, but lacks chronic, multi-generation reproductive, developmental toxicity, and developmental neurotoxicity studies. Additionally, the studies supporting the critical effect predominantly come from the same research institute. **Overall confidence in the RfC for 1,2,3-TMB is low to medium.**

1.1.6. Comparison of Candidate Reference Concentrations for 1,2,3-TMB

The predominant noncancer effect observed following acute, short-term, and subchronic inhalation exposures to 1,2,3-TMB is neurotoxicity, although respiratory toxicity is also observed following acute and subchronic exposures, while hematological effects are observed after subchronic exposures. Figure 1-2 provides a graphical display of all candidate PODs and RfCs derived from the two subchronic studies considered in the selection of the inhalation RfC for 1,2,3-TMB.

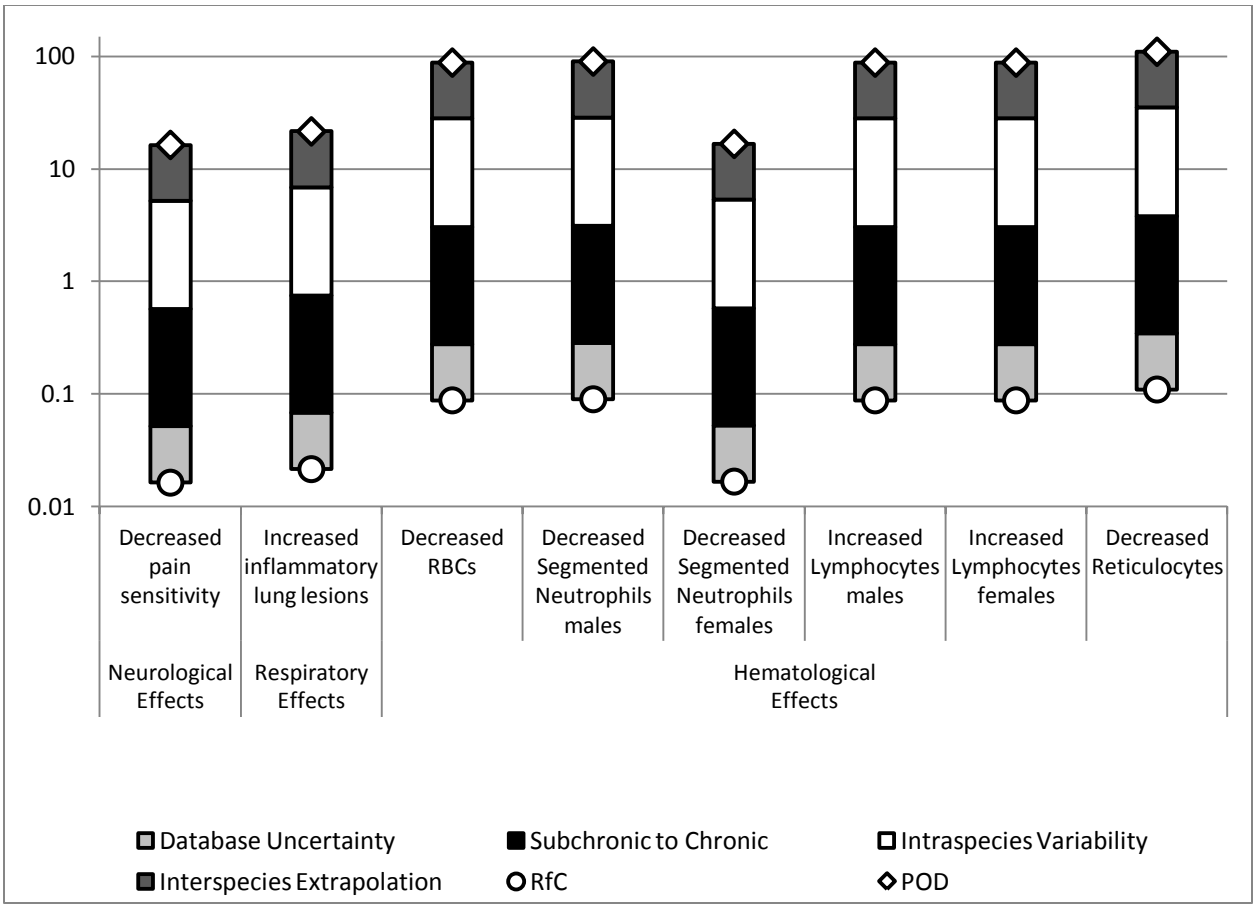


Figure 1-2. Array of candidate POD_{HEC} values with applied UFs and candidate RfCs for neurological, respiratory, and hematological effects resulting from inhalation exposure to 1,2,3-TMB

1.2. Oral Reference Dose for Effects other than Cancer for 1,2,3-TMB

The available oral database is inadequate to derive an RfD for 1,2,3-TMB. No chronic, subchronic, or short-term oral exposure studies were found in the literature. However, as discussed in Section 1, there are sufficient similarities between isomers regarding observed toxicological effects that support adopting the RfD for 1,2,4-TMB as the RfD for 1,2,3-TMB. Specifically, the qualitative pattern of neurotoxic effects following short-term and subchronic inhalation exposures is similar between isomers. Particularly important to this determination is that, although 1,2,3-TMB is observed to decrease pain sensitivity at lower concentrations than 1,2,4-TMB (LOAEL values of 123 vs. 492 mg/m^3), the magnitude of decreased pain sensitivity is similar for 1,2,4-TMB and 1,2,3-TMB, especially in the low and mid exposure concentrations. This similarity of effect in the low dose region of the dose-response curve is exhibited by equal RfC values derived from isomer-specific data: 2×10^{-2} mg/m^3. Although a PBPK model exists for 1,2,4-TMB that allows for route-to-route extrapolation from inhalation to oral exposure, no such model exists for 1,2,3-TMB.

However, similarities in blood:air and tissue:air partition coefficients and degree of absorption into the bloodstream between the 1,2,4-TMB and 1,2,3-TMB isomers support the conclusion that internal blood dose metrics for 1,2,3-TMB would be similar to those calculated for 1,2,4-TMB using that isomer's available PBPK model. Also, the qualitative metabolic profiles for the two isomers are similar, with dimethylbenzyl hippuric acids being the major terminal metabolite for both isomers, such that first-pass metabolism through the liver is not expected to differ greatly between 1,2,4-TMB and 1,2,3-TMB.

Therefore, given the above similarities in toxicokinetics and toxicity, the RfD derived for 1,2,4-TMB, 6×10^{-3} mg/kg-day, was adopted for the RfD for 1,2,3-TMB.

1.2.1. Uncertainties in the Derivation of the Reference Dose for 1,2,3-TMB

The uncertainties regarding adopting the RfD for 1,2,4-TMB as the RfD for 1,2,3-TMB encompass previous areas of uncertainty involved in the derivation of the RfD for 1,2,4-TMB (see Section 2). Additionally, there is uncertainty in this adoption regarding the assumptions made about the similarity in toxicity and toxicokinetics between the two isomers. However, as discussed above and in Sections 1 and in B.2, there is strong evidence that both isomers share multiple commonalities and similarities regarding their toxicokinetic and toxicological properties that support adopting one isomer's value for the other.

1.2.2. Confidence Statement for the Reference Dose for 1,2,3-TMB

As noted previously, a confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in U.S. EPA (1994b), Section 4.3.9.2. The chronic RfD of 6×10^{-3} mg/kg-day derived for 1,2,4-TMB was adopted as the RfD for 1,2,3-TMB based on the conclusion that the two isomers were sufficiently similar regarding chemical properties, kinetics, and toxicity. **Thus, confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996) is medium (see above). Confidence in the database is low to medium** as the database includes acute, short-term, and subchronic studies in rats and mice. The database lacks a multi-generation reproductive, developmental toxicity, or developmental neurotoxicity study, and the studies supporting the critical effect predominantly come from the same research institute. **Overall confidence in the RfD for 1,2,3-TMB is low** due to uncertainties surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,2,3-TMB.

1.3. Cancer Assessment for 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005), the database for 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB provides **"inadequate information to assess the carcinogenic potential"**. This characterization is based on the limited and equivocal genotoxicity findings, and the lack of data indicating carcinogenicity in experimental animal species. Information available on which to base a cancer assessment is lacking, and thus, no cancer risk values are derived.

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