EPA/635/R-11/012C
www.epa.gov/iris

Toxicological review of 1,2,4- and 1,3,5-Trimethylbenzene

(CAS No. 95-63-6 and 108-67-8)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

JANUARY 2012

NOTICE

This document is an **Interagency Science Consultation draft**. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

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

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

CONTENTS

LIST OF TABLES AND FIGURES

ABBREVIATIONS AND ACRONYMS

1,2,4-TMB	1,2,4-trimethylbenzene
1,3,5-TMB	1,3,5-trimethylbenzene
AAQC	Ambient air quality criterion
ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, distribution, metabolism and excretion
AEGL	Acute exposure guideline limit
AIC	Akaike Information Criterion
BAL	bronchoalveolar lavage
BMD	benchmark dose
BMDL	lower confidence limit on the benchmark dose
BMDS	benchmark dose software
BMR	benchmark response
BW	body weight
C	Celsius
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
CNS	central nervous system
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DMBA	dimethylbenzoic acid
DMHA	dimethylhippuric acid
DNA	deoxyribonucleic acid
EC_{50}	half maximal effective concentration
EEG	Electroencephalogram
EPA	U.S. Environmental Protection Agency
g	gram
GD	gestational day
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HEK	Human epidermal keratinocytes
HERO	Health Effects Research Online
HEV	human epithelial keratinocytes
HSDB	Hazardous Substance Database
IL-8	interleukin-8
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
JP-8	jet propulsion fuel 8
k	kilogram
K_m	Michaelis-Menten constant
L	liter
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
m^3	meter cubed
mg	milligram
n	nanogram
NCEA	National Center for Environmental Assessment
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NOAEL	No-observed-adverse-effect level
OMOE	Ontario Ministry of the Environment
OSHA	Occupational Safety and Health Administration
p	probability value
p-RfC	Provisional RfC
PBPK	physiologically based pharmacokinetic (model)
PEL	permissible exposure limit
POD	point of departure
POD_{ADJ}	duration adjusted POD
POI	Point of impingement
ppm	parts per million
RBC	red blood cell
RD_{50}	50% respiratory rate decrease
REL	Recommended exposure limit
RfC	reference concentration
RfD	reference dose
RGDR	regional gas dose ratio
ROS	reactive oxygen species
SCE	sister chromatid exchange
SD	standard deviation
SOA	Secondary organic aerosol
t	time
TLV	threshold limit value
TMB	trimethylbenzene
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_D	database deficiency uncertainty factor
µg	microgram
µl	microliter
µmol	micromol
UV	ultraviolet
V	volt
VOC	volatile organic compound
W	watt
WBC	white blood cell
WS	white spirit

χ^2	chi-squared

PREAMBLE

1. Scope of the IRIS Program

Soon after EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in decisions to protect human health and the environment. The Clean Air Act, for example, mandates that EPA provide "an ample margin of safety to protect public health"; the Safe Drinking Water Act, that "no adverse effects on the health of persons may reasonably be anticipated to occur, allowing an adequate margin of safety." Accordingly, EPA relies on health assessments to identify adverse effects and exposure levels below which these effects are not anticipated to occur.

IRIS assessments critically review the publicly available studies to identify adverse health effects of chemicals and to characterize exposure-response relationships. Exceptions are chemicals currently used exclusively as pesticides, ionizing and non-ionizing radiation, and criteria air pollutants listed under section 108 of the Clean Air Act (carbon monoxide, lead, nitrogen oxides, ozone, particulate matter, and sulfur oxides; EPA evaluates these in Integrated Science Assessments). An assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture.

Once a year, the IRIS Program asks EPA programs and regions, other federal agencies, state governments, and the general public to nominate chemicals and mixtures for future assessment or reassessment. These agents may be found in air, water, soil, or sediment. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. IRIS can assess other agents as an urgent public health need arises. IRIS also reassesses agents as significant new data are published.

2. Process for developing and peer-reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009) involves systematic review of the pertinent studies, opportunities for public input, and multiple levels of scientific review. EPA revises draft assessments after each review, and external drafts and comments become part of the public record (EPA 2009).

Step 1. Development of a draft Toxicological Review (usually about 11-1/2 months duration). The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate the studies, identify health effects, weigh the evidence of causation for each effect, identify mechanistic events and pathways, and derive toxicity values.

Step 2. Internal review by scientists in EPA programs and regions (2 months). The draft assessment is revised to address comments from within EPA.

Step 3. Interagency science consultation with other federal agencies and White House offices (1-1/2 months). The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and EPA's response to major comments become part of the public record.

Step 4. External peer review, after public review and comment (3-1/2 months or more, depending on the review process). EPA releases the draft assessment for public review and comment, followed by external peer review. The peer review meeting is open to the public and includes time for oral public comments. The peer reviewers also receive the written public comments. The peer reviewers assess whether the evidence has been assembled and evaluated according to guidelines and whether the conclusions are justified by the evidence. The peer review draft, peer review report, and written public comments become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary (2 months). The draft assessment is revised to reflect the peer review comments, public comments, and newly available studies. The disposition of peer review comments and public comments becomes part of the public record.

Step 6. Final EPA review and interagency science discussion with other federal agencies and White House offices (1-1/2 months). The draft assessment and summary are revised to address EPA and interagency comments. The science discussion draft, written interagency comments, and EPA's response to major comments become part of the public record.

Step 7. Completion and posting (1 month). The Toxicological Review and IRIS summary are posted on the IRIS website (http://www.epa.gov/iris/).

The remainder of this Preamble addresses step 1, the development of a draft Toxicological Review. IRIS assessments follow standard practices of evidence evaluation and peer review, many of which are discussed in EPA guidelines (EPA 1986a, 1986b, 1991, 1996, 1998, 2000a, 2005a, 2005b) and other descriptions of "best practices" (EPA 1994, 2000b, 2002, 2006, 2011). Transparent application of scientific judgment is of paramount importance. To provide a harmonized approach across IRIS assessments, this Preamble summarizes concepts from these guidelines and emphasizes principles of general applicability.

3. Identifying and selecting pertinent studies

3.1 Identifying studies

Before beginning an assessment, EPA conducts a comprehensive search of the primary scientific literature. The literature search follows standard practices and includes the PubMed and ToxNet databases of the National Library of Medicine and other databases listed in EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. EPA posts the results of the literature search on the IRIS website and requests information from the public on additional studies and ongoing research.

Each assessment also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted to EPA under the Toxic Substances Control Act. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, EPA will have it peer-reviewed.

EPA also examines the toxicokinetics of the agent to identify other chemicals (for example, major metabolites of the agent) to include in the assessment if adequate information is available, in order to more fully explain the toxicity of the agent and to suggest dose metrics for subsequent modeling.

In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components (EPA 1986a, 2000a). The literature search seeks, in decreasing order of preference:

- Studies of the mixture being assessed.
- Studies of a sufficiently similar mixture. In evaluating similarity, the assessment considers the alteration of mixtures in the environment through partitioning and transformation.
- Studies of individual chemical components of the mixture, if there are not adequate studies of sufficiently similar mixtures.

3.2 Selecting pertinent epidemiologic studies

Study design is the key consideration for selecting pertinent epidemiologic studies from the results of the literature search.

- Cohort studies and case-control studies provide the strongest epidemiologic evidence, as they collect information about individual exposures and disease.
- Cross-sectional studies provide useful evidence if they relate exposures and disease at the individual level and it is clear that exposure preceded the onset of disease.
- Ecologic studies (geographic correlation studies) relate exposures and disease by geographic area. They can provide strong evidence if there are large exposure contrasts between geographic areas, relatively little exposure variation within study areas, and population migration is limited.
- Case reports of high or accidental exposure lack definition of the population at risk and the expected number of cases. They can provide information about a rare disease or about the relevance of analogous results in animals.

The assessment briefly reviews ecologic studies and case reports but includes details only if they suggest effects not identified by other epidemiologic studies.

3.3 Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental studies from the results of the literature search.

- Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier and are considered most pertinent to human environmental exposure.
- Injection or implantation studies are often considered less pertinent but may provide valuable toxicokinetic or mechanistic information. They also may be useful for identifying effects in animals if deposition or absorption is problematic (for example, for particles and fibers).

Exposure duration is also a key design consideration for selecting pertinent experimental studies.

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.
- Studies of effects from subchronic exposure are pertinent but less preferred than studies of chronic exposure.
- Short-term and acute studies are less pertinent but are useful for obtaining toxicokinetic or mechanistic information. The assessment reviews short-term and acute studies if they suggest distribution or effects at a site not identified by longer-term studies.
- For developmental toxicity and reproductive toxicity, irreversible effects may result from a brief exposure during a critical period of development. Accordingly, specialized study designs are used for these effects (EPA 1991, 1996, 1998).

4. Evaluating the quality of individual studies

4.1 Evaluating the quality of epidemiologic studies

The assessment evaluates design and methodologic aspects that can increase or decrease the weight given to each epidemiologic study in the overall evaluation (EPA 1991, 1994, 1996, 1998, 2005a):

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study and comparison populations.
- Ascertainment of exposure and the potential for misclassification.
- Ascertainment of disease or effect and the potential for misclassification.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects, including latent effects.
- Characterization of exposure during critical periods for the development of effects.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and the resulting potential for selection bias.
- Potential confounding and other sources of bias are identified and addressed in the study design or in the analysis of results. The basis for consideration of confounding is a reasonable expectation that the confounder is prevalent in the population and is related to both exposure and outcome.

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating epidemiologic studies of these effects (EPA 1991, 1996, 1998, 2005a).

4.2 Evaluating the quality of experimental studies

The assessment evaluates design and methodologic aspects that can increase or decrease the weight given to each experimental study in the overall evaluation (EPA 1991, 1994, 1996, 1998, 2005a):

- Documentation of study design, animals or study population, methods, basic data, and results.
- Relevance of the animal model or study population and the experimental methods.
- Characterization of the nature and extent of impurities and contaminants of the administered chemical or mixture.
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects.
- Sample sizes and statistical power to detect dose-related differences or trends.
- Ascertainment of survival, vital signs, disease or effects, and cause of death.
- Control of other variables that could influence the occurrence of effects.

The assessment uses statistical tests to evaluate whether the observations may be due to chance. The standard for determining statistical significance of a response is a trend test or

comparison of outcomes in the exposed groups against those of concurrent controls. In some situations, examination of historical control data from the same laboratory within a few years of the study may improve the analysis. For an uncommon effect that is not statistically significant compared with concurrent controls, historical controls may show that the effect is unlikely to be due to chance. For a response that appears significant against a concurrent control response that is unusual, historical controls may offer a different interpretation (EPA 2005a).

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (EPA 1991, 1996, 1998, 2005a). In multi-generation studies, agents that produce developmental effects at doses that are not toxic to the maternal animal are of special concern. Effects that occur at doses associated with mild maternal toxicity are not assumed to result only from maternal toxicity. Moreover, maternal effects may be reversible, while effects on the offspring may be permanent (EPA 1991, 1998).

4.3 Reporting study results

The assessment uses evidence tables to report details of the design and key results of pertinent studies. There may be separate tables for each site of toxicity or type of study.

If a large number of studies observe the same effect, the assessment considers the study characteristics in this section to identify the strongest studies or types of study. The tables report details from these studies, and the assessment explains the reasons for not reporting details of other studies or groups of studies that do not add new information. Supplemental material provides references to all studies considered, including those not summarized in the tables.

The assessment discusses strengths and limitations that affect the interpretation of each study. If the interpretation of a study in the assessment differs from that of the study authors, the assessment discusses the basis for the difference.

As a check on the selection and evaluation of pertinent studies, EPA asks peer reviewers to identify studies that were not adequately considered.

5. Weighing the overall evidence of each effect

5.1 Weighing epidemiologic evidence

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole to determine the extent to which any observed associations may be causal. Positive, negative, and null results are given weight according to study quality. This evaluation considers aspects of an association that suggest causality, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998) (EPA 1994, 2002, 2005a; DHHS 2004).

Strength of association: The finding of a large relative risk with narrow confidence intervals strongly suggests that an association is not due to chance, bias, or other factors. Modest relative risks, however, may reflect a small range of exposures, an agent of low potency, an increase in a disease that is common, exposure misclassification, or other sources of bias.

Consistency of association: An inference of causality is strengthened if elevated risks are observed in independent studies of different populations and exposure scenarios. Reproducibility of findings constitutes one of the strongest arguments for causality. Discordant results sometimes reflect differences in exposure or in confounding factors.

Specificity of association: As originally intended, this refers to one cause associated with one disease. Current understanding that many agents cause multiple diseases and many diseases have multiple causes make this a less informative aspect of causality, unless the effect is rare or unlikely to have multiple causes.

Temporal relationship: A causal interpretation requires that exposure precede development of the disease.

Biologic gradient (exposure-response relationship): Exposure-response relationships strongly suggest causality. A monotonic increase is not the only pattern consistent with causality. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

Biologic plausibility: An inference of causality is strengthened by data demonstrating plausible biologic mechanisms, if available.

Coherence: An inference of causality is strengthened by supportive results from animal experiments, toxicokinetic studies, and short-term tests. Coherence may also be found in other lines of evidence, such as changing disease patterns in the population.

"Natural experiments": A change in exposure that brings about a change in disease frequency provides strong evidence of causality.

Analogy: Information on structural analogues or on chemicals that induce similar mechanistic events can provide insight into causality.

These considerations are consistent with contemporary guidelines that evaluate the quality and weight of evidence. Confidence is increased if the magnitude of effect is large, if there is evidence of an exposure-response relationship, or if an association was observed and the plausible biases would tend to decrease the magnitude of the reported effect. Confidence is decreased for study limitations, inconsistency of results, indirectness of evidence, imprecision, or reporting bias (Guyatt et al 2008a,b).

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may choose a descriptor such as *sufficient evidence*, *suggestive evidence*, *inadequate evidence*, or *evidence suggestive of no causal relationship* to characterize the epidemiologic evidence of each effect (DHHS 2004).

5.2 Weighing experimental evidence

For each effect, the assessment evaluates the evidence from the animal experiments as a whole to determine the extent to which they indicate a potential for effects in humans. Consistent results across various species and strains increase confidence that similar results would occur in humans. Although causality is not at issue in controlled experiments, several concepts discussed by Hill (1965) affect the weight of experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (EPA 1994, 2002, 2005a).

In weighing evidence from multiple experiments, EPA (2005a) distinguishes

Conflicting evidence (that is, mixed positive and negative results in the same sex and strain using a similar study protocol) from

Differing results (that is, positive results and negative results are in different sexes or strains or use different study protocols).

Negative or null results do not invalidate positive results in a different experimental system. EPA regards all as valid observations and looks to mechanistic information, if available, to reconcile differing results.

It is well established that there are critical periods for some developmental and reproductive effects. Accordingly, the assessment determines whether critical periods have been adequately investigated (EPA 1991, 1996, 1998, 2005a, 2005b). Similarly, the assessment determines whether the database is adequate to evaluate other critical sites and effects.

5.3 Characterizing modes of action

For each effect, the assessment discusses the available information on its *modes of action* and associated *key events* (*key events* being empirically observable, necessary precursor steps or biologic markers of such steps; *mode of action* being a series of key events involving interaction with cells, operational and anatomic changes, and resulting in disease). Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms. The assessment addresses several questions about each hypothesized mode of action (EPA 2005a).

(a) **Is the hypothesized mode of action sufficiently supported in test animals?** Strong support for a key event being necessary to a mode of action can come from experimental challenge to the hypothesized mode of action, where suppressing a key event suppresses the disease. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, but not by replicate experiments in the same model. The assessment may consider various aspects of causality in addressing this question.

(b) **Is the hypothesized mode of action relevant to humans?** The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed between animals and humans, though it may hold for certain modes of action. Information suggesting quantitative differences is considered in dose-response analyses but is not used to determine relevance. Similarly, anticipated levels of human exposure are not used to determine relevance.

(c) **Which populations or life-stages can be particularly susceptible to the**

hypothesized mode of action? The assessment reviews the key events to identify populations and life-stages that might be susceptible to their occurrence. Quantitative differences may result in separate toxicity values for susceptible populations or life-stages.

The assessment discusses the likelihood that an agent operates through multiple modes of action. An uneven level of support for different modes of action can reflect disproportionate resources spent investigating them (EPA 2005a). It should be noted that in clinical reviews, the quality of evidence may be reduced if evidence is limited to studies funded by one interested sector (Guyatt et al 2008b).

Studies of genetic toxicity are often available, and the assessment evaluates the evidence of a mutagenic mode of action.

- Demonstration of gene mutations, chromosome aberrations, or aneuploidy in humans or experimental mammals (*in vivo*) provides the strongest evidence.
- This is followed by positive results in lower organisms or in cultured cells (*in vitro*) or for other genetic events.
- Negative results carry less weight, partly because they cannot exclude the possibility of effects in other tissues (IARC 2006).

For germ-cell mutagenicity, EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (EPA 1986b).

5.4 Characterizing the overall weight of the evidence

After weighing the epidemiologic and experimental studies pertinent to each effect, the assessment may select a standard descriptor to characterize the overall weight of the evidence. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (EPA 2005a).

Carcinogenic to humans: There is convincing epidemiologic evidence of a causal association (that is, there is reasonable confidence that the association cannot be fully explained by chance, bias, or confounding); or there is strong human evidence of cancer or its precursors, extensive animal evidence, identification of key precursor events in animals, and strong evidence that they are anticipated to occur in humans.

Likely to be carcinogenic to humans: The evidence demonstrates a potential hazard to humans but does not meet the criteria for *carcinogenic*. There may be a plausible association in humans, multiple positive results in animals, or a combination of human, animal, or other experimental data.

Suggestive evidence of carcinogenic potential: The data raise concern for effects in humans but are not sufficient for a stronger conclusion. This descriptor covers a range of evidence, from a positive result in the only available study to a single positive result in an extensive database that includes negative results in other species.

Inadequate information to assess carcinogenic potential: No other descriptors apply. *Conflicting evidence* can be classified as *inadequate information* if all positive results are opposed by negative studies of equal quality in the same sex and strain. *Differing results*, however, can be classified as *suggestive evidence* or as *likely to be carcinogenic*.

Not likely to be carcinogenic to humans: There are robust data for concluding that there is no basis for concern. There may be no effects in both sexes of at least two appropriate animal species; positive animal results and strong, consistent evidence that each mode of action in animals does not operate in humans; or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

6. Selecting studies for derivation of toxicity values

For each effect associated with an agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The derivation of toxicity values may be linked to the weight-of-evidence descriptor. For example, EPA typically derives toxicity values for agents classified as *carcinogenic to humans* or *likely to be carcinogenic*, but not for agents with *inadequate information* or that are *not likely to be carcinogenic* (EPA 2005a).

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal (EPA 1994, 2005a):

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and effects can be attributed to the agent.

- Among experimental animal models, those that respond most like humans are preferred, if the comparability of response can be determined.
- Studies by a route of human environmental exposure are preferred, although a validated toxicokinetic model can be used to extrapolate across exposure routes.
- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects are representative of lifetime exposure.
- Studies with multiple exposure levels are preferred for their ability to provide information about the shape of the exposure-response curve.
- Studies that show an exposure-response gradient are preferred, as long as lack of a monotonic relationship at higher exposure levels can be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, misclassification bias, or selection bias.
- Among studies that show an exposure-response gradient, those with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

If a large number of studies are suitable for dose-response analysis, the assessment considers the study characteristics in this section to focus on the most informative data. The assessment explains the reasons for not analyzing other groups of studies. As a check on the selection of studies for dose-response analysis, EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1 General framework for dose-response analysis

EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (EPA 2005a).

Within the observed range, the preferred approach is to use modeling to incorporate a wide range of data into the analysis. The modeling yields a *point of departure* (an exposure level near the lower end of the observed range, without significant extrapolation to lower doses) (sections 7.2-7.3).

Extrapolation to lower doses considers what is known about the modes of action for each effect (sections 7.4-7.5). An alternative to low-dose extrapolation is derivation of reference values, which are calculated by adjusting the point of departure by factors that account for several sources of uncertainty and variability (section 7.6).

Increasingly, EPA is making use of multiple data sets or combining multiple responses in deriving toxicity values. EPA also considers multiple dose-response approaches when they can be supported by robust data.

7.2 Modeling dose

The preferred approach for analysis of dose is toxicokinetic modeling because of its ability to incorporate a wide range of data. The preferred dose metric would refer to the active agent at the site of its biologic effect or to a close, reliable surrogate measure. The active agent may be the administered chemical or a metabolite. Confidence in the use of a toxicokinetic model depends on the robustness of its validation process and on the results of sensitivity analyses (EPA 1994, 2005a, 2006).

Because toxicokinetic modeling can require many parameters and more data than are typically available, EPA has developed standard approaches that can be applied to typical data sets. These standard approaches also facilitate comparison across exposure patterns and species.

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a longer duration (EPA 1991, 1996, 1998, 2005a).
- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.
  - Oral doses are scaled allometrically using $mg/kg^{3/4}$-d as the equivalent dose metric across species. As allometric scaling is typically based on adult body weight, it is not used for early-life exposure or for developmental effects (EPA 2005a, 2011).
  - Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the effect occurs at the site of first contact or after systemic circulation (EPA 1994).

It can be informative to convert doses across exposure routes. If this is done, the assessment describes the underlying data, algorithms, and assumptions (EPA 2005a).

7.3 Modeling response in the range of observation

Toxicodynamic ("biologically based") modeling can incorporate data on biologic processes leading to a disease. Such models require sufficient data to ascertain a mode of action and to quantitatively support model parameters associated with its key events. Because different models may provide equivalent fits to the observed data but diverge substantially at lower doses, critical biologic parameters should be measured from laboratory studies, not by model fitting. Confidence in the use of a toxicodynamic model depends on the robustness of its validation process and on the results of sensitivity analyses. Peer review of the scientific basis and performance of a model is essential (EPA 2005a).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, EPA has developed a standard set of empirical ("curve-fitting") models (http://www.epa.gov/ncea/bmds/) that can be applied to typical data sets, including those that are nonlinear. EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (EPA 2000b). Additional judgment or alternative analyses are used when the procedure fails to yield reliable results, for example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses (EPA 2005a).

Modeling is used to derive a point of departure (EPA 2000b, 2005a). (See section 7.6 for alternatives if a point of departure cannot be derived by modeling.)

- For dichotomous responses, the point of departure is the 95% lower bound on the dose associated with a small increase of a biologically significant effect.
    - If linear extrapolation to lower doses will be used, a standard value near the low end of the observable range is used (10% response for animal data, 1% for epidemiologic data, depending on the observed response rates).
    - If nonlinear extrapolation will be used, both statistical and biologic factors are considered (10% response for minimally adverse effects, 5% or lower for more severe effects or for developmental toxicity data on individual offspring).
- For continuous responses, the point of departure is ideally a level where the effect is considered minimally adverse. In the absence of such definition, both statistical and biologic factors are considered in selecting a response level.

7.4 Extrapolating to lower doses

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation is typically used for known and likely carcinogens. Low-dose extrapolation considers what is known about modes of action (EPA 2005a).

(1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model beyond the observed range if significant model uncertainty can be ruled out with reasonable confidence. Below the range where confidence bounds on the predictions are reasonably precise, extrapolation may continue using a linear model.

(2) Linear extrapolation is used if the dose-response curve is expected to have a linear component below the point of departure. This includes:

- Agents or their metabolites that are DNA-reactive and have direct mutagenic activity.
- Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect.

Linear extrapolation is also used if the evidence is insufficient to establish a mode of action.

The result of linear extrapolation is described by an *oral slope factor* or an *inhalation unit risk*, which is the slope of the dose-response curve at lower doses.

(3) Nonlinear extrapolation is used if there are sufficient data to ascertain the mode of action and to conclude that it is not linear at lower doses, and the agent does not demonstrate mutagenic or other activity consistent with linearity at lower doses. If nonlinear extrapolation is appropriate but no model is developed, a default is to calculate reference values.

If linear extrapolation is used, the assessment develops a candidate slope factor or unit risk for each suitable data set. These results are arrayed, using common dose metrics, to show the distribution of relative potency across various effects and experimental systems. The assessment then derives an overall slope factor and an overall unit risk for the agent, considering the various dose-response analyses, the study preferences discussed in section 6, and the possibility of basing a more robust result on multiple data sets.

7.5 Considering susceptible populations and life-stages

The assessment analyzes the available information on populations and life-stages that may be particularly susceptible to each effect. A tiered approach is used (EPA 2005a).

(1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or life-stage, these data are analyzed to derive separate toxicity values for susceptible individuals.

(2) If data on risk-related parameters allow comparison of the general population and susceptible individuals, these data are used to adjust the general-population toxicity values for application to susceptible individuals.

(3) In the absence of chemical-specific data, application of *age-dependent adjustment factors* is recommended for early-life exposure to suspected carcinogens. There is evidence of early-life susceptibility to various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life exposure. To address the potential for early-life susceptibility, EPA recommends:

   – 10-fold adjustment for exposures before age 2 years.

   – 3-fold adjustment for exposures between ages 2 and 16 years.

   These adjustments are generally applied only for a mutagenic mode of action, though early-life susceptibility has been observed for several carcinogens that are not mutagenic (EPA 2005b).

7.6 Reference values and uncertainty factors

An *oral reference dose* or an *inhalation reference concentration* is an estimate of an exposure (including in susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime (EPA 2002). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a threshold can be based on prevention of an early key event. Reference values provide no information about risks at exposures above the reference value.

The assessment characterizes effects that form the basis for reference values as adverse, considered to be adverse, or a precursor to an adverse effect. For developmental, reproductive, and neurotoxicity there is guidance on adverse effects and their biologic markers (EPA 1991, 1996, 1998).

To account for uncertainty and variability in the derivation of a lifetime human exposure where effects are not anticipated to occur, reference values are calculated by adjusting the point of departure by a series of *uncertainty factors*. If a point of departure cannot be derived by modeling, a no-observed-adverse-effect level or a lowest-observed-adverse-effect level is substituted. The assessment discusses scientific considerations involving several areas of variability or uncertainty.

Human variation. A factor of 10 is applied to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. This factor is reduced only if the point of departure is derived specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (EPA 1991, 1994, 1996, 1998, 2002).

Animal-to-human extrapolation. A factor of 10 is applied if animal results are used to make inferences about humans. This factor is often regarded as comprising toxicokinetics and toxicodynamics in equal parts. Accordingly, if the point of departure is based on toxicokinetic modeling, dosimetry modeling, or allometric scaling across species, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicodynamic differences. An animal-to-human factor is not applied if a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences and residual uncertainty across species (EPA 1991, 1994, 1996, 1998, 2002).

Adverse-effect level to no-observed-adverse-effect level. If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such effects are not expected. This can be a matter of great uncertainty, especially if there is no

evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (EPA 1991, 1994, 1996, 1998, 2002).

Subchronic-to-chronic exposure. If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure would have effects at lower levels. A factor of 10 is applied to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure. This factor may also be applied for developmental or reproductive effects if exposure covered less than the full critical period. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response (EPA 1994, 1998, 2002).

Incomplete database. If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or life-stage, the assessment may apply a database uncertainty factor (EPA 1991, 1994, 1996, 1998, 2002). EPA typically follows the suggestion that a factor of 10 be applied if both a prenatal toxicity study and a two-generation reproduction study are missing, and a factor of $10^{1/2}$ if either is missing (EPA 2002).

In this way, the assessment derives candidate reference values for each suitable data set and effect that is plausibly associated with the agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures (EPA 1994). The assessment then selects an overall reference dose and an overall reference concentration for the agent to represent lifetime human exposure levels where effects are not anticipated to occur.

The assessment may also report reference values for each effect. This would facilitate subsequent cumulative risk assessments, where it may be important to consider the combined effect of chemicals acting at a common site or operating through common mechanisms (EPA 2002).

7.7 Confidence and uncertainty in the reference values

The assessment selects a standard descriptor to characterize the level of confidence in each reference value, based on the likelihood that the value would change with further testing. Confidence in reference values is based on quality of the studies used and completeness of the database, with more weight given to the latter. The level of confidence is increased for reference values based on human data supported by animal data (EPA 1994).

High confidence: The reference value is not likely to change with further testing, except for mechanistic studies that might affect the interpretation of prior test results.

Medium confidence: This is a matter of judgment, between high and low confidence.

Low confidence: The reference value is especially vulnerable to change with further testing.

These criteria are consistent with contemporary guidelines that evaluate the quality of evidence. These also focus on whether further research would be likely to change confidence in the estimate of effect (Guyatt et al 2008a).

All assessments discuss the significant uncertainties encountered in the analysis. EPA provides guidance on characterization of uncertainty (EPA 2005a). For example, the discussion distinguishes model uncertainty (lack of knowledge about the most appropriate experimental or analytic model), parameter uncertainty (lack of knowledge about the parameters of a model), and human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

AUTHORS | CONTRIBUTORS | REVIEWERS

Authors

J. Allen Davis, M.S.P.H. (Chemical Manager)
Eva McLanahan, Ph.D. (LCDR, USPHS)
Paul Schlosser, Ph.D.
John Cowden, Ph.D.
Gary Foureman, Ph.D. (Currently ICF Int.)

U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
Research Triangle Park, NC

Andrew Kraft, Ph.D

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

Ray Antonelli, B.S.

Oak Ridge Institute for Scientific Education
Research Triangle Park, NC

Contributors

Reeder Sams, Ph.D.
John Stanek, Ph.D.
Rob Dewoskin, Ph.D.
George Woodall, Ph.D.
Geniece Lehmann, Ph.D.
Connie Meacham, M.S.

U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
Research Triangle Park, NC

Technical Support

Ellen Lorang, M.S.
Deborah Wales
Gerald Gurevich

U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
Research Triangle Park, NC

Contractor Support

Battelle Memorial Institute, Pacific Northwest Division, Richmond, WA
Karla D. Thrall, Ph.D.

Battelle Memorial Institute, Columbus, OH
Jessica D. Sanford, Ph.D.
Maureen A. Wooton
Robert A. Lordo, Ph.D.
Anthony Fristachi

Toxicology Excellence for Risk Assessment (TERA)
Under Battelle Memorial Institute Contract EP-C-09-006
Lisa M. Sweeney, Ph.D., DABT
Melissa J. Kohrman-Vincent, B.A.

Executive Direction

Reeder Sams, Ph.D.
John Vandenberg, Ph.D.
Debra Walsh, M.S.

U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
Research Triangle Park, NC

Vincent Cogliano, Ph.D.
Samantha Jones, Ph.D.
Lynn Flowers, Ph.D.
Jamie Strong Ph.D.

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

Reviewers

This document has been provided for review to EPA scientists.

PREFACE

Background of Current Toxicological Review

There is currently no entry on the IRIS Database for either 1,2,4-TMB or 1,3,5-TMB. The current assessment has undergone draft development in which an RfC and RfD were derived for both 1,2,4-TMB and 1,3,5-TMB. No cancer values are derived for either isomer.

Chemical and Physical Information

The commercially available substance known as trimethylbenzene (TMB), CAS No. 25551-13-7, is a mixture of three isomers in various proportions, namely CAS No. 526-73-8 (1,2,3-trimethylbenzene or hemimellitene), CAS No. 108-67-8 (1,3,5-trimethylbenzene or mesitylene), and CAS No. 95-63-6 (1,2,4-trimethylbenzene or pseudocumene). The focus of this EPA review is two of these isomers: 1,2,4-trimethylbenzene (1,2,4-TMB) and 1,3,5-trimethylbenzene (1,3,5-TMB).

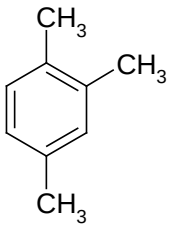
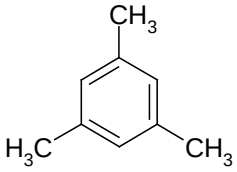
The TMBs are aromatic hydrocarbons with three methyl substituents attached to a benzene ring and the chemical formula C9H12. The chemical and physical properties of the TMB isomers are similar to one another. TMB is a colorless, flammable liquid with a strong aromatic odor; an odor threshold of 0.4 parts per million (ppm) of air has been reported (U.S. EPA, 1994). It is insoluble in water but miscible with organic solvents such as ethyl alcohol, benzene, and ethyl ether (OSHA, 1996).

Vehicle emissions are a major anthropogenic source of 1,2,4-TMB and 1,3,5-TMB, due to the widespread use of the C9 fraction as a gasoline additive (U.S. EPA, 1994). Other uses of 1,2,4-TMB and 1,3,5-TMB include solvents in research and industry, uses as a dyestuff intermediate, paint thinner, and as a UV oxidation stabilizer for plastics (HSDB, 2011a, b). Production and use of 1,2,4-TMB and 1,3,5-TMB may result in their release to the environment through various waste streams. If released to the atmosphere, 1,2,4-TMB and 1,3,5-TMB will exist solely in the vapor phase in the ambient atmosphere, based on measured vapor pressures of 2.10 and 2.48 mm Hg at 25ºC, respectively (HSDB, 2011a, b). Both isomers are expected to have limited mobility through soil based on their Log KOC -values, but are expected to volatilize from both moist and dry soil surfaces and surface

waters based on their respective Henry's Law constants and vapor pressures (see Table A.1-1). Degradation of both isomers in the atmosphere occurs by reaction with hydroxyl radicals, the half-life of which is 11-12 hours (HSDB, 2011a, b). Non-volatilized 1,2,4-TMB and 1,3,5-TMB may be subject to biodegradation under aerobic conditions (HSDB, 2011a, b). The estimated bio-concentration factors (439 and 234) and high volatility of 1,2,4-TMB and 1,3,5-TMB suggest that bioaccumulation of these chemicals will not be significant (U.S. EPA, 1987).

Additional information on the chemical identities and physicochemical properties of 1,2,4-TMB and 1,3,5-TMB are listed in Table A.1-1.

Table 1. Physical properties and chemical identity of 1,2,4-TMB and 1,3,5-TMB

CAS Registry Number	95-63-6	108-67-8
Synonym(s)	1,2,4-Trimethylbenzene, pseudocumene, asymmetrical trimethylbenzene	1,3,5-Trimethylbenzene, mesitylene, symmetrical trimethylbenzene
Molecular formula	C_9H_{12}	
Molecular weight	120.19	
Chemical structure		
Melting point, °C	-43.8	-44.8
Boiling point, °C @ 760 mm Hg	168.9	164.7
Vapor pressure, mm Hg @ 25°C	2.10	2.48
Density, g/mL at 20 °C relative to the density of H2O at 4 °C	0.8758	0.8637
Flashpoint, °C	44	50
Water solubility, mg/L at 25 °C	57	48.2
Other solubilities	Miscible with ethanol, benzene, ethyl ether, acetone, carbon tetrachloride, petroleum ether	Miscible with alcohol, ether, benzene, acetone, and oxygenated and aromatic solvents
Henry's Law Constant, atm-m3/mole	6.16×10^{-3}	8.77×10^{-3}
Log KOW	3.78	3.42
Log KOC	2.73	2.70-3.13
Bioconcentration Factor	439	234

CAS Registry Number	95-63-6	108-67-8
Synonym(s)	1,2,4-Trimethylbenzene, pseudocumene, asymmetrical trimethylbenzene	1,3,5-Trimethylbenzene, mesitylene, symmetrical trimethylbenzene
Conversion factors	1 ppm = 4.92 mg/m^3 1 mg/m^3 = 0.2 ppm	

Source: (HSDB, 2011a, b; U.S. EPA, 1987)

Programmatic Interest

1,2,4-trimethylbenzene (1,2,4-TMB) and 1,3,5-trimethybenzene (1,3,5-TMB) are industrial solvents found at Superfund sites. This IRIS assessment is being developed due to the potential for human environmental exposure to these compounds. The related isomer, 1,2,3-trimethylbenzene, is not included in this Toxicological Review.

Other Agency and International Assessments

Table 2. Other Agency and International Assessments

Agency	Inhalation value
National Institue of Occupational Safety and Health (NIOSH, 1992)	Recommended Exposure Limit (REL) for TMBs– 25 ppm (123 mg/m^3) time weighted average for up to a 10 hour work day and a 40 hour work week, based on the risk of skin irritation, central nervous system depression, and respiratory failure (reference not provided)
American Conference of Governmental Industrial Hygienists (ACGIH, 2002)	Threshold Limit Value (TLV) for VOC mixture containing 1,2,4-TMB and 1,3,5-TMB– 25 ppm (123 mg/m^3) time weighted average for a normal 8-hour work day and a 40-hour work week, based on the risk of irritation and central nervous system effects (Battig et al., 1956)
National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (U.S. EPA, 2007)	For TMBs: AEGL-1 (nondisabling) – 180 ppm (890 mg/m^3) to 45 ppm (220 mg/m^3) (10 minutes to 8 hours, respectively) (Korsak and Rydzyński, 1996) AEGL-2 (disabling) – 460 ppm (2300 mg/m^3) to 150 ppm (740 mg/m^3) (10 minutes to 8 hours, respectively) (Gage, 1970)
Ontario Ministry of the Environment (MOE, 2006)	For TMBs: 24 hour Ambient Air Quality Criterion (AAQC) – 0.3 mg/m^3 based on CNS effects; half-hour Point of Impingement (POI) – 0.9 mg/m^3 based on CNS effects (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996)

EXECUTIVE SUMMARY

Effects other than cancer observed following inhalation exposure to 1,2,4-TMB

The relationship between exposure to 1,2,4-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 and controlled human exposures to 1,2,4-TMB or solvent mixtures containing 1,2,4-TMB. Health effects noted in these studies were limited to irritative (eye irritation) and neurological effects (hand tremble, abnormal fatigue, lack of coordination) (Lammers et al., 2007; Chen et al., 1999; Norseth et al., 1991; Battig et al., 1956). No human studies were found for 1,3,5-TMB.

Animal inhalation studies included acute and short-term studies for both isomers that reported respiratory irritative (decreased respiration rates) and neurological effects (decreased pain sensitivity and decreased neuromuscular function and coordination) that supported effects seen in human studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a; Gralewicz et al., 1997b; Korsak et al., 1995). Three subchronic inhalation studies were found for 1,2,4-TMB that observed exposure-response effects in multiple organ systems, including the nervous, hematological, and pulmonary systems (Korsak et al., 2000; Korsak et al., 1997; Korsak and Rydzyński, 1996). In these studies, disturbances in CNS function, including decreased pain sensitivity and decreased neuromuscular function and coordination appear to be the most sensitive endpoints following exposure to 1,2,4-TMB. No subchronic studies were found that investigated exposure to 1,3,5-TMB. One developmental study that was found (Saillenfait et al., 2005) that observed similar levels of maternal and fetal toxicity (i.e., decreased maternal weight gain and fetal weight) following exposure to either isomer; other indices of fetal toxicity (i.e., fetal death and malformations) were not affected by exposure.

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

The chronic RfC of 2 × 10-2 mg/m3 for 1,2,4-TMB was calculated from a BMDL of 84.0 mg/m3 (resulting in an PODADJ of 0.085 mg/L blood) for decreased pain sensitivity in

male rats exposed to 1,2,4-TMB via inhalation for 90 days (6 hours/day, 5 days/week) (Korsak and Rydzyński, 1996). A PBPK model was then used to estimate a human equivalent concentration of 15.6 mg/m3, which was used as the POD to derive the RfC. A total UF of 1000 was used: 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), 10 to account for subchronic-to-chronic extrapolation due the lack of a suitable chronic study, and 3 to account for deficiencies in the database (no chronic study, no two-generation reproductive/developmental toxicity study).

Table II. Reference concentration (RfC) for 1,2,4-TMB

Critical Effect	Point of Departure (mg/m^3)	UF	Chronic RfC (mg/m^3)
Decreased pain sensitivity 90 day rat study Korsak and Rydzyński (1996)	POD_{HEC} = 15.6 A PBPK model was used to calculate an internal blood dose from the rat inhalation study and then a human equivalent concentration was calculated. This HEC served as the POD.	1,000	2×10^{-2}

Confidence Levels for the Derivation of the RfC 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, Korsak and Rydzyński (1996) is medium. The study is a well-conducted peer-reviewed study that utilized three dose groups plus untreated controls, an appropriate number of animals per dose group, and performed statistical analyses. The critical effect on which the RfC is based is well-supported as the weight of evidence for 1,2,4-TMB-induced neurotoxicity is coherent across multiple animals species (i.e., human, mouse, and rat) and consistent across multiple exposure durations (i.e., acute, short-term, and sub-chronic) (Gralewicz and Wiaderna, 2001; Chen et al., 1999; Wiaderna et al., 1998; Gralewicz et al., 1997a; Gralewicz et al., 1997b; Korsak and Rydzyński, 1996; Norseth et al., 1991). Confidence in the database for 1,2,4-TMB is low to medium as the database includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. The database lacks a chronic and multigenerational reproductive study, and the studies

supporting the critical effect predominately come from the same research institute. Overall confidence in the RfC for 1,2,4-TMB is low to medium.

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

No chronic or subchronic studies exist that would support the derivation of an RfC for 1,3,5-TMB, however two short-term neurotoxicity studies and one developmental toxicity study were identified as potential studies from which to identify a critical effect for RfC derivation. Ultimately, the two short-term neurotoxicity studies were inappropriate for the derivation of an RfC due to the magnitude of uncertainty associated with those data sets: in order to use the endpoints from these studies, a total uncertainty factor of 10,000 would be necessary: 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), 10 to account for extrapolation from a LOAEL to a NOAEL, 10 to account for subchronic-to-chronic extrapolation due the lack of a suitable chronic study, and 3 to account for deficiencies in the database (no chronic study, no two-generation reproductive/developmental toxicity study). Using the Saillenfait et al. (2005) study and the most sensitive endpoint in that study, decreased maternal weight gain, would result in an RfC 15-fold greater than that derived for 1,2,4-TMB (3 × 10-1 vs. 2 × 10-2 mg/m3). This was deemed to not be scientifically justified as the toxicological database for 1,2,4-TMB and 1,3,5-TMB, demonstrates that the two isomers are similar to one another regarding respiratory, neurological, and developmental toxicity in acute and developmental studies (Saillenfait et al., 2005; Korsak and Rydzyński, 1996; Korsak et al., 1995), although 1,3,5-TMB was observed to induce neurotoxicity at a slightly greater magnitude and earlier onset of effect compared to 1,2,4-TMB at the same exposure concentration (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001). Additionally, available toxicokinetic data regarding blood:air partition coefficients, respiratory uptake, and absorption into the bloodstream in humans and rats do not suggest any appreciable differences can be expected between the two isomers (Meulenberg and Vijverberg, 2000; Järnberg et al., 1996; Dahl et al., 1988). Therefore, given the apparent similarities between the two isomers (see Section 2.1.6), it was determined that the scientific database supported adopting the RfC for 1,2,4-TMB for the RfC for 1,3,5-TMB. **Thus, the chronic RfC of 2 × 10-2 mg/m3 derived for 1,2,4-TMB was adopted as the RfC for 1,3,5-TMB based on the determination of sufficient similarity regarding chemical properties, kinetics, and toxicity between the two isomers.**

Confidence Levels for the Derivation of the RfC for 1,3,5-TMB

As noted previously, 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 EPA (1994), Section 4.3.9.2. The chronic RfC of 2 × 10-2 mg/m3 derived for 1,2,4-TMB was adopted as the RfC for 1,3,5-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 developmental toxicity studies in rats and mice. The database lacks a chronic, subchronic, and multigenerational reproductive study. Additionally, the studies supporting the critical effect predominately come from the same research institute Overall confidence in the RfC for 1,3,5-TMB is low due to uncertainties surrounding the adoption of the RfC derived for 1,2,4-TMB as the RfC for 1,3,5-TMB.

Effects other than cancer observed following oral exposure

No chronic, subchronic, or short-term studies were identified that examined the noncancer effects of oral exposure to 1,2,4-TMB or 1,3,5-TMB. A series of oral or i.p. injection studies were identified that investigated the acute neurotoxic effects of 1,2,4-TMB and 1,3,5-TMB exposure (Tomas et al., 1999a; Tomas et al., 1999b; Tomas et al., 1999c). In these studies exposed rats demonstrated changes in electrocortical arousal, altered EEG activity in the cortical and hippocampal regions of the brain, and increase locomotor activity (possible due to difficulty maintain balance due to motor ataxia). As these effects were only observed in studies investigating acute exposures, they were not deemed sufficient for derivation of human health values.

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

A PBPK model (Hissink et al., 2007), modified by EPA to include an oral compartment was available for estimating the oral dose that would yield a blood concentration equal to the blood concentration at the POD used in the derivation of the RfC for 1,2,4-TMB. Under the assumption of constant oral ingestion and 100% absorption of 1,2,4-TMB via constant infusion rate into the liver, a PODHED of 6.2 mg/kg-day was derived. Hepatic first pass metabolism was also evaluated in humans using the modified PBPK model; at low daily doses, inhalation doses were estimated to result in steady state venous blood

concentrations 4-fold higher than blood concentrations resulting from equivalent oral doses, due to hepatic first pass metabolism.

The same total UF of 1,000 as was used for the RfC derivation was applied: 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), 10 to account for subchronic-to-chronic extrapolation due the lack of a suitable chronic study, and 3 to account for deficiencies in the database (no multigeneration reproductive/developmental toxicity study).

Table 3. Reference dose (RfD) for 1,2,4-TMB

Critical Effect	Point of Departure (mg/kg-day)	UF	Chronic RfD (mg/kg-day)
Decreased pain sensitivity 90 day rat study Korsak and Rydzyński (1996)	Route to route extrapolation using Korsak and Rydzyński (1996) subchronic inhalation study in Wistar rats	1000	6×10^{-3}

Confidence Levels for the Derivation of the RfD for 1,2,4-TMB

A PBPK model was utilized to perform a route-to-route extrapolation to determine a POD for the derivation of the RfD from the Korsak and Rydzyński (1996) inhalation study and corresponding critical effect. The confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996) is medium (see above). Confidence in the database for 1,2,4-TMB is low to medium as the database includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. The database lacks a multigenerational reproductive study, and the studies supporting the critical effect predominately come from the same research institute. Overall confidence in the RfD for 1,2,4-TMB is low due to uncertainties surrounding the application of the available PBPK model for the purposes of a route-to-route extrapolation.

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

The oral database for 1,3,5-TMB includes no chronic, subchronic, or short-term oral exposure studies. However, as determined for the RfC derivation for 1,3,5-TMB, the toxicokinetic and toxicological similarities between 1,3,5-TMB and 1,2,4-TMB demonstrate sufficient similarity between the two isomers to support adopting the RfD for 1,2,4-TMB for

the RfD for 1,3,5-TMB. In addition to the previously discussed similarities in toxicokinetics, the qualitative metabolic profiles for the two isomers are similar to such a degree that first-pass metabolism through the liver is not expected to differ greatly between 1,2,4-TMB and 1,3,5-TMB. **Therefore, the chronic RfD of 6 × 10-3 mg/kg-day derived for 1,2,4-TMB was adopted as the RfD for 1,3,5-TMB based on the determination of sufficient similarity regarding toxicokinetics and toxicity between the two isomers.**

Confidence Levels for the Derivation of the RfD for 1,3,5-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 EPA (1994), 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 1,3,5-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 developmental toxicity studies in rats and mice. The database lacks a multigenerational reproductive study, and the studies supporting the critical effect predominately come from the same research institute. Overall confidence in the RfD for 1,3,5-TMB is low due to uncertainties surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,3,5-TMB.

Evidence for human carcinogenicity

No chronic inhalation studies that investigated cancer outcomes were identified in the literature for 1,2,4-TMB or 1,3,5-TMB. One oral cancer study was found in which rats were exposed via oral gavage to one experimental dose of 800 mg/kg-day (Maltoni et al., 1997). This study observed marginal increases in total malignant tumors and head tumors (e.g., neuroesthesioepithelioma) and provided no statistical analyses of results. A number of methodological issues limit the utility of this study (only one dose group, no discussion of histopathological analyses). When Fisher's exact test was performed by EPA on the incidences calculated from the reported percentages of animals bearing tumors in the control and 800 mg/kg dose groups no statistically significant associations were observed. Therefore, in accordance with the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), the database for 1,2,4-TMB was deemed to provide inadequate information to assess carcinogenic potential, and thus, no cancer values for 1,2,4-TMB are derived in this document. No studies of carcinogenicity were available for 1,3,5-TMB and thus the

database was deemed inadequate to assess carcinogenic potential; no cancer values were derived for 1,3,5-TMB.

LITERATURE SEARCH STRATEGY AND STUDY EVALUATION

Literature Search Strategy and Study Selection

The literature search strategy employed for 1,2,4-TMB and 1,3,5-TMB was conducted with the keywords listed in Table VI. Primary, peer-reviewed literature identified through December 2011 was included where that literature was determined to be relevant to the assessment. Potentially relevant publications on 1,2,4-TMB and 1,3,5-TMB were identified through a literature search conducted with the EBSCO Discovery Service feature of Health and Environmental Research On-Line (HERO), a meta-search engine with access to numerous databases including the Science Citation Index (SCI), Toxicology Literature Online (TOXLINE), The National Library of Medicine (NLM, PubMed/Medline), and Web of Science (WOS). Other peer-reviewed information, including health assessments developed by other organizations, review articles, literature necessary for the interpretation of TMB-induced health effects, and independent analyses of the health effects data was retrieved and included in the assessment where appropriate. A data call-in was announced by EPA in April, 2008 and any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

Table IV: Details of the search strategy employed for 1,2,4-TMB and 1,3,5-TMB

Databases	Keywords	Limits
EBSCO DISCOVERY SERVICE: HERO SCI NLM TOXLINE WOS	1,2,4-trimethylbenzene OR pseudocumene OR 95-63-6 1,3,5-trimethylbenzene OR mesitylene OR 108-67-8 **Additional search terms:** neurotoxicity, genotoxicity, developmental toxicity inflammation, irritation, toxicokinetics, pbpk, mode of action, white spirit, C9, C9 fraction, JP-8 **Also, specific searches were performed on specific metabolites:** 2,4-dimethylbenzoic acid OR 611-01-8; 2,4-dimethylhippuric acid OR 41859-41-0; 2,5-dimethylbenzoic acid OR 610-72-0; 2,5-dimethylhippuric acid OR 41859-40-9; 3,4-dimethylbenzoic acid OR 619-04-5; 3,4-dimethylhippuric acid OR 23082-12-4; 2,4,5-trimethylphenol OR 496-78-6; 2,3,5-	Search constraints: none Last search: December 2011

	trimethylphenol OR 697-82-5; 2,3,6-trimethylphenol OR 2416-94-6; 2,4,6-trimethylphenol OR 527-60-6; 3,5-dimethylbenzoic acid OR 499-06-9; 3,5-dimethylhippuric acid OR 23082-14-6	

The first step in the review of the available literature is the identification of studies pertinent to the development of the document. These references include, but are not limited to, studies related to: toxicokinetics, toxicity, carcinogenicity, mode of action, and physiologically-based pharmacokinetic modeling. The pertinent studies are then identified for inclusion in the hazard identification based on a preliminary review of the overall study design, with particular attention to the exposure route and duration. The available epidemiological and toxicological studies are further evaluated and identified for consideration for quantitative analysis based on a more specific evaluation of the study design, methods, and data quality.

Approximately 2500 references were obtained from the literature searches for 1,2,4-TMB and 1,3,5-TMB including references retrieved from specific literature searches necessary for the interpretation of TMB-induced health effects (e.g., literature on specific modes of action, PBPK analysis). The comprehensive, unedited list of studies captured in the literature search can be found on the HERO website. From this full list of references, there are 583 references that were considered for inclusion in the Toxicological Review. From this list of "considered" references, 112 full text publications were identified as providing relevant information for use in the development of this document.

The references that are cited in the document, as well as those that were considered but not included in the Toxicological Review of 1,2,4-TMB and 1,3,5-TMB, can be found within the HERO website (http(s):hero.epa.gov/tmb). This site contains HERO links to lists of references, including bibliographic information and abstracts, which were considered for inclusion in the Toxicological Review of 1,2,4-TMB and 1,3,5-TMB. This document is not intended to be a comprehensive treatise on the chemical or toxicological nature of 1,2,4-TMB and 1,3,5-TMB.

Study Evaluation for Hazard Identification

In general, the quality and relevance of health effects studies were evaluated as outlined in the Preamble to this assessment. In addition, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA,

1994) were consulted for guidance in evaluating scientific quality of the available studies. All studies that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the totality of the evidence for health effects in humans. The hazard identification analyses for each health endpoint in Section 1 discuses the breadth of the available literature and the extent to which the studies informed the conclusions concerning hazard. The available studies examining health effects of 1,2,4-TMB and 1,3,5-TMB exposure in humans (three occupational exposure studies, one cross-section residential study, and two controlled experiments of acute exposures) are discussed and evaluated in the hazard identification sections of the assessment (Section 1), with specific limitations of individual studies and of the collection of studies noted. The evaluation of the effects seen in the experimental animal studies focuses on the available acute, short-term, subchronic, and developmental toxicity studies, as no chronic inhalation exposure studies were found and the only identified chronic oral exposure study did not include data on effects other than cancer.

1. HAZARD IDENTIFICATION

1.1. Synthesis of Major Toxicological Effects

1.1.1. Neurotoxic Effects

There is evidence in humans and animals that inhalation exposure to TMBs induces neurotoxic effects. Occupational exposure studies in humans provide evidence of neurotoxicity following inhalation exposure to complex volatile organic compound (VOC) mixtures containing 1,2,4-TMB and 1,3,5-TMB. Prevalence rates of neuropsychological symptoms increased with exposure duration in dockyard painters (Chen et al., 1999); similarly, a significant positive association between 1,2,4-TMB exposure and exposure symptoms (e.g., abnormal fatigue) was reported in asphalt workers (Norseth et al., 1991). Nervousness, tension, headaches, vertigo, and anxiety were reported in paint shop workers exposed to 49-295 mg/m^3 of a solvent mixture containing 50% 1,2,4-TMB and 30% 1,3,5-TMB (Battig et al. (1956), as reviewed by (MOE, 2006)). Increased fatigue, decreased vigor, and increased reaction time were noted in controlled, acute volunteer studies in which humans were exposed to mixtures containing 1,2,4-TMB (Lammers et al., 2007), although it is unclear whether 1,2,4-TMB or other constituents within the mixtures were responsible for the observed effects. Uptake of 1,2,4-TMB and 1,3,5-TMB was reported in volunteers exposed for 2 hours to 11 or 123 mg/m^3 1,2,4-TMB, or 123 mg/ m^3 1,3,5-TMB; however, effects on the CNS, based on measures of overt CNS depression (heart rate during exposure and pulmonary ventilation) and a subjective rating of CNS symptoms (data not reported), were not observed (Järnberg et al., 1996). The Järnberg et al. (1997a; 1996) studies are limited for evaluating neurotoxicity to TMBs due to a lack of methods to adequately assess CNS function and lack of no-exposure controls, short exposure duration, and exposure of individual subjects to multiple, different concentrations of TMB isomers and/or mixtures containing TMBs.

In animals, there is consistent evidence of neurotoxicity following inhalation exposure to either 1,2,4-TMB or 1,3,5-TMB. Decreased pain sensitivity has been observed following inhalation exposure to 1,2,4-TMB and 1,3,5-TMB in multiple studies conducted in male Wistar rats. To test pain responses following TMB exposure, animal studies have employed the hot plate test. In this test, a thermal stimulus is applied to determine pain sensitivity, as indicated by the animals' latency to paw-lick following introduction of the stimulus.

Decreases in pain sensitivity have been observed at concentrations ≥ 492 mg/m^3 following subchronic and short-term exposure to 1,2,4-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Korsak and Rydzyński, 1996) and short-term exposure to 1,3,5-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001). In the subchronic study (Korsak and Rydzyński, 1996), 1,2,4-TMB inhalation resulted in reduced pain sensitivity which occurred in a concentration-dependent manner. In short-term studies that examined a range of concentrations (Wiaderna et al., 2002; Gralewicz et al., 1997a) these decreases in pain sensitivity following exposure to 1,2,4-TMB or 1,3,5-TMB were non-monotonic. Differences in experimental design may account for the lack of monotonicity in these short-term studies, in contrast to the observations in Korsak and Rydzyńsky, (1996). Similar to the subchronic study, acute exposures to 1,2,4-TMB or 1,3,5-TMB induced concentration-dependent decreases in pain sensitivity, with EC_{50} values of 5,682 and 5,963 mg/m^3, respectively (Korsak and Rydzyński, 1996; Korsak et al., 1995).

A second, somewhat different measure of pain sensitivity was reported in studies evaluating performance in the hot plate test (before and after footshock) several weeks following short-term, inhalation exposure to 1,2,4-TMB or 1,3,5-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a). In these studies, treatment-related, statistically significant changes in pain sensitivity at ≥ 492 mg/m^3 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 exposure concentration were observed prior to or immediately following footshock. These findings indicate that inhalation exposure to TMBs may prolong footshock-induced reductions in pain sensitivity. It is also plausible that an amplification of responses associated with classically conditioned analgesia (i.e., decreased pain sensitivity) occurs following 1,2,4-TMB and 1,3,5-TMB exposure. Specifically, footshock can cause contextual cues (e.g., hot plate test) to become associated with the noxious stimulus (footshock), inducing stress or fear-related responses in the shocked animal such that, subsequently, both footshock itself as well as the contextual cues associated with footshock, can reduce sensitivity to pain (possibly via the release of endogenous opiods). Thus, exposure to the hot plate apparatus immediately following footshock may associate this test environment with the footshock, such that subsequent re-exposure to the hot plate apparatus can, itself, produce analgesia. From the data available, the relative contribution of these behaviors to the observed effects cannot be easily distinguished.

The decreases in pain sensitivity measured in the subchronic and acute studies were observed immediately after exposure, with no significant effects persisting two weeks after exposures were terminated (Korsak and Rydzyński, 1996; Korsak et al., 1995). In contrast, performance in the hot plate test was significantly impaired following short-term exposure

to either 1,2,4-TMB or 1,3,5-TMB when tested 50-51 days after exposure (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a), indicating a persistence of these effects. The ability of male Wistar rats to respond to a thermal stimulus in the hot plate test was consistently impaired following inhalation exposure to TMBs. In these studies, 1,2,4-TMB and 1,3,5-TMB similar in their capacity to decrease pain sensitivity (Table 1-1). Pain sensitivity was not examined following oral exposure.

Human exposures to solvent mixtures containing 1,2,4-TMB (Lammers et al., 2007) or 1,2,4-TMB and 1,3,5-TMB (Battig et al., 1956), as reviewed by MOE (2006) suggest possible effects on the neuromuscular system, as effects reported included increased reaction time and vertigo, respectively. Animal studies using rotarod performance, which tests motor coordination, balance, and overall neuromuscular function, indicate that inhalation of 1,2,4-TMB or 1,3,5-TMB can affect neuromuscular system function. Significant decreases in rotarod performance were observed at 1230 mg/m^3 1,2,4-TMB when tested immediately after exposure for either 8 or 13 weeks (Korsak and Rydzyński, 1996). This impaired function was still evident at 2 weeks post-exposure and, while not statistically significant, may indicate long-lasting neuromuscular effects of subchronic exposures to 1,2,4-TMB. Acute inhalation exposure studies support this observation. Effects such as loss of reflexes and righting responses have been observed following acute inhalation exposure to 1250-45,000 mg/m^3 of 1,2,4-TMB (MOE, 2006; Henderson, 2001). Similarly, acute exposure to 1,2,4-TMB or 1,3,5-TMB resulted in decreased performance in rotarod tests immediately following exposure, with EC_{50} values of 4693 mg/m^3 and 4738 mg/m^3, respectively (Korsak and Rydzyński, 1996; Korsak et al., 1995); these results indicate the 1,2,4-TMB and 1,3,5-TMB may be similar in their ability to impair neuromuscular function, balance, and coordination following acute inhalation exposure. No studies evaluating oral exposure to TMB isomers address this endpoint.

The neurobehavioral tests administered (i.e., hot plate and rotarod) in the subchronic and acute studies by Korsak and Rydzyńsky, (1996) and Korsak et al. (1995) appear to have been administered on the same days; however, it is unclear whether the tests were performed sequentially in the same cohorts of animals. Performing the hot plate test immediately following the rotarod test could introduce a potential confounder, as shock alone (such as that used as negative reinforcement following rotarod failure) can cause reductions in pain sensitivity. Thus, if the tests were performed sequentially in the same animals, TMB-exposed animals failing more often in the rotarod test may exhibit increases in paw-lick latency unrelated to treatment, as compared to controls receiving less shock reinforcement. However, the observations by Korsak and Rydzyńsky, (1996) and Korsak et al. (1995) are supported by 2.8 and 2.9-fold increases in latency to paw-lick that, although

not statistically significant, were observed weeks subsequent to short-term exposures to 492 mg/m^3 1,2,4-TMB or 1,3,5-TMB (Gralewicz and Wiaderna, 2001).

Effects in open field testing have been consistently reported in oral and inhalation studies of exposure to 1,2,4-TMB and 1,3,5-TMB in male rats. Altered behaviors in open field tests can involve contributions not only from elevated anxiety due to open spaces and bright light but also from changes in motor function. Decreased anxiety and/or increased motor function at ≥ 492 mg/m^3 1,2,4-TMB or 1,3,5-TMB has been reported in short-term studies, as evidenced by increases in horizontal locomotion or grooming activities (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a). Statistically significant increases in horizontal locomotion were observed in short-term studies assessing open field behavior following inhalation exposure to either isomer (Gralewicz and Wiaderna, 2001). Non-monotonic increases in grooming were reported following short-term exposure to 1,2,4-TMB (with a statistically significant increase only in the mid-exposure group); changes in horizontal locomotion were not statistically significant (increases of 3-35% were non-monotonic) (Gralewicz et al., 1997a). As open field testing was conducted 25 days after termination of exposure in these studies, the results suggest latency for the effects on anxiety and/or motor function.

Slight increases in locomotor activity were also observed in open field tests immediately following acute, oral exposure to 1,2,4-TMB or 1,3,5-TMB. Significant increases were observed at 3850 mg/kg for 1,2,4-TMB and at ≥ 1,920 mg/kg for 1,3,5-TMB, with minimal dose-effect or time-effect relationships and negligible differences in the magnitude of the change in activity between isomers (Tomas et al., 1999b). The study authors attributed these changes to discomfort arising from difficulty in breathing and/or maintaining balance due to the onset of motor ataxia; notably, by 90 minutes following exposure, the rats were reported to be completely inactive and several rats in the high dose group died within 24 hours (Tomas et al., 1999b). Open field tests cannot easily distinguish between anxiety-related responses and changes in motor activity. However, effects on motor activity were observed following inhalation exposure to elevated concentrations of TMBs in several acute studies, although the results are somewhat inconsistent with observations in open field tests. Decreased motor activity was observed in male rats immediately after exposure at 5000 mg/m^3 1,2,4-TMB (McKee et al., 2010). Decreased motor activity was also reported in rats acutely exposed to a mixture containing TMB isomers (Lammers et al., 2007), but the use of a mixture precludes a determination of the toxicity specifically associated with 1,2,4-TMB or 1,3,5-TMB. As biphasic changes in activity are frequently observed following exposures to solvents, it is likely that the timing of the evaluations conducted in the short-term versus acute studies may influence the

consistency of these results. Overall, exposure to 1,2,4-TMB and 1,3,5-TMB affects anxiety and/or motor function at concentrations above 492 mg/m^3, although the exact, potentially biphasic, concentration-response relationship remains unclear.

Cognitive function following exposure to 1,2,4-TMB or 1,3,5-TMB has not been evaluated in humans or following oral exposure in animals; exposure of human volunteers to mixtures containing TMBs did not indicate any effects on short-term learning and memory tests (Lammers et al., 2007). Similarly, short-term spatial memory (radial maze performance) was unaffected by exposure to either TMB isomer in animal studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a). In contrast, effects on cognitive function in different neurobehavioral tests, observed as altered conditioning behaviors, were consistently observed in multiple studies in male rats weeks following short-term inhalation exposure to 1,2,4-TMB or 1,3,5-TMB, although clear concentration-effect relationships were not observed. Comparing the results of the behavioral tests reveals that there are differences in these neurological effects reported for 1,2,4-TMB and 1,3,5-TMB, as well as differences in the exposure concentrations at which the cognitive effects were observed. Decreased step-down latency in passive avoidance tests 35-45 days after short-term inhalation exposure to ≥ 492 mg/m^3 1,2,4-TMB or ≥ 123 mg/m^3 1,3,5-TMB was observed in treated male rats (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a); decreases may be attributed to a reduced ability to inhibit motor reactions (or a lowered motor threshold) in response to stress. These responses were consistently observed and similar in magnitude across all studies at 7 days post footshock. Statistically significant changes were not observed ≤ 24 hours following footshock and were not consistently observed 3 days following footshock, suggesting that 1,2,4-TMB and 1,3,5-TMB exposure may affect adaptive behaviors associated with the persistence of stress or fear-related responses. Reduced active avoidance learning was also observed in male rats following short-term inhalation exposure to 492 mg/m^3 1,2,4-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001); however, these changes were not observed in the other 1,2,4-TMB short-term study (Gralewicz et al., 1997a). Decreased performance in active avoidance tests was consistently observed following short-term exposure to ≥ 492 mg/m^3 1,3,5-TMB(Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001). Similar to 1,2,4-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001), the effects of 1,3,5-TMB were particular to the learning component of the test (acquisition training), rather than the memory component (retention session 7 days later). It is unclear whether potential TMB-induced alterations in locomotor activity would affect performance in these tests. Acute inhalation exposure studies provide some support for the observed effects of TMBs on learned behaviors. Significant increases in response latency in

psychomotor tasks, observed immediately after exposure (effects did not persist to 24 hours later), were reported in male rats following acute exposure to 5000 mg/m^3 1,2,4-TMB (McKee et al., (2010)) or to 4800 mg/m^3 of a mixture containing TMBs (Lammers et al., 2007). The effects on active and passive avoidance behaviors indicate that learning and/or long-term memory processes are affected by exposure to 1,2,4-TMB or 1,3,5-TMB. The data suggest that 1,3,5-TMB may be a more potent inducer of toxic effects on cognitive function than 1,2,4-TMB, as the effects following exposure to 1,3,5-TMB occurred at lower concentrations (123 mg/m^3 vs. 492 mg/m^3, were of greater intensity and occurred earlier than those reported following exposure to 1,2,4-TMB.

Controlled human exposure studies suggest that exposures of ≤ 123 mg/m^3 1,2,4-TMB or 1,3,5-TMB do not cause overt CNS depression (Järnberg et al., 1996), although symptoms related to this effect (e.g., lightheadedness, fatigue) have been reported in workers occupationally exposed to mixtures containing TMBs. In animals, CNS depression has been observed following acute inhalation exposure to 25,000-44,000 mg/m^3 1,3,5-TMB (ACGIH, 2002). Neurophysiological evidence from short-term inhalation studies, as well as supportive evidence from acute oral and injection studies, suggests that exposures to 1,2,4-TMB or 1,3,5-TMB at lower concentrations (at least for 1,2,4-TMB) may affect parameters associated with brain arousal. Concentration-dependent decreases (statistically significant at 1230 mg/m^3) in electrocortical arousal (i.e., spike-wave discharge activity in recordings from cortical-hippocampal electroencephalograms, EEGs) were observed in male rats 120 days after short-term exposure to ≥ 492 mg/m^3 1,2,4-TMB, suggesting persistent functional changes in the rat CNS (Gralewicz et al., 1997b). In recordings from rats that were awake, but immobile (not exhibiting pronounced exploratory activity, as determined by EEG morphology), statistically significant decreases in spike wave discharge activity were observed at 24 hours following short-term exposure to 492 mg/m^3 1,2,4-TMB (Gralewicz et al., 1997b). Dose-related decreases in spike wave discharges were observed at ≥ 240 mg/kg 1,2,4-TMB or 1,3,5-TMB subsequent to acute oral exposure (Tomas et al., 1999a); stronger and more persistent effects on electrocortical arousal were observed following oral exposure to 1,3,5-TMB compared with 1,2,4-TMB (Tomas et al., 1999a). Similar effects were observed following i.p. injection of 1,2,4-TMB and 1,3,5-TMB (Tomas et al., 1999c). The observed EEG abnormalities following inhalation and oral exposure to 1,2,4-TMB or 1,3,5-TMB provide supportive evidence of the CNS depressant effects of these compounds (Gralewicz et al., 1997b).

A summary of the observed neurotoxicity for 1,2,4-TMB and 1,3,5-TMB is shown in Tables 1-1 and 1-2 for inhalation and oral exposures, respectively.

Table 1-1: Summary of observed in vivo neurotoxicity in subchronic and short-term studies of male Wistar rats following inhalation exposure to 1,2,4-TMB or 1,3,5-TMB

Health Effect	Study Design and Reference	Results	Lowest Level at which Significant Effects were observed (mg/m^3)[a]
1,2,4-TMB			
Decreased Pain Sensitivity	0, 123, 492, 1,230 mg/m^3 recovery (1,230 mg/m^3 at 2 weeks post-exposure) 90 days; 10/group Korsak & Rydzynsky (1996)	Exposure-dependent increases in paw-lick latency which recover by two weeks post-exposure. *Response relative to control:* 0, 18, 79*, 95*% (recovery= 12%)	**492**
	0, 492 mg/m^3 4 weeks; 11/group Gralewicz & Wiaderna (2001)	Increased paw-lick latency 24 hours after intermittent footshock[b]. *Response relative to control:* 0, 191*%	**492**
	0, 123, 492, 1,230 mg/m^3 4 weeks; 15/group Gralewicz et al. (1997a)	Non-monotonic increases in paw-lick latency 24 hours after intermittent footshock[b]. *Response relative to control:* 0, 9, 61*, 46*%	**492**
Impaired Neuromuscular Function and Coordination	0, 123, 492, 1,230 mg/m^3 recovery (1,230 mg/m^3 at 2 weeks post-exposure) 90 days; 10/ group Korsak & Rydzynsky (1996)	Exposure-dependent increases in rotarod failures which do not recover by two weeks post exposure. *Response relative to control:* 0, 10, 20, 40*% (recovery= 30%)	**1,230**
Decreased Anxiety and/or Increased Motor Function	0, 492 mg/m^3 4 weeks; 11/group Gralewicz & Wiaderna (2001)	Increased horizontal locomotion in open field activity tests. *Response relative to control:* 0, 62*%	**492**
	0, 123, 492, 1,230 mg/m^3 4 weeks; 15/group Gralewicz et al. (1997a)	Non-monotonic increases in grooming in open field activity tests at middle concentration; no change in horizontal locomotion. *Response relative to control:* 0, 82, 147*, 76%	**492**
Altered Cognitive Function	0, 492 mg/m^3 4 weeks; 11/group Gralewicz & Wiaderna (2001)	Decreased step down latency in passive avoidance tests and decreased performance in active avoidance tests. *Response relative to control:* 0, 43*%[c] ; 0, 60*%[d]	**492**
	0, 123, 492, or 1,230 mg/m^3 4 weeks; 15/group Gralewicz et al. (1997a)	Non-monotonic decreases in step down latency in passive avoidance tests. *Response relative to control:* 0, 21, 81*, 49*%[c] ;	**492**

		0, 30, 27, 34% [e]	
Decreased Cortico-Hippocampal Activity	0, 123, 492, 1,230 mg/m^3 4 weeks; 9/group Gralewicz et al. (1997b)	Non-monotonic decreases in spike-wave discharges at 24 hours post-exposure (EEG) at middle concentration. *Response relative to control:* 0, 31, 64*, 19%	**492**

1,3,5-TMB			
Decreased Pain Sensitivity	0, 492 mg/m^3 4 weeks; 11/group Gralewicz & Wiaderna (2001)	Increased paw-lick latency 24 hours after intermittent footshock[b]. *Response relative to control:* 0, 250*%	**492**
	0, 123, 492, 1,230 mg/m^3 4 weeks; 12/group Wiaderna et al. (2002)	Non-monotonic increases in paw-lick latency 24 hours after intermittent footshock at middle concentration[b]. *Response relative to control:* 0, 4, 70*, 17%	**492**
Decreased Anxiety and/or Increased Motor Function	0, 492 mg/m^3 4 weeks; 11/group Gralewicz & Wiaderna (2001)	Increased horizontal locomotion in open field activity tests. *Response relative to control:* 0, 70*%	**492**
Altered Cognitive Function	0, 123, 492, 1,230 mg/m^3 4 weeks; 12/group Wiaderna et al. (2002)	Non-monotonic decreases in step down latency in passive avoidance tests and in performance in active avoidance tests. *Response relative to control:* 0, 48*, 55*, 46*%[c] ; 0, 40*, 35*, 50*%[d]	**123**
	0, 492 mg/m^3 4 weeks; 11/group Gralewicz & Wiaderna (2001)	Decreased step down latency in passive avoidance tests and decreased performance in active avoidance tests. *Response relative to control:* 0, 57*%[c] ; 0, 70*% [d]	**492**

* Significantly different from controls

For studies other than Korsak and Rydzynsky, 1996, % change from control calculated from digitized data using GrabIt software

[a] Lowest effect level at which statistically significant changes were observed

[b] This effect was only observed 24 hours following intermittent foot shock; no significant effects at any exposure were observed prior to or immediately following foot shock

[c] Decreased step down latency in passive avoidance tests at 7 days post footshock

[d] Increased number of trials to reach avoidance criteria

[e] Decreased avoidance response % in trials 25-30 of training

Tables 1-2: Summary of observed in vivo neurotoxicity for 1,2,4-TMB and 1,3,5-TMB — oral exposures

Health Effect	Study Design and Reference	Results	Lowest Level at which Significant Effects were observed (mg/kg)[a]
		1,2,4-TMB	
Decreased Cortico-Hippocampal Activity	240, 960, 3,850 mg/kg, single oral gavage Rat, Wag/Rij, male, 18/group Tomas et al. (1999a)	Inhibition of the number and duration of high voltage spindle activity in the hippocampus and cortex. *Response relative to control:* All doses produced differences from control during at least one of the measured time points	**240**
Decreased Anxiety and/or Increased Motor Function	960, 1920, 3850 mg/kg single oral gavage Rat, Wag/Rij, male, 10/group Tomas et al. (1999b)	Slight increase in spontaneous locomotor activity in open field test. *Response relative to control:* Significant difference from control reported at 3,850 mg/kg when data were considered by time points (i.e., dose × time interaction)	**3,850**
Decreased Cortico-Hippocampal Activity	790 mg/kg, single i.p injection Rat, Wistar, Male, 4 Tomas et al. (1999c)	Significant differences in hippocampal and cortical brain wave amplitude following injection. *Response relative to control:* cortical wave amplitude decreased up to 6.5%; hippocampal wave amplitude decreased up to 59.6%	**790**
		1,3,5-TMB	
Decreased Cortico-Hippocampal Activity	240, 960, 3,850 mg/kg, single oral gavage Rat, Wag/Rij, male, 18/group Tomas et al. (1999a)	Inhibition of high voltage spindle activity in the hippocampus and cortex. *Response relative to control:* All doses produced differences from control during at least one of the measured time points	**240**
Decreased Anxiety and/or Increased Motor Function	960, 1920, 3850 mg/kg single oral gavage Rat, Wag/Rij, male, 10/group Tomas et al. (1999b)	Slight increase in spontaneous locomotor activity in open field test. *Response relative to control:* Significant difference from control reported at 1,920 and 3,850 mg/kg	**1920**
Decreased Cortico-Hippocampal Activity	790 mg/kg, single i.p injection Rat, Wistar, Male, 4 Tomas et al. (1999c)	Significant differences in hippocampal and cortical brain wave amplitude following injection. *Response relative to control:* cortical wave amplitude decreased up to 13.9%; hippocampal wave amplitude decreased up to 38%	**790**

* Significantly different from controls

[a] Lowest effect level at which statistically significant changes were observed

- **Mode of Action Analysis for neurotoxicity**

The observation of neurotoxicity following acute-, short-term-, and subchronic-duration exposure to TMB (Lammers et al., 2007; Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997a; Gralewicz et al., 1997b; Korsak and Rydzyński, 1996; Korsak et al., 1995) may indicate that TMB perturbs normal neurotransmission in exposed animals (see Table 1-3), although the specific key events necessary for TMB-induced neurotoxicity are not established. Although limited mechanistic data for TMBs exists, structurally similar compounds like toluene and xylene have been more thoroughly characterized and it is hypothesized that TMBs would operate through a similar mechanism in producing the resultant neurotoxicological effect. Aromatic hydrocarbons are known to interact with catecholaminergic systems (Kyrklund, 1992). Inhalation exposures to toluene and xylene have been shown to significantly change concentration and turnover rate of both dopamine and norepinephrine in various regions of the rat brain (Rea et al., 1984; Andersson et al., 1983; Andersson et al., 1981; Andersson et al., 1980). These changes have been hypothesized to be due to potential metabolites with affinity to catecholamine receptors that would, in turn, influence the uptake and release of neurotransmitters (Andersson et al., 1983; Andersson et al., 1981; Andersson et al., 1980).

Catecholaminergic changes with toluene have been reported and are similar to that observed with TMBs which would therefore increase the plausibility that the mechanisms of neurotoxicity are similar between the two compounds. For example, subchronic inhalation exposures of rats to low concentrations of toluene (as low as 80 ppm [300 mg/m3]) have been shown to decrease spatial learning and memory, increase dopamine-mediated locomotor activity, increase in the number of dopamine D2 receptors, and increase dopamine D2 agonist receptor binding (Hillefors-Berglund et al., 1995; von Euler et al., 1994; von Euler et al., 1993). These effects were observed to persist up to four weeks after the termination of the toluene exposure. Activation of the dopaminergic system may also result in an inability to inhibit locomotor responses normally suppressed by punishment (Jackson and Westlind-Danielsson, 1994). Direct application of dopamine to the nucleus accumbens of rats has been observed to result in retardation of the acquisition of passive avoidance learning at concentrations that also stimulated locomotor activity (Bracs et al., 1984). Increases in catecholaminergic neurotransmission (through exposure to norepinephrine or dopamine agonists) result in dose-dependent reductions in the duration of spike wave discharges in rats (Snead, 1995; Warter et al., 1988). These observations and findings are in concordance with those resulting from exposure to

1,2,4-TMB and 1,3,5-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a; Gralewicz et al., 1997b) (Tomas et al., 1999a; Tomas et al., 1999c). Additionally, with regards to toluene and related aromatic hydrocarbons, it is known that there is direct interaction with these compounds on various ion channels (ligand and voltage gated) that are present in the central nervous system (Bowen et al., 2006; Balster, 1998). There is not enough information to ascertain the specific molecular sites and how the changes correlate to the observed neurotoxicological effects. However, it is widely believed that the interactions with the neuronal receptors in the brain (e.g. ion channels, catecholaminergic systems) may influence these changes.

Aromatic hydrocarbons may also affect the phospholipids in the nerve cell membrane (Andersson et al., 1981). Pertubation of the phospholipids on the cell membrane could indirectly affect the binding of neurotransmitters to the catecholamine receptors and potentially lead to alterations in receptor activity or uptake-release mechanisms. Uneven distribution of metabolites within differing regions of the brain, or spatial variations in phospholipid composition of nerve cell membranes may explain the differential effects seen in regard to catecholamine levels and turnover. Based on effect levels with other related solvents (e.g., toluene – see Balster (1998)), it is overall hypothesized that with TMBs there may be an initial interaction with the neuronal receptors (e.g., catecholaminergic systems, ion channels) followed by, at much higher exposures, interaction with the lipid membrane when the available sites on the neuronal receptors are completely occupied.

Additional mechanisms that may play a role in TMB neurotoxicity include production of reactive oxygen species (ROS). Myhre et al. (2000) found increased respiratory burst in neutrophils after 1,2,4-TMB exposure demonstrated by fluorescence spectroscopy, hydroxylation of 4-hydroxybenzoic acid, and electron paramagnetic resonance spectroscopy. The authors suggest the observation of solvent-induced ROS production may relevant to brain injury as microglia cells have a respiratory burst similar to neutrophils. Stronger evidence of potential ROS-related mechanisms of neurotoxicity was observed in a related study by Myhre and Fonnum (2001) in which rat neural synaptosomes exposed to 1,2,4-TMB produced a dose-dependent increase in reactive oxygen and nitrogen species demonstrated by the formation of the fluorescence of 2'7'-dichlorofluorescein (DCF). This observation of ROS production in rat synaptosomes may explain the observed TMB-induced neurotoxicity in acute, short-term, and subchronic inhalation studies.

Is the hypothesized mode of action sufficiently supported in test animals?

The hypothesis that TMB exposure results in abnormal neurotransmission in animals is supported by the available literature, including the observation that related methylated aromatic compounds (i.e., toluene and xylene) perturb the catecholaminergic system and elicit similar neurological effects as TMB isomers.

Is the hypothesized mode of action relevant to humans?

The observed neurotoxic effects in animals are relevant to humans, especially given the observation of similar neuropsychological effects in humans exposed to complex solvent mixtures containing TMB isomers.

In summary, neurotoxicity is associated with exposure to 1,2,4-TMB and 1,3,5-TMB based on evidence in humans and animals. The information regarding neurotoxicity in humans is limited and most of the available studies evaluating these effects involve exposure to mixtures containing TMB isomers and not specific exposure to the individual isomers. Additionally, none of the available studies have addressed the potential for latent neurological effects or effects in sensitive populations. However, the available information shows uptake of 1,2,4-TMB and 1,3,5-TMB in humans and suggests an association between TMB exposure in humans and neurotoxic effects. The observation of neurotoxicity in 1,2,4-TMB-exposed male Wistar rats was consistent across multiple exposure concentrations, including subchronic, short-term, and acute exposures. Similar indices of neurotoxicity were observed in male rats exposed to 1,3,5-TMB for short-term and acute durations. Although the oral database is limited, similar effects were observed (e.g., EEG; open field) across inhalation and oral study paradigms.

All of the available animal studies were conducted in male rats (Wistar or Wag/Rij) and by the same research group (The Nofer Institute of Occupational Medicine, Lodz Poland). No chronic studies are available, although there is consistent evidence of neurotoxicity following inhalation and oral exposure to 1,2,4-TMB and 1,3,5-TMB. Most of the neurotoxicity tests incorporated the application of footshock which could involve multiple neurological functions. The spectrum of effects suggests that TMBs affect multiple, possibly overlapping, CNS systems rather than a single brain region or neuronal nuclei (suggested by the solvent activity of the compounds). Almost all tests (other than pain) involve a contributing component of motor system function. Some endpoints exhibited clear exposure-response relationships (e.g., pain sensitivity and rotarod, although the former was not consistent across studies with different experimental design (i.e., varying exposure

durations and timing of endpoint analyses). Other endpoints did not show a clear concentration-effect relationship, suggesting either that exposures below a threshold value were not tested or do not exist, or that the presence of TMBs alone was sufficient to elicit a response in these tests, possibly via irritation or stress-related phenomena. However, irritation is highly unlikely given the latency between the exposures and the effects.

Multiple neurotoxic effects were observed weeks to months after cessation of inhalation exposure despite rapid clearance of these chemicals from blood and CNS tissues (see Appendix A), indicating that these effects are persistent. Although the reported human symptoms do not directly parallel the animal data, some similar effects were observed in both humans and rats. The majority of the neurotoxicity evidence available for TMBs was observed in laboratory animals as neurobehavioral effects including decreased pain sensitivity, impaired neuromuscular function and coordination, altered cognitive function, and decreased anxiety and/or increased motor function; and neurophysiological effects including decreased cortico-hippocampal activity. These effects are recognized in the U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) as possible indicators of neurotoxicity. EPA considered the neurotoxic effects to be biologically plausible and analogous to effects that could occur in humans; and concluded that the available evidence for 1,2,4-TMB and 1,3,5-TMB identified neurotoxicity as a toxicity hazard.

1.1.2. Respiratory Effects

There is evidence in humans and animals that inhalation exposure to TMBs induces respiratory toxicity. Occupational and residential exposure studies in humans provide evidence of respiratory toxicity following inhalation exposure to complex VOC mixtures containing 1,2,4-TMB and 1,3,5-TMB. While controlled human exposures (Jones et al., 2006; Järnberg et al., 1997a; Järnberg et al., 1996) have failed to observe substantial irritative symptoms following acute (less than 4 hours) inhalation exposures of up to 25 ppm (123 mg/m^3) 1,2,4-TMB or 1,3,5-TMB, occupational exposures have been shown to be associated with increased measures of sensory irritation, such as laryngeal and/or pharyngeal irritation (Norseth et al., 1991) and asthmatic bronchitis (Battig et al., 1956), as reviewed in (MOE, 2006). Residential exposures have demonstrated significant associations between 1,2,4-TMB and asthma (Billionnet et al., 2011). However, these studies evaluated TMB exposures occurring as exposures to complex solvent or VOC mixtures, thereby precluding a determination of the ultimate etiological agent.

In animals, there is consistent evidence of respiratory toxicity following inhalation exposure of rodents to 1,2,4-TMB (Table 1-3). Markers of pulmonary inflammation and irritation in the lungs of rats have been observed following subchronic inhalation exposures of Wistar rats to 1,2,4-TMB. Increases in populations of immune and inflammatory cells in bronchoalveolar lavage (BAL) fluid have been observed at concentrations ≥ 123 mg/m^3 following subchronic exposures of male Wistar rats to 1,2,4-TMB (Korsak et al., 1997). Specifically, the amount of cells in the BAL fluid of exposed rats was increased for total cells (≥ 123 mg/m^3, increased 2.3-3.0 fold) and macrophages (≥ 492 mg/m^3, increased 2.1-2.7 fold). However, some attenuation of these effects was observed at high concentrations (i.e., 1230 mg/m3) compared to lower doses. For example, the number of macrophages was increased 2.7-fold relative to control at 492 mg/m3, but only 2.2-fold at 1,230 mg/m3. This may indicate either adaptation to the respiratory irritation effects of 1,2,4-TMB during exposure, saturation of metabolic pathways, or immune suppression at higher doses. Subchronic exposure of male Wistar rats also significantly increased the BAL numbers of polymorphonuclear leukocytes and lymphocytes; however the specific exposure concentrations eliciting these significant increases were not reported by study authors. A small, but not significant, decrease in cell viability was observed at ≥ 123 mg/m3 following subchronic exposure to 1,2,4-TMB (Korsak et al., 1997).

In addition to increased populations of immune and inflammatory cells, histopathological alterations described as peribronchial, lung parenchymal, and perivascular lymphocytic infiltration in the lower respiratory tract have also been observed following subchronic exposures of 1,2,4-TMB to male and female Wistar rats (Korsak et al., 2000). Significant proliferation of peribronchial lymphatic tissue and interstitial lymphocytic infiltrations were observed in male rats exposed to 492 mg/m3, although trend-analysis demonstrated these increases were not concentration-dependent. The bronchial epithelium lost its cuboidal shape in some rats with peribronchial lymphatic proliferation, and was observed to form lymphoepithelium. Interstitial lymphocytic infiltrations were also observed in female rats exposed to 1230 mg/m3. Although unlike male rats, this increase in females was concentration-dependent as determined by a trend analysis. Alveolar macrophages were increased in both sexes at 1,230 mg/m3 with trend analysis demonstrating concentration-dependence across the entire concentration range. However, when the incidences of all pulmonary lesions were analyzed in aggregate, trend-analysis demonstrated significant increases in pulmonary lesions in both sexes across the entire concentration range. Pulmonary lesions were significantly increased in males at ≥ 492 mg/m3, but not at any exposure concentration in females. Studies on the respiratory effects of subchronic exposures to 1,3,5-TMB were not available.

Additional effects on clinical chemistry including increased total protein (37% increase at both 123 and 492 mg/m3), decreased mucoprotein (13% decrease, 123 mg/m3), increased lactate dehydrogenase (170% and 79% increase at 123 and 492 mg/m3, respectively) and increased acid phosphatase activity (47-75% increase at ≥ 123 mg/m3) were observed in 1,2,4-TMB-exposure animals; suggesting pulmonary irritation or inflammation. All of these effects also exhibited either some attenuation of effect at high concentrations compared to lower concentrations, or no increase in effect as exposure concentration increased. Therefore, some adaptation to the respiratory irritation effects of 1,2,4-TMB may be occurring.

Tables 1-3: Summary of observed in vivo respiratory toxicity for 1,2,4-TMB and 1,3,5-TMB — inhalation exposures

Health Effect	Study Design and Reference	Results	Lowest Level at which Significant Effects were observed (mg/m^3)[a]
1,2,4-TMB			
Pulmonary inflammation/ irritation	123-1,230 mg/m^3, 90 days (6h/day, 5 days/week) Rat, Wistar, male, 6-7 Korsak et al. (1997)	Increased total bronchoalveolar cell count with evidence of attenuation at high exposure *Response relative to control:* 0, 202*, 208*, 231*%	**123**
	123-1,230 mg/m^3, 90 days (6h/day, 5 days/week) Rat, Wistar, male, 6-7 Korsak et al. (1997)	Increased macrophage count with evidence of attenuation at high exposure *Response relative to control:*0, 107, 170*, 116*%	**492**
	123-1,230 mg/m^3, 90 days (6h/day, 5 days/week) Rat, Wistar, male and female, 6-7 Korsak et al. (2000)	Increase in number of pulmonary lesions *Response relative to control:* Incidences not reported, calculation of response relative to control not possible; authors report statistically significant increases at 492 and 1,230 mg/m^3	**492**
Clinical Chemistry Effect	123-1,230 mg/m^3, 90 days (6h/day, 5 days/week) Rat, Wistar, male, 6-7 Korsak et al. (1997)	Increased acid phosphatase activity with evidence of attenuation at high exposure *Response relative to control:*0, 47*, 74*, 45*%	**123**
Sensory Irritation (Decreased respiration)	1,245-9,486 mg/m^3, single exposure, 6 minutes Mouse, BALB/C, male, 8-10 Korsak et al. (1997); Korsak et al. (1995)	Decreased respiratory rate as measured during first minute of exposure *Response relative to control:*RD_{50} = 2,844	**2,844**
1,3,5-TMB			
Sensory Irritation (Decreased respiration)	1,245-9,486 mg/m^3, single exposure, 6 minutes Mouse, BALB/C, male, 8-10 Korsak et al. (1997)	Decreased respiratory rate as measured during first minute of exposure *Response relative to control:* RD_{50} = 2,553	**2,553**

* Significantly different from controls

[a] Lowest effect level at which statistically significant changes were observed

Decreased respiration, a symptom of sensory irritation, has been observed in male BALB/C mice following acute inhalation exposures either 1,2,4-TMB or 1,3,5-TMB for six minutes. These acute exposures were observed to result in dose-dependent depression of

respiratory rates, with the maximum decrease in respiration occurring in the first one or two minutes of exposure (Korsak et al., 1997; Korsak et al., 1995). The concentration of 1,2,4-TMB and 1,3,5-TMB that was observed to result in a 50% depression in the respiratory rate (RD_{50}) was similar between the two isomers: 578 and 519 ppm (2,844 and 2,553 mg/m3), respectively.

- **Mode of Action Analysis for respiratory toxicity**

Data regarding the potential mode of action for the respiratory effects resulting from TMB exposure are limited and the key events for TMB-induced respiratory toxicity are not established. However, the available toxicological data suggest that TMB isomers act as potent acute respiratory irritants and induce inflammatory responses following longer exposures (i.e., subchronic) in animals. The study authors (Korsak et al., 1997; Korsak et al., 1995) suggested that the decreased respiratory rate is indicative of irritation, and proposed that respiratory irritants such as TMB may activate a "sensory irritant receptor" on the transgeminal nerve ending in the nasal mucosa leading to an inflammatory response. Korsak et al. (1997; 1995) further suggest that activation of this irritant receptor follows either adsorption of the agonist, or adsorption and chemical reaction with the receptor. The authors reference a proposed model for the receptor protein that includes two main binding sites for benzene moieties and a thiol group. Further, the study authors suggest that in the case of organic solvents (i.e., toluene, xylene, TMB) a correlation between the potency of the irritating effect and the number of methyl groups is likely given the observation that RD_{50} values for depressed respiratory rates following exposure to TMB isomers is approximately 8-fold lower than toluene and 4-fold lower than xylene.

Following subchronic exposure of rats to 1,2,4-TMB, inflammatory cell (macrophages, polymorphonuclear leukocytes, and lymphocytes) numbers were increased along with markers of their activation (total lactate dehydrogenase and acid phosphatase activity in BAL) (Korsak et al., 1997), further indicating the inflammatory nature of responses in the respiratory tract of TMB-exposed animals. Inflammatory pulmonary lesions were also observed following subchronic exposures in rats. However, many of these effects were not observed to be concentration-dependent in repeated exposure studies (i.e., no progression of effect over an order of magnitude of doses), suggesting that there may be adaptation to respiratory irritation that occurs following extended exposure to TMB. The processes responsible for the respiratory inflammatory responses in subchronically exposed animal are unknown. However, a major inflammatory mediator, interleukin 8 (IL-8), was increases following exposure of porcine and human macrophages to secondary organic

aerosol (SOA) particles derived from 1,3,5-TMB (Gaschen et al., 2010). The observation that IL-8 levels increase following exposure to 1,3,5-TMB-derived SOA is noteworthy as a major function of IL-8 is to recruit immune cells to sites of inflammation. Therefore, the observation of inflammatory lesions involving immune cells (i.e., macrophages, leukocytes) may be partially explained by increases in inflammatory cytokines following TMB exposures. Additionally, ROS-generation has been observed in cultured neutrophil granulocytes and rat neural synaptosomes exposed to TMB (Myhre and Fonnum, 2001; Myhre et al., 2000), and the related compounds benzene and toluene have been shown to induce oxidative stress in cultured lung cells (Mögel et al., 2011). Although pulmonary ROS-generation has not been observed following in vivo or in vitro TMB exposures, there is suggestive evidence that it could play a role in the irritative and inflammatory responses seen in exposed animals.

In a study investigating jet fuel-induced cytotoxicity in human epidermal keratinocytes (HEK), aromatic hydrocarbons were more potent inducers of cell death than aliphatic constituents, even though the aromatic compounds only accounted for less than one-fourth of aliphatic constituents (Chou et al., 2003). Of the single aromatic ring hydrocarbons, 1,2,4-TMB and xylene were the most lethal to HEK. Increased cytotoxicity may explain the small, but insignificant, decrease in BAL cell viability observed in Korsak et al. (1997).

Is the hypothesized mode of action sufficiently supported in test animals?

Data that would allow for the determination of a mode of action for respiratory effects due to TMB exposure are limited. However, the observation of ROS generation in cultured neutrophils and ROS generation in lung cells exposed to related aromatic compounds (i.e., benzene and toluene), suggests that oxidative stress may play a role in the observed TMB-induced respiratory effects in exposed animals.

Is the hypothesized mode of action relevant to humans?

The respiratory effects in animals are relevant to humans, especially given the observation of irritative and inflammatory respiratory effects (e.g., asthma) in humans exposed to complex solvent mixtures containing TMB isomers.

In summary, respiratory toxicity is associated with exposure to 1,2,4-TMB and 1,3,5-TMB based on evidence in humans and animals. The information in humans is limited for a number of reasons including: all studies investigating exposure to 1,2,4-TMB and 1,3,5-TMB were conducted using a complex VOC mixture. However, the available information

demonstrates uptake of 1,2,4-TMB and 1,3,5-TMB by humans, and suggests an association between TMB exposure in humans and respiratory toxicity. The observation of respiratory irritation and inflammation in Wistar rats and BALB/C mice following exposure to 1,2,4-TMB was consistent across multiple exposure concentrations, and subchronic and acute exposure durations. All of the available animal studies were conducted in rodents (Wistar rats or BALB/C mice) and by the same research group (The Nofer Institute of Occupational Medicine, Lodz Poland). No chronic studies are available. Although some endpoints (BAL macrophages, alkaline phosphatase) showed dose-dependence at low and mid exposure concentrations, all effects were observed to exhibit some attenuation of effect at high doses, potentially indicating either adaptation to the respiratory irritation effects, saturation of metabolic and/or toxicity pathways, or immune suppression at higher doses.

Although the reported human symptoms (laryngeal and/or pharyngeal irritation, asthmatic bronchitis, asthma) do not directly parallel the effects observed in animal studies, the observation of irritative and/or inflammatory responses in multiple species (including humans) demonstrates a consistency in TMB-induced respiratory toxicity. EPA considered the observed respiratory effects in animals to be biologically plausible and analogous to effects that could occur in humans; and concluded that the available evidence for 1,2,4-TMB identified respiratory toxicity as a toxicity hazard.

1.1.3. Reproductive and Developmental Toxicity

There are no studies in humans that investigated the reproductive or maternal toxicity of either 1,2,4-TMB or 1,3,5-TMB. Maternal toxicity in the form of decreased corrected body weight (i.e., maternal body weight minus the weight of the gravid uterus) was observed in dams following exposure during gestational exposure to 1,2,4-TMB or 1,3,5-TMB (Saillenfait et al., 2005). Dams exposed to 2,952 mg/m3 1,2,4-TMB gained only 50% of the weight gained by control animals, whereas dams exposed to 2,952 mg/m3 1,3,5-TMB gained only 25% of the weight gained by controls. Decreased maternal food consumption (across GD 6-21) was also observed at 2,952 mg/m3 1,2,4-TMB and 1,476 mg/m3 1,3,5-TMB, although the difference compared to controls (9-13%) was modest compared to the observed decreases in maternal weight gain. The decrease in food consumption at 1,476 mg/m3 1,3,5-TMB was determined to not be a marker of adversity given no accompanying decrease in maternal weight gain at that concentration.

There are no studies in humans that investigated the developmental toxicity of either 1,2,4-TMB or 1,3,5-TMB. Developmental toxicity (reported as decreased fetal body weight) has been observed in male and female rat fetuses following gestational exposure to 1,2,4-

TMB and 1,3,5-TMB on gestational days 6 through 20 via inhalation for 6 hours a day (Saillenfait et al., 2005) (Table 1-4). Fetal body weights were decreased (statistically significant) by 5-13% at concentrations of > 2,952 mg/m3 of 1,2,4-TMB and 1,3,5-TMB. No adverse effects were noted on embryo/fetal viability and no increase in skeletal, visceral, or external morphology (i.e., teratogenesis) was observed up to the highest concentrations for either isomer.

Table 1-4: Summary of observed developmental toxicity for 1,2,4-TMB and 1,3,5-TMB — inhalation exposures

Health Effect	Study Design and Reference	Results	Lowest Level at which Significant Effects were observed (mg/m^3)[a]
1,2,4-TMB			
Developmental Toxicity	0, 492, 1,476, 2,952, 4,428 mg/m^3, GD 6-20 (6h/day) Rat, Sprague-Dawley, female & male, 24-25 dams Saillenfait et al. (2005)	Decreased fetal body weight of male and female fetuses *Response relative to control:* Male: 0, 1, 2, 5*, 11*% Female: 0, 1, 3, 5*, 12*%	**2,952**
Maternal Toxicity	0, 492, 1,476, 2,952, 4,428 mg/m^3, GD 6-20 (6h/day) Rat, Sprague-Dawley, female & male, 24-25 dams Saillenfait et al. (2005)	Decreased corrected maternal weight gain *Response relative to control:* 0, +7, 7, 51*, 100*% (weight gain = 0 g)	**2,952**
1,3,5-TMB			
Developmental Toxicity	0, 492, 1,476, 2,952, 4,428 mg/m^3, GD 6-20 (6h/day) Rat, Sprague-Dawley, female & male, 24-25 dams Saillenfait et al. (2005)	Decreased fetal body weight of male and female *Response relative to control:* Male: 0, 1, 5, 7*, 12*% Female: 0, 1, 4, 6, 13*%	**2,952**
Maternal Toxicity	0, 492, 1,476, 2,952, 4,428 mg/m^3, GD 6-20 (6h/day) Rat, Sprague-Dawley, female & male, 24-25 dams Saillenfait et al. (2005)	Decreased corrected maternal weight gain *Response relative to control*: 0, +3, 31, 76*, 159*% (weight gain = -12 g)	**2,952**

* Significantly different from controls

[a] Lowest effect level at which statistically significant changes were observed

- **Mode of Action Analysis for developmental toxicity**

The mode of action for 1,2,4-TMB- and 1,3,5-TMB-induced developmental toxicity is unknown. The database for developmental toxicity following exposure to 1,2,4-TMB is limited to one animal study; no studies in humans are available. Although there is only one study available, 1,2,4-TMB and 1,3,5-TMB demonstrated effects on fetal and maternal body weights. The developmental and maternal toxicity in animals was considered by the Agency to be biologically plausible and potentially analogous to effects that could occur in humans. EPA concluded that the available evidence for 1,2,4-TMB and 1,3,5-TMB identified maternal and developmental toxicity as a toxicity hazard.

1.1.4. Hematological Toxicity and Clinical Chemistry Effects

There is limited evidence in humans, and stronger evidence in animals, that exposure to TMBs induces hematological toxicity. Alterations in blood clotting and anemia in workers exposed to a paint solvent containing 1,2,4-TMB and 1,3,5-TMB was reported by Battig et al. (1956), as reviewed by MOE (2006). A LOAEL of 295 mg/m3 was identified from this study. However, as workers were exposed to a solvent mixture containing TMB isomers, it is impossible to ascertain the ultimate contribution of either isomer to the observed health effects.

In animals, there is evidence of hematological toxicity following subchronic exposure to 1,2,4-TMB and short-term exposure to 1,3,5-TMB (Table 1-5). Subchronic exposures to 1,2,4-TMB have been shown to result in hematological effects and changes in serum chemistry in exposed rats (Korsak et al., 2000). In male rats at termination of exposure, RBC counts were decreased by 23% and WBC counts increased 180% at 1,230 mg/m3; the observed alteration in blood cell counts were exposure concentration-dependent as determined by trend analysis. A concentration-dependent increase in WBC count was also observed in female rats, pair-wise comparisons of individual doses failed to reach statistical significance at any concentration. WBC counts were observed to be slightly decreased (18%) relative to controls two weeks after the termination of exposure, whereas RBC counts were still decreased by 24% relative to control (although this decreased failed to reach statistical significance). Significant decreases in reticulocytes (71% of controls) and clotting time (37% of controls) were observed in female rats exposed to 1,230 mg/m3 and 492 mg/m3 1,2,4-TMB, respectively. Both of these effects were concentration-dependent when analyzed over the entire range of exposure concentrations with values 60-65% greater than controls after end of exposure; animals fully recovered within two weeks after termination of exposure. The only clinical chemistry parameter significantly

altered was an increase in sorbitol dehydrogenase at ≥ 123 mg/m3 in exposed male rats, although these increases were not exposure-dependent. Sorbitol dehydrogenase activity was also higher in exposed female rats, but the increases in activity were not significantly higher when compared to controls.

An increase in aspartate aminotransferase, but no other substantial hematological effects, was observed in rats 14 days following short-term exposure (6 hours/day, 6 days/week for five weeks) (Wiglusz et al., 1975a; Wiglusz et al., 1975b). The adversity of aspartate aminotransferase is unclear given the lack of a clear pattern in temporality (effects at some days post-exposure, but not others) and the lack of accompanying liver histopathology.

Tables 1-5: Summary of observed in vivo hematological toxicity and clinical chemistry effects for 1,2,4-TMB and 1,3,5-TMB — inhalation exposures

Health Effect	Study Design and Reference	Results	Lowest Level at which Significant Effects were observed (mg/m^3)[a]
1,2,4-TMB			
Hematological toxicity	123-1,230 mg/m^3, 90 days (6h/day, 5days/week) Rat, Wistar, female & male, 6-7 Korsak et al. (2000)	Decreased red blood cells in males only. *Response relative to control:*0, 1, 15, 23*% (recovery = 24%)	**1,230**
	123-1,230 mg/m^3, 90 days (6h/day, 5days/week) Rat, Wistar, female & male, 6-7 Korsak et al. (2000)	Increased white blood cells in males only. *Response relative to control:*0, 2, 4, 80*% (recovery = 18% decrease)	**1,230**
	123-1,230 mg/m^3, 90 days (6h/day, 5days/week) Rat, Wistar, female & male, 6-7 Korsak et al. (2000)	Decreased reticulocytes in females only. *Response relative to control:*0, 51, 49,71*% (recovery = 65% increase)	**1,230**
	123-1,230 mg/m^3, 90 days (6h/day, 5days/week) Rat, Wistar, female & male, 6-7 Korsak et al. (2000)	Non-monotonic decreases in clotting time in females only. *Response relative to control:*0, 23,37*,27*% (recovery = 60% increase)	**492**
Clinical Chemistry Effects	123-1,230 mg/m^3, 90 days (6h/day, 5days/week) Rat, Wistar, female & male, 6-7	Non-monotonic increases in sorbitol dehydrogenase in males only. *Response relative to control:0, 73*, 74*,73*%*	**123**

	Korsak et al. (2000)		
1,3,5-TMB			
Hematological Effect	1,500-6,000 mg/m^3, single exposure, 6 hours Samples collected 0, 1, 7, 14, and 28 days post exposure Rat, Wistar, male, 6 Wiglusz et al. (Wiglusz et al., 1975a)	Increased segmented neutrophilic granulocytes (1-28 days post exposure). *Response relative to control:* Increased across all days of exposure.	**3,000**
Clinical Chemistry Effects	3,000 mg/m^3, 5 weeks (6 h/day, 6days/week) Samples collected 1, 3, 7, 14, and 28 days during exposure Rat, Wistar, male, 6 Wiglusz et al. (1975b)	Increased aspartate aminotransferase on day 14 *Response relative to control (day 14):*12*%	**3,000**
	300-3,000 mg/m^3, single exposure, 6 hours Samples collected 0, 2, 7, 14 and 28 days post exposure Rat, Wistar, male, 6 Wiglusz et al. (1975b)	Increased alkaline phosphatase on day 7 post-exposure *Response relative to control (on day 7):*0, -0.1, 0.03, 84*%	**3,000**

* Significantly different from controls
[a] Lowest effect level at which statistically significant changes were observed

Acute exposures of male Wistar rats to 1,500-6,000 mg/m3 1,3,5-TMB for six hours did not result in substantial effects on hemoglobin or RBC or WBC count (Wiglusz et al., 1975a). However, the number of segmented neutrophilic granulocytes was increased in 1,3,5-TMB-exposed rats up to 28 days following exposure (statistics not reported). The greatest increase in granulocyte numbers (100%) was observed the day of exposure and one day following in rats exposed to 6,000 mg/m3, although attenuation was seen 7-28 days following exposure, possibly indicating induction of metabolizing enzymes or saturation of toxicity pathways. Investigation of clinical chemistry parameters in rats acutely exposed to 300- 3,000 mg/m3 for six hours did not reveal any consistent pattern in the levels of aspartate or alanine aminotransferases, although alkaline phosphatase was statistically increased 84% in rats seven days following exposure to 3,000 mg/m3 (Wiglusz et al., 1975b). NOAELs and LOAELs determined from these acute exposure studies are provided in Table 1-5.

- **Mode of Action Analysis for hematological toxicity and clinical chemistry effects**

The mode of action for 1,2,4-TMB-induced hematological and clinical chemistry effects is currently unknown. Increased sorbitol dehydrogenase activity is a marker for hepatic

injury (Ramaiah, 2007) and therefore, underlying hepatotoxicity could explain its increase in exposed males. However, absolute and relative liver weights were not observed to increase with exposure, and microscopic histopathological analysis of the liver did not demonstrate any observable changes. The increases in WBC counts in exposed animals could be secondary to the observed respiratory irritative and inflammatory effects of 1,2,4-TMB exposure (2000; 1997).

In summary, hematological toxicity was observed with exposure to 1,2,4-TMB and 1,3,5-TMB in humans and animals. The information regarding hematological toxicity in humans is limited to one study involving exposure to a complex VOC mixture containing both 1,2,4-TMB and 1,3,5-TMB, but it did report hematological effects (alterations in clotting an anemia) that are roughly analogous to those effects (decreased RBCs and decreased clotting time) observed in rats following subchronic exposure to 1,2,4-TMB. Although both databases are limited (there are no chronic studies in animals), there is some consistency in effects across species (i.e., rats and humans). EPA considered the hematological effects to be biological plausible and analogous to effects that could occur in humans; and concluded that the available evidence for 1,2,4-TMB identified hematological toxicity as a toxicity hazard.

1.1.5. Other Toxicological Effects

One animal study was identified that investigated the association of chronic oral exposure (via gavage) to 1,2,4-TMB and cancer endpoints (Maltoni et al., 1997). Male and female Sprague-Dawley rats were exposed to a single dose of 800 mg/kg-day of 1,2,4-TMB in olive oil by stomach tube for four days/week starting at 7 weeks of age. Exposures were terminated at the end of 104 weeks (i.e. at 111 weeks of age) and the animals were kept under observation until natural death. The authors report that chronic oral exposure to 1,2,4-TMB resulted in an "intermediate" reduction of survival in male rats and a "slight" reduction in females (no quantitative information on survival was reported). A slight increase in total malignant tumors in both sexes of rats was observed, with the incidence of head cancers being specifically increased in male rats. The predominant type of head cancer identified was neuroesthesioepithelioma, which arises from the olfactory neuroepithelium and is normally rare in Sprague-Dawley rats. Other head cancers observed included those in the Zymbal gland, ear duct, and nasal and oral cavities. No tests of statistical significance were reported for these data. When Fisher's exact test was performed (by EPA) on the incidences calculated from the reported percentages of animals

bearing tumors in the control and exposed animals, no statistically significant associations were observed.

Janik-Spiechowicz et al. (1998) investigated the genotoxicity of the trimethylbenzene isomers 1,2,4-TMB, and 1,3,5-TMB by measuring three genotoxic endpoints: mutation frequency in bacteria, micronucleus formation in mice, and sister chromatid exchanges in mice. Neither isomer induced gene mutations in any Salmonella typhimurium strain tested (TA102, TA100, TA98, and TA97a). Both isomers were also negative for the formation of micronuclei in Imp:BALB/c mice following i.p. injection. Males in the high dose groups for 1,2,4-TMB and 1,3,5-TMB exposures had a statistically significant reduction in the ratio of PCEs to NCEs indicating bone marrow cytotoxicity. However, both isomers significantly increased the frequency of sister chromatid exchanges (SCEs) in Imp:BALB/c mice following i.p. injection, with 1,2,4-TMB having the more significant response. These results appeared to have occurred at doses that did not induce significant bone marrow cytotoxicity except at the highest dose.

In summary, although very little genotoxicity data are available on 1,2,4-TMB and 1,3,5-TMB, Janik-Spiechowicz et al. (1998) observed negative results in several key mutagenicity assays, including the Ames mutation assay in Salmonella and in vivo assays for micronucleus formation in mouse bone marrow cells. However, Janik-Spiechowicz et al. (1998) did observe increased incidence of SCE in mice exposed to both TMB isomers. Increased frequency of SCEs indicates that DNA damage has occurred as a result of exposure to these isomers, but it does not provide a specific indication of mutagenic potential, as there is no known mechanistic association between SCE induction and a transmissible genotoxic effect. With only one positive SCE result, and all other data showing negative results for gene and chromosomal mutation in vitro and in vivo, there is not enough evidence to conclude that either isomer is directly genotoxic.

1.1.6. Similarities between 1,2,4-TMB and 1,3,5-TMB regarding observed inhalation and oral toxicity

In the existing toxicological database for 1,2,4-TMB and 1,3,5-TMB, important similarities have been observed in the potency and magnitude of effect resulting from exposure to the two isomers, although some important differences also exist. In acute studies investigating respiratory irritative effects, the RD_{50} of the two isomers were very similar: 2,844 mg/m3 for 1,2,4-TMB and 2,553 mg/m3 for 1,3,5-TMB (Korsak et al., 1997). The similarity regarding toxicity was also observed in acute inhalation neurotoxicity studies: the EC_{50} for decreased coordination, balance, and neuromuscular function was

4,694 mg/m3 for 1,2,4-TMB and 4,738 mg/m3 for 1,3,5-TMB. The EC_{50} for decreased pain sensitivity were also similar for both isomers: 5,683 mg/m3 for 1,2,4-TMB and 5,963 mg/m3 for 1,3,5-TMB (Korsak and Rydzyński, 1996). Neurotoxic endpoints were also similarly affected by oral exposure to individual isomers: increased electrocortical arousal was observed in rats exposed to 240 mg/kg 1,2,4-TMB or 1,3,5-TMB, and altered brain EEGs were observed in rats exposed to 790 mg/kg 1,2,4-TMB or 1,3,5-TMB (Tomas et al., 1999a; Tomas et al., 1999c). Although these effects were seen at the same dose levels in animals exposed to either isomer, these doses were LOAELs, and it is unclear whether this similar potency would be observed at lower doses. Additionally, there were differences in the magnitude of effect between the isomers, with 1,2,4-TMB inducing larger abnormalities in brain EEGs and 1,3,5-TMB affecting electrocortical arousal to a greater degree than 1,2,4-TMB.

In short-term neurotoxicity studies, a similar pattern of effects (inability to learn passive and/or active avoidance and decreased pain sensitivity) indicating altered neurobehavioral function were observed for both isomers (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a). In these studies, 1,3,5-TMB was shown be more toxic than 1,2,4-TMB: rats exposed to 123 mg/m3 1,3,5-TMB displayed significantly decreased abilities to learn passive or active avoidance (Wiaderna et al., 2002), whereas 1,2,4-TMB elicited the inability to learn passive avoidance in rats exposed to 492 mg/m3 1,2,4-TMB and did not affect active avoidance at any exposure concentration (Gralewicz et al., 1997a). Additionally, in animals exposed to either isomer at 492 mg/m3, exposure to 1,3,5-TMB decreased the ability to learn passive avoidance to a greater degree than 1,2,4-TMB (approximately 50% decrease vs. 40%), and the effect of 1,3,5-TMB manifested at earlier time points than 1,2,4-TMB (three vs. seven days) (Gralewicz and Wiaderna, 2001).

Lastly, similarities were observed in 1,2,4-TMB- and 1,3,5-TMB-induced developmental and maternally toxic effects (Saillenfait et al., 2005). Male fetal weights were significantly reduced in animals exposed gestationally to 2,952 mg/m3 1,2,4-TMB (5% decrease) or 1,3,5-TMB (7% decrease). 1,2,4-TMB also significantly decreased female fetal weights by approximately 5% in animals exposed to the same concentration. Although, 1,3,5-TMB significantly reduced female fetal weights by 13% in animals exposed to 5,904 mg/m3, female weights were decreased at 2,952 mg/m3 to a similar magnitude (6%) as animals exposed to the same concentration of 1,2,4-TMB. Maternal toxicity, measured as decreased corrected maternal weight gain, was significantly decreased in animals exposed to 2,952 mg/m3 1,2,4-TMB or 1,3,5-TMB. However, 1,3,5-TMB exposure resulted in a 75%

reduction of maternal weight gain compared to controls, whereas 1,2,4-TMB exposure reduced maternal weight gain by 50%.

1.1.7. Susceptible Populations or Lifestages

Although there are no chemical-specific data that would allow for the definitive identification of susceptible subpopulations, the reduced metabolic and elimination capacities in children relative to adults may be a source of susceptibility (Ginsberg et al., 2004). TMB isomers are metabolized via side-chain oxidation to form alcohols and aromatic carboxylic/mercapturic acids or by hydroxylation to form phenols, which are then conjugated with glucuronic acid, glycine, or sulfates for urinary excretion. The activities of multiple cytochrome P450 (CYP P450) mono-oxygenase isozymes have been shown to be reduced in children up to 1 year of age compared to adult activities (Ginsberg et al., 2004). Additionally, the rate of glucuronidation and sulfation is decreased in children. Therefore, as both CYP P450 mono-oxygenase activities and the rate of glucuronidation and sulfation appear to be decreased in early life, newborns and young infants may experience higher and more persistent blood concentrations of 1,2,4-TMB, 1,3,5-TMB, and/or their respective metabolites compared with adults at similar exposure levels. Reduced renal clearance in children may be another important source of potential susceptibility. TMB isomers and their metabolites are excreted in the urine of exposed laboratory animals and occupationally exposed humans. Data indicating reduced renal clearance for infants up to 2 months of age (Ginsberg et al., 2004) may suggest a potential to affect TMB excretion, thus possibly prolonging its toxic effects. Additionally, those with pre-existing respiratory diseases (e.g., asthma) may be more sensitive to the respiratory irritative and inflammatory effects of TMB isomers.

1.2. Selection of Candidate Principal Studies and Critical Effects for Derivation of Reference Values

1.2.1. Inhalation Exposure – Effects Other Than Cancer – 1,2,4-TMB

While literature exists on the noncancer effects of 1,2,4-TMB exposure in humans, including neurological, respiratory, and hematological toxicities, no human studies are available that would allow for the quantification of subchronic or chronic noncancer effects. The available human studies evaluated TMB exposures occurring as 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, limit their utility in derivation of quantitative human health toxicity values. However, these studies provide supportive evidence for the neurological, respiratory, and hematological toxicity of TMB isomers in humans and determination of coherency of effect in both humans and laboratory animals for deriving toxicity values.

Studies investigating 1,2,4-TMB noncancer effects in experimental animal models were identified in the literature. Acute inhalation studies observing neurotoxicity and respiratory toxicity in exposed rodents were identified, but the high exposure concentrations used and acute exposure duration of these studies limit their applicability for quantitation of chronic human health effects. However, as with the human mixture studies, these studies do provide qualitative information regarding consistency and coherence of effect that is supportive of the development of quantitative human health risk values.

1,2,4-TMB-induced toxicity across several organ systems was observed in three subchronic studies by Korsak et al., (2000; 1997) and Korsak and Rydzyński (1996). Data from these studies were considered as candidate critical effects for the purpose of determining the point of departure (POD) for derivation of the inhalation RfC for 1,2,4-TMB. These studies were determined to be adequate as evaluated using study quality characteristics related to study populations (studies used rats as an appropriate laboratory animal species and utilized appropriate sham-exposed controls), exposure (the purity of 1,2,4-TMB was reported as ≥ 97% pure (impurities not reported), the studies utilized an appropriate route [inhaled air] and duration [subchronic] of exposure, and the studies used a reasonable range of appropriately-spaced dose levels to facilitate dose-response analysis), and data (appropriate latency between exposure and development of toxicological outcomes was used, the persistence of some outcomes after termination of exposure was investigated, adequate numbers of animals per dose group were used, and appropriate statistical tests including pair-wise and trend analyses were performed). When considered together, these subchronic studies examined 1,2,4-TMB-induced toxicity in multiple organ systems including CNS, hematological, and pulmonary effects (Table 1-6).

Table 1-6: Non-cancer endpoints resulting from subchronic inhalation exposure to 1,2,4-TMB considered for the derivation of the RfC

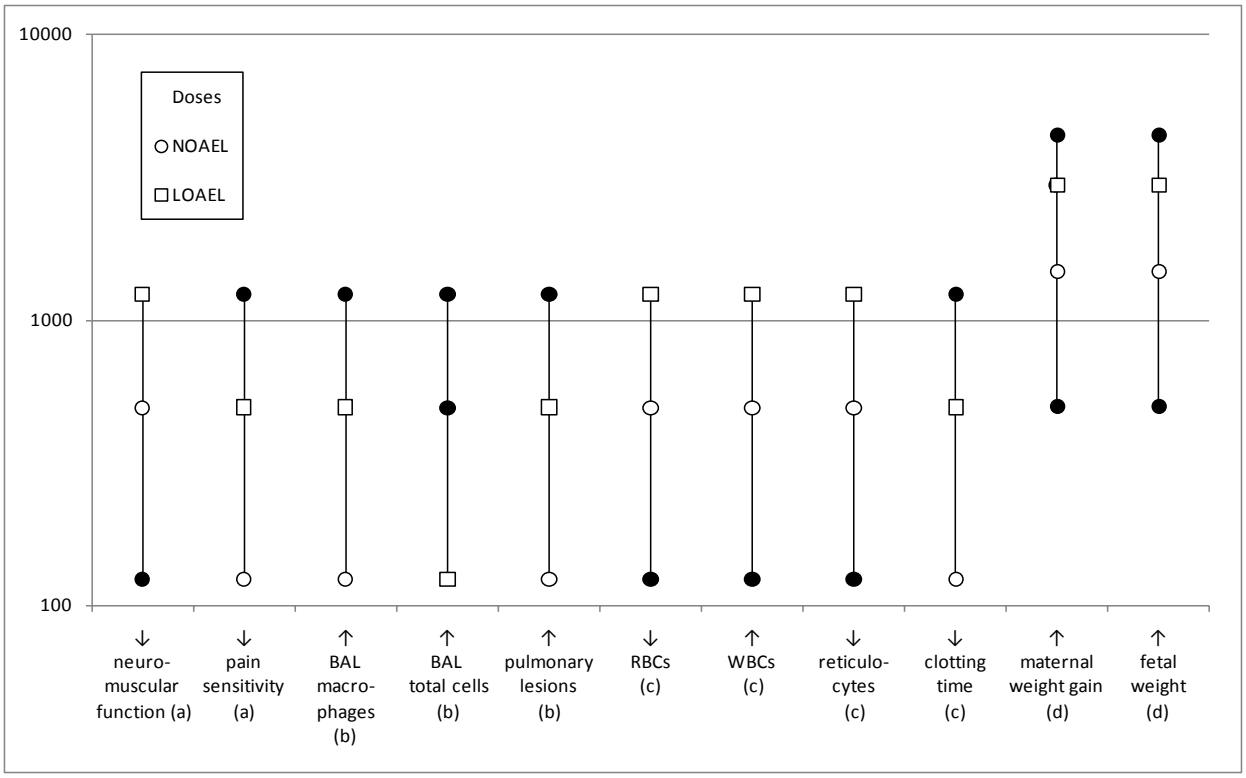
Endpoint	Species/ Sex	Exposure Concentration (mg/m^3)			
		0	123	492	1,230
Neurotoxicological Endpoints					

Decreased pain sensitivity (measured as latency to paw-lick in seconds)	Rat, male	15.4 ± 5.8[a] (n = 9)	18.2 ± 5.7 (n = 10)	27.6 ± 3.2[d] (n = 9)	30.1 ± 7.9[d] (n = 10)
Impaired neuromuscular function and coordination (% failures on rotarod)		0 (n = 10)	10 (n = 10)	20 (n = 10)	40[e] (n = 10)
Hematological Endpoints					
Decreased RBCs (10^6/cm^3)	Rat, male	9.98 ± 1.68 (n = 10)	9.84 ± 1.82 (n = 10)	8.50 ± 1.11 (n = 10)	7.70 + 1.38[d] (n = 10)
Increased WBCs (10^6/cm^3)		8.68 ± 2.89 (n = 10)	8.92 ± 3.44 (n = 10)	8.30 ± 1.84 (n = 10)	15.89 ± 5.74[d] (n = 10)
Decreased reticulocytes (%)	Rat, female	3.5 ± 2.6 (n = 10)	1.7 ± 2.0 (n = 10)	1.8 ± 0.9 (n = 10)	1.0 ± 0.6[c] (n = 20)
Decreased clotting time (seconds)		30 ± 10 (n = 10)	23 ± 4 (n = 10)	19 ± 5[d] (n = 10)	22 ± 7[c] (n = 20)
Pulmonary Endpoints					
Increased BAL macrophages (10^6/cm^3)	Rat, male	1.83 ± 0.03 (n = 6)	3.78 ± 0.8 (n = 6)	4.95 ± 0.2[d] (n = 7)	3.96 ± 0.3[d] (n = 6)
Increased BAL total cells (10^6/cm^3)		1.93 ± 0.79 (n = 6)	5.82 ± 1.32[f] (n = 6)	5.96 ± 2.80[d] (n = 7)	4.45 ± 1.58[c] (n = 7)
Increased inflammatory lung lesions		[b] (n = 10)	[b] (n =10)	[b] (n = 10)	[b] (n = 20)

[a] Values are expressed as mean ± one standard deviation
[b] Incidences for individual dose groups not reported in the study. NOAEL for males identified by EPA was 123 mg/m^3
[c] $p < 0.05$; [d] $p < 0.01$; [e] $p < 0.005$; [f] $p < 0.001$

Adapted from Korsak et al., (2000; 1997) and Korsak and Rydzyński (1996)

Endpoints from these studies that demonstrated statistically significant pair-wise increases or decreases relative to control were considered for the derivation of the RfC for 1,2,4-TMB. These endpoints included decreased neuromuscular function and coordination, and decreased pain sensitivity in male rats (Korsak and Rydzyński, 1996), increased BAL total cells and increased BAL macrophages in male rats (Korsak et al., 1997), and decreased RBCs, increased WBCs, and increased pulmonary inflammatory lesions in male rats and decreased reticulocytes and clotting time in female rats (Korsak et al., 2000). Increases in BAL polymorphonuclear leukocytes and lymphocytes were not considered for RfC derivation due to a lack of reporting at which doses statistically significant increases occurred. Changes in BAL protein and enzyme activity level were not considered due to non-monotonically increasing dose-responses, and increases in sorbitol dehydrogenase were not further considered due to the lack of accompanying hepatocellular histopathological alterations in exposed animals. Endpoints carried forward for derivation of an RfC for 1,2,4-TMB, along with their NOAEL and LOAEL values, are graphically represented in Figure 1-1.



Solid lines represent range of exposure concentrations. (a) Korsak and Rydzyński (1996); (b) Korsak et al. (1997); (c) Korsak et al. (2000); (d) Saillenfait et al. (2005)

Figure 1-1. Exposure response array for inhalation exposure to 1,2,4-TMB.

Although the Saillenfait et al. (2005) study was a well conducted developmental toxicity study, data from this study were not considered for identification of candidate critical effects for 1,2,4-TMB due to the fact that maternal and developmental toxicities were observed at doses 6- to 24-fold higher than the doses that resulted neurological, hematological, and pulmonary effects observed in the subchronic Korsak studies.

1.2.2. Inhalation Exposure – Effects Other Than Cancer – 1,3,5-TMB

No human studies are available that would allow for the quantification of subchronic or chronic noncancer effects resulting from inhalation exposure to 1,3,5-TMB. The available human studies evaluated TMB exposures occurring as complex solvent or VOC mixtures, and this consideration along with similar uncertainties as discussed for 1,2,4-TMB limit their utility in derivation of quantitative human health toxicity values. As for 1,2,4-TMB, the human studies do provide supportive evidence for the neurological toxicity of 1,3,5-TMB in humans and strengthen the determination of consistency and coherency of effect in humans and laboratory animals.

No suitable chronic or subchronic inhalation studies investigating 1,3,5-TMB noncancer effects in experimental animal models were identified in the literature that would support the derivation of the RfC. Two short-term inhalation studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001) investigating neurotoxicity outcomes were identified in the literature and data from these studies were considered as candidate critical effects for the purpose of derivation of an RfC for 1,3,5-TMB in the absence of a suitable chronic or subchronic study. Additionally, one developmental toxicity study was identified in the literature; Saillenfait et al. (2005). Data from these studies were considered as candidate critical effects for the purpose of determining the POD for derivation of the inhalation RfC for 1,3,5-TMB. Based on the noncancer database for 1,3,5-TMB, these studies were determined to be adequate as evaluated using study quality characteristics related to study populations (studies used rats as an appropriate laboratory animal species and utilized appropriate sham-exposed controls), exposure (the purity of 1,3,5-TMB was reported as 99% pure, the studies utilized an appropriate route [inhaled air] duration [short-term and gestational] of exposure (although the duration for short-term studies was not optimal), and the studies used a reasonable range of appropriately-spaced dose levels to facilitate dose-response analysis), and data (appropriate latency between exposure and development of toxicological outcomes was used, the persistence of some outcomes after termination of exposure was investigated, adequate numbers of animals per dose group were used, and appropriate pair-wise statistical tests were performed).

When considered together, these short-term and developmental studies examined 1,3,5-TMB-induced toxicity in multiple organ systems in adult, pregnant, and developing organism. Endpoints from these studies that demonstrated statistically significant pair-wise increases or decreases relative to control were considered for the derivation of the RfC for 1,3,5-TMB. Gralewicz and Wiaderna (2001) and Wiaderna et al. (2002) both indicate altered cognitive function, decreased pain sensitivity, and decreased anxiety and/or increased motor function following inhalation exposure to 1,3,5-TMB (see Table 1-1). Wiaderna et al. (2002) reported that 123 mg/m3 was the LOAEL for altered cognitive function the NOAEL for decreased pain sensitivity. As altered cognitive function was observed at a lower exposure concentration than decreased pain sensitivity, only altered cognitive function was further considered for derivation of an RfC for 1,3,5-TMB from the Wiaderna et al. (2002) study. All three neurotoxic effects (altered cognitive function, decreased pain sensitivity, and decreased anxiety and/or increased motor function) were observed at the only exposure concentration utilized in the Gralewicz and Wiaderna (2001) (i.e., 492 mg/m3); these LOAELs were further considered for derivation of an RfC for 1,3,5-

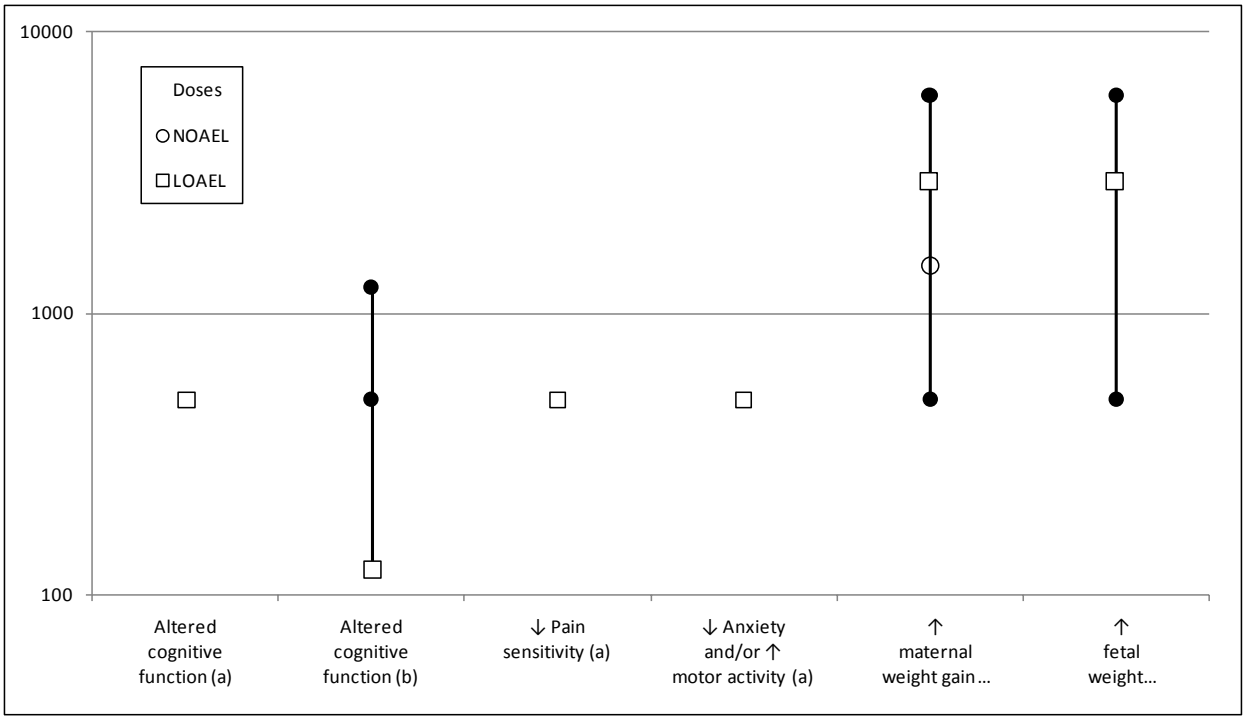
TMB. From the Saillenfait et al. (2005) study, decreased male and female fetal weights and decreased corrected maternal weight gain were considered for derivation of the RfC (Table 1-7). Changes in serum chemistry parameters in rats exposed subchronically to 1,3,5-TMB were not considered for derivation of the RfC due to inconsistent temporal patterns of effect and the lack of accompanying histopathology.

Table 1-7: Non-cancer endpoints resulting from gestational inhalation exposure (GD 6-20) to 1,3,5-TMB considered for the derivation of the RfC

Endpoint	Species/ Sex	Exposure Concentration (mg/m^3)				
		0 (n = 21)[a]	492 (n = 22)	1,476 (n = 21)	2,952 (n = 17)	5,904 (n = 18)
Developmental Endpoints						
Decreased fetal weight (g)	Rat, male	5.80 ± 0.41[b]	5.76 ± 0.27	5.50 ± 0.31	5.39 ± 0.55[c]	5.10 ± 0.57[d]
	Rat, female	5.50 ± 0.32	5.74 ± 0.21	5.27 ± 0.47	5.18 ± 0.68	4.81 ± 0.45[d]
Maternal Endpoints						
Decreased maternal weight gain (g)	Rat, female	29 ± 14	30 ± 9	20 ± 12	7 ± 20[c]	-12 ± 19[d]

[a] Number of dams with live litters, numbers of live fetuses not explicitly reported
[b] Values are expressed as mean ± one standard deviation
[c] $p < 0.05$; [d] $p < 0.01$

Adapted from Saillenfait et al. (2005)



Solid lines represent range of exposure concentrations. (a) Gralewicz and Wiaderna (2001); (b) Gralewicz et al. (1997a); (c) Saillenfait et al. (2005)

Figure 1-2. Exposure response array for inhalation exposure to 1,3,5-TMB.

1.2.3. Oral Exposure – Effects Other Than Cancer – 1,2,4-TMB and 1,3,5-TMB

No human studies are available that would allow for the quantification of subchronic or chronic noncancer effects resulting from oral exposure to either 1,2,4-TMB or 1,3,5-TMB. Additionally, no suitable chronic or subchronic oral studies investigating 1,2,4-TMB or 1,3,5-TMB noncancer effects in experimental animal models were identified in the literature that would support the derivation of an RfD. Although the oral database for 1,2,4-TMB and 1,3,5-TMB are inadequate to support the derivation of an RfD, a PBPK model is available to perform a route-to-route extrapolation (Hissink et al., 2007). The Hissink model was chosen as an appropriate model because it was the only published 1,2,4-TMB model that included parameterization for both rats and humans, the model code was available, and the model adequately predicted experimental data in the dose range of concern. The use of inhalation toxicity data to derive an oral RfD is supported by the 1,2,4-TMB and 1,3,5-TMB database: 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.

Further, no evidence exists that would suggest toxicity profiles would differ to a substantial degree between oral and inhalation exposures.

1.3. Carcinogenicity Analysis

Synthesis and Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (2005a), the database for 1,2,4-TMB and 1,3,5- TMB provides "inadequate information to assess the carcinogenic potential" of these isomers. This characterization is based on the fact that there is no information regarding the carcinogenicity of TMB in humans and that the only animal study on the carcinogenicity of 1,2,4-TMB observed no statistically significant carcinogenic effects. No studies regarding the carcinogenicity of 1,3,5-TMB were identified in the available scientific literature.

Only one animal carcinogenicity study was identified (Maltoni et al., 1997), involving exposure to 1,2,4-TMB by oral gavage. Although an increased incidence of total malignant tumors in both sexes and head cancers (predominately neuroethesioepithelioma) in males was observed in exposed rats, no statistical analyses were reported. When EPA independently performed the Fisher's exact test on the reported data, no statistically significant effects were observed.

2. DOSE-RESPONSE ANALYSIS

2.1. Inhalation Reference Concentration for Effects other than Cancer

2.1.1. Dose-Response Assessment for RfC derivation for 1,2,4-TMB

As discussed in Section 1.2.1, endpoints observed in Korsak et al., (2000; 1997) and Korsak and Rydzyński (1996) that demonstrated statistically significant (at $p < 0.05$ or greater) pair-wise increases or decreases relative to control for at least one dose group were considered for the derivation of the RfC for 1,2,4-TMB; these effects are listed in Table 1-6 above. This assessment used the benchmark dose (BMD) approach, when possible, to estimate a point of departure (POD) for the derivation of an RfC for 1,2,4-TMB (Table 2-1; see Section C.1 of Appendix B (U.S. EPA, 2011c) for detailed methodology). The BMD approach involves fitting a suite of mathematical models to the observed dose-response data using EPA's Benchmark Dose Software (BMDS, version 2.2). Each fitted model estimates aBMD and its associated 95% lower confidence limit (BMDL) corresponding to a selected benchmark response (BMR). For dichotomous data (i.e., impaired neuromuscular function and coordination, measured as % failure on rotarod) from Korsak and Rydzyński (1996), no information is available regarding the change in this response that would be considered biologically significant, and thus a BMR of 10% extra risk was used to model this endpoint, consistent with the Benchmark Dose Technical Guidance (U.S. EPA, 2000a). For continuous data (i.e., decreased pain sensitivity, increased BAL macrophages, decreased RBCs, decreased reticulocytes, and decreased clotting time) from the Korsak and Rydzyński (1996) and Korsak et al. (2000; 1997) studies, no information is available regarding the change in these responses that would be considered biologically significance, 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, 2000a). 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 1,2,4-TMB were not amenable to BMD modeling for a variety of reasons, including equal responses at all exposure groups (e.g., increased BAL total cells), responses only in the high dose group with no significant changes in responses in lower dose groups (e.g., increased WBCs), and

absence of incidence data (e.g., increased inflammatory lung lesions). Additionally, some datasets were deemed adequate for BMD modeling, but no model provided an adequate fit to the data (e.g., increased BAL macrophages), estimated BMDs were greater than the highest exposure concentration (e.g., decreased reticulocytes), or estimated BMDLs were determined to be biologically implausibly low (e.g., decreased clotting time). In these cases, the NOAEL/LOAEL approach was used to identify a POD. Detailed modeling results are provided in Section C.2 of Appendix B (U.S. EPA, 2011c) (detailed modeling results for maternal and fetal endpoints observed in Saillenfait et al. (2005) are provided in Appendix B (U.S. EPA, 2011c) for comparison to endpoints observed in the Korsak et al., (2000; 1997) and Korsak and Rydzyński (1996) studies).

Table 2-1: Summary of dose-response analysis and point of departure estimation for endpoints resulting from subchronic inhalation exposure to 1,2,4-TMB

Reference	Endpoint	Sex/ Species	POD Basis	Best-fit Model; BMR	Candidate POD (mg/m^3)	BW[a] (kg)	POD_{ADJ}[b] (mg/L)
Neurotoxicological Endpoints							
Korsak and Rydzyński (1996)	Decreased pain sensitivity	Male, rat	BMDL	Exponential 4; 1 SD	84.0	0.387	0.085
	Impaired neuromuscular function and coordination	Male, rat	BMDL	Log-logistic; 10% ER	93.9	0.387	0.096
Hematological Endpoints							
Korsak et al. (2000)	Decreased RBCs	Male, rat	BMDL	Exponential 4; 1 SD	174.1	0.390	0.187
	Increased WBCs	Male, rat	NOAEL	n/a	492	0.399	0.867
	Decreased reticulocytes	Female, rat	NOAEL	n/a	492	0.230	0.890
	Decreased clotting time	Female, rat	NOAEL	n/a	123	0.243	0.127
Pulmonary Endpoints							
Korsak et al. (1997)	Increased BAL macrophages	Male, rat	NOAEL	n/a	123	0.383	0.127
	Increased BAL total cells	Male, rat	LOAEL	n/a	123	0.383	0.127
Korsak et al. (2000)	Increased inflammatory lung lesions	Male and female, rat	NOAEL	n/a	123	0.390	0.127

[a] Group specific mean body weight (BW) reported in Korsak et al. (2000; Korsak et al., 1997). For endpoints from these studies using a NOAEL or LOAEL for the POD, the reported group specific mean BW for that dose group was used in PBPK POD_{ADJ} calculations. For decreased RBCs from Korsak et al. (2000), the group specific mean BW for the dose group closest to the BMDL was used. For decreased pain sensitivity and coordination, balance, and neuromuscular function from Korsak and Rydzyński (1996), the average of the group specific mean BWs from Korsak et al. (2000; Korsak et al., 1997) for the dose group closest to the BMDL was used.
[b] Weekly average venous blood TMB concentration (mg/L) estimated for a rat exposed to the corresponding candidate POD for 6 h/day, 5 d/wk. See Appendix A (U.S. EPA, 2011d) for details on PBPK modeling.

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. For 1,2,4-TMB, a PBPK model (Hissink et al., 2007) was employed to make this adjustment. This PBPK model (described in Appendix A (U.S. EPA, 2011d); Section A.1) was used to estimate the steady-state weekly average venous blood concentration (mg/L) of 1,2,4-TMB for rats exposed to 1,2,4-TMB for 6 h/d, 5 d/wk. For each exposure concentration, once the model reached steady-state, the resulting weekly average venous blood concentration (mg/L) of 1,2,4-TMB was employed as the dose metric for these endpoints. This dose metric was considered the duration-adjusted POD (POD_{ADJ}) for each candidate critical effect (Table 2-1).

2.1.2. RfC Derivation for 1,2,4-TMB

For the derivation of an RfC based upon an animal study, the calculated POD_{ADJ} values are further adjusted to reflect the human equivalent concentration (HEC) (Table 2-2).

Table 2-2: Candidate POD_{ADJ} values, human equivalent concentrations (HECs), and applied uncertainty factors used in the derivation of RfCs for 1,2,4-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}	
Neurotoxicological Endpoints										
Korsak and Rydzyński (1996)	Decreased pain sensitivity	0.085	15.6	3	10	1	10	3	1,000	1.56×10^{-2}
	Impaired neuromuscular function and coordination	0.096	17.6	3	10	1	10	3	1,000	1.76×10^{-2}
Hematological Endpoints										
Korsak et al. (2000)	Decreased RBCs	0.187	33.7	3	10	1	10	3	1,000	3.37×10^{-2}
	Increased WBCs	0.867	131.5	3	10	1	10	3	1,000	1.31×10^{-1}
	Decreased reticulocytes	0.890	134.0	3	10	1	10	3	1,000	1.34×10^{-1}

	Decreased clotting time	0.127	23.2	3	10	1	10	3	1,000	2.32×10^{-2}
Pulmonary Endpoints										
Korsak et al. (1997)	Increased BAL macrophages	0.127	23.2	3	10	1	10	3	1,000	2.32×10^{-2}
	Increased BAL total cells	0.127	23.2	3	10	10	10	3	10,000	n/a[c]
Korsak et al. (2000)	Increased inflammatory lung lesions	0.127	23.2	3	10	1	10	3	1,000	2.32×10^{-2}

[a] Human equivalent concentration
[b] As calculated by application of uncertainty factors, not rounded.
[c] Endpoint excluded for further consideration due to a UF_{TOTAL} of 10,000. The 2002 report, "A Review of the Reference Dose and Reference Concentration Processes" (U.S. EPA, 2002) recommends a maximum total UF of 3000 for derivation of an RfC.

The HEC was derived using a human PBPK model (Hissink et al., 2007) to account for interspecies differences in toxicokinetics. The human PBPK model was run (as described in Appendix A (U.S. EPA, 2011d)), assuming a continuous (24 h/day, 7 day/week) exposure, to estimate a human PODHEC that would result from the same weekly average venous blood concentration reflected in the PODADJ in animals (Table 2-2).

Neurotoxicity is the most consistently observed endpoint in the toxicological database for 1,2,4-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). The observation of decreased pain sensitivity was observed in multiple studies across multiple exposure durations (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996; Korsak et al., 1995), and in the presence of other metrics of altered neurobehavior, including impaired neuromuscular function and coordination and altered cognitive function. Additionally, neurotoxicological endpoints (hand tremble, weakness) are observed in human worker populations exposed to complex VOC mixtures containing 1,2,4-TMB. In consideration of the recommendations in the U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (1998) and given the consistency of effect across independent studies, multiple durations of exposure in animal studies, the consistency of observed neurotoxicity in animals and humans, the EPA concluded that neurotoxicity represents strong evidence of toxicity hazard and that decreased pain sensitivity is an adverse effect, and the most appropriate effect on which to base the RfC. Therefore, decreased pain sensitivity was selected as the

critical effect and Korsak and Rydzyński (1996) as the principal study for the 1,2,4-TMB RfC.

A PODHEC of 15.6 mg/m3 for decreased pain sensitivity (Korsak and Rydzyński, 1996) was used as the POD to derive the chronic RfC for 1,2,4-TMB. The uncertainty factors, selected based on 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 total UF of 1,000. This composite uncertainty factor 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 1,2,4-TMB. In this assessment, the use of a PBPK model to convert internal doses in rats to administered doses in humans reduces toxicokinetic uncertainty in extrapolating from the rat to humans, but does not account for interspecies differences due to toxicodynamics. A default UF_A of 3 was thus applied to account for this remaining toxicodynamic 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 1,2,4-TMB. No information is currently available to predict potential variability in human susceptibility, including variability in the expression of enzymes involved in 1,2,4-TMB metabolism. Due to this lack of data on variability within the human population, a default 10-fold UF_H was applied.

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 minimally, 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.

A database uncertainty factor, UF_D, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for database deficiencies due to the lack of a multi-generation reproductive toxicity study. The database contains three subchronic studies that are well-designed and observe exposure-response effects in multiple organ systems in rats exposed to 1,2,4-TMB via inhalation (nervous, hematological, and pulmonary systems). The database additionally

contains a well-designed developmental toxicity study that investigated standard measures of maternal and fetal toxicity in a different strain of rat. Although there is no information regarding the potential for developmental neurotoxicity and the critical effect for the RfC is altered CNS function, this raises concern regarding possible neurodevelopmental effects of 1,2,4-TMB exposure. However, in the absence of information regarding the magnitude of transfer of 1,2,4-TMB or its metabolites across the placenta, and given the observation that other developmental effects occur at concentrations 6- to 24-fold greater than those eliciting neurotoxicity in adult animals, it may be unlikely that neurodevelopmental data would result in a lower RfC.

Application of this 1000-fold composite UF to the PODHEC yields the following chronic RfC for 1,2,4-TMB:

$RfC = POD_{HEC} \div UF = 15.6\ mg/m^3 \div 1{,}000 = 0.02\ mg/m^3 = 2 \times 10^{-2}\ mg/m^3$ (rounded to one significant digit)

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, Korsak and Rydzyński (1996) is medium. The study is a well-conducted peer-reviewed study that utilized three dose groups plus untreated controls, an appropriate number of animals per dose group, and performed statistical analyses. The critical effect on which the RfC is based is well-supported as the weight of evidence for 1,2,4-TMB-induced neurotoxicity is coherent across multiple animals species (i.e., human, mouse, and rat) and consistent across multiple exposure durations (i.e., acute, short-term, and sub-chronic) (Gralewicz and Wiaderna, 2001; Chen et al., 1999; Wiaderna et al., 1998; Gralewicz et al., 1997a; Gralewicz et al., 1997b; Korsak and Rydzyński, 1996; Norseth et al., 1991). Confidence in the database for 1,2,4-TMB is low to medium as the database includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. The database lacks a chronic and multigenerational reproductive study, and the studies supporting the critical effect predominately come from the same research institute. Overall confidence in the RfC for 1,2,4-TMB is low to medium.

2.1.3. Comparison of Candidate RfCs for 1,2,4-TMB

The predominant noncancer effect observed following acute, short-term, and subchronic inhalation exposures to 1,2,4-TMB is neurotoxicity, although respiratory

toxicity is also observed following acute and subchronic exposures, while hematological effects are observed after subchronic exposures. Figure 2-1 provides a graphical display of all of the candidate PODs and RfCs derived from the three subchronic studies considered in the selection of the final POD for the inhalation RfC.

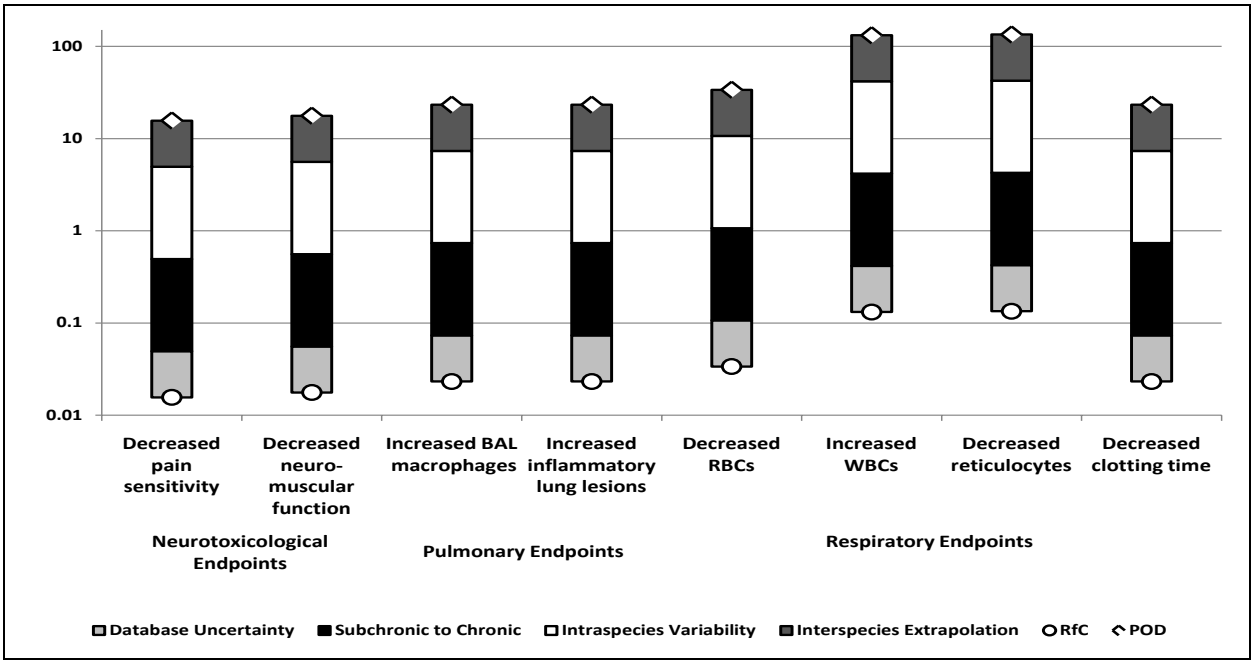


Figure 2-1: Array of candidate POD_{HEC} values with applied UFs and candidate RfCs for CNS, hematological, and pulmonary effects resulting from inhalation exposure to 1,2,4-TMB

2.1.4. Uncertainties in the Derivation of the RfC for 1,2,4-TMB

As presented above, the UF approach following EPA practices and RfC guidance (U.S. EPA, 2002, 1994), was applied to the PODHEC in order to derive the chronic RfC for 1,2,4-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 uncertainty into the RfC calculation as selection of alternative CNS, hematological, or pulmonary effects would result in an equivalent RfCs (i.e., 2 × 10-2 mg/m3, see Figure 2-1). Some uncertainty does exist regarding the selection of the BMRs for use in BMD modeling, but selection of 10% extra risk for dichotomous endpoints and 1 SD for continuous endpoints is supported by current EPA guidance (U.S. EPA, 2000a). Uncertainty regarding

the selection of particular models for individual endpoints does exist as selection of alternative models could decrease or increase the RfC. However, the best-fit model is the most appropriate for RfC derivation based on current EPA guidance (U.S. EPA, 2000a). Uncertainty may exist in the PBPK model estimates of internal blood dose metrics for the rat, and subsequent HEC calculations for the human, including parameter uncertainty, but such uncertainties would apply equally to all endpoints. Lastly, the extent of inter-individual variation of 1,2,4-TMB metabolism in humans and potential susceptible subpopulations have not been well characterized, and thus these two considerations remain sources of some uncertainty in the current assessment.

2.1.5. Dose-Response Assessment for RfC derivation for 1,3,5-TMB

As discussed above in Section 1.2.2, endpoints observed in Saillenfait et al. (2005) that demonstrated statistically significant (at $p < 0.05$ or greater) pair-wise increases or decreases relative to control for at least one dose group were considered for the derivation of the RfC for 1,3,5-TMB; these effects are listed in Table 1-7. Additionally, altered cognitive function, decreased pain sensitivity, and decreased anxiety and/or increased motor function observed in Gralewicz et al. (2001) and Wiaderna et al. (2002) were also considered as the basis for the derivation of the RfC for 1,3,5-TMB. This assessment used the BMD approach, when possible, to estimate a POD for the derivation of an RfC for 1,3,5-TMB (Table 2-4; see Section C.1 of Appendix B (U.S. EPA, 2011c) 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 male and female fetal weight) from the Saillenfait et al. (2005) study, a BMR equal to 5% relative deviance from the control mean was used. A decrease in body weight of 10% is generally assumed to be a minimally biologically significant response in adult animals. Because the developing organism may be more sensitive to decreases in body weight, a 5% decrease in fetal body weight relative to control was assumed to be a minimally biologically significant response for the fetuses in the Saillenfait et al. (2005) study. As recommended by EPA's Benchmark Dose Technical Guidance (2000a), a BMR equal to a change in the mean of 1 standard deviation of the model estimated control mean was also used in modeling the fetal endpoints for comparison purposes. No information is available regarding the magnitude of response that would be considered biologically significant for decreased maternal weight gain. 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 this endpoint. 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. The data for neurotoxicity (i.e., altered cognitive function, decreased pain sensitivity, and decreased anxiety and/or increased motor function) for 1,3,5-TMB were not amenable to BMD modeling. Gralewicz and Wiaderna (2001) only employed one exposure concentration when investigating the neurotoxic effects of 1,3,5-TMB following short-term inhalation exposures. Thus, the observed neurotoxic effects in this study were not amenable to modeling according to EPA guidance (2000a). For altered cognitive function (as measured as decreased passive and active avoidance) reported in Wiaderna et al. (2002), responses were observed to be equal in all exposure groups. Therefore, for the neurotoxic effects observed in Gralewicz and Wiaderna (2001) and Wiaderna et al. (2002) the NOAEL/LOAEL approach was used to determine a POD. In the Saillenfait et al. (2005) study, although decreased fetal body weight in females was considered appropriate for BMD modeling, BMDS was unable to adequately model the variance in response for this endpoint. Therefore, the NOAEL/LOAEL approach was also used in this case to identify a POD. Detailed modeling results are provided in Section B.2 of Appendix B (U.S. EPA, 2011c).

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 Gralewicz and Wiaderna (2001) and Wiaderna et al. (2002) studies, rats were exposed to 1,3,5-TMB for 6 hours/day, 5 days/week for 4 weeks. Because no PBPK model exists for 1,3,5-TMB, the duration-adjusted PODs for neurobehavioral 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 altered cognitive function from Gralewicz and Wiaderna (2001), the PODADJ would be calculated as follows:

POD_{ADJ} (mg/m^3) = 492 mg/m^3× 6 hours/24 hours × 5 days/7 days

POD_{ADJ} (mg/m^3) = 87.9 mg/m^3

In the Saillenfait et al. (2005) study, rats were exposed to 1,3,5-TMB for 6 hours/day for 15 consecutive days (GDs 6-20). Therefore, the duration-adjusted PODs for developmental/ maternal effects were calculated as follows:

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

For example, for decreased fetal weight in males, the PODADJ would be calculated as follows:

POD_{ADJ} (mg/m³) = 1649 mg/m³ × 6 hours/24 hours

POD_{ADJ} (mg/m³) = 412.0 mg/m³

The calculated PODADJ (mg/m3) values for all neurotoxicity and developmental endpoints considered for RfC derivation are presented in Table 2-4.

Table 2-4: Duration adjusted point of departure (POD_{ADJ}) estimates from short-term and gestational inhalation exposures to 1,3,5-TMB

Reference	Endpoint	Sex/ Species	POD Basis	Best-fit Model; BMR	Candidate POD (mg/m³)	POD_{ADJ} (mg/L) [a]
Neurotoxicological Endpoints						
Gralewicz and Wiaderna (2001)	Altered cognitive function	Male, rat	LOAEL	n/a	492	87.9
	Decreased pain sensitivity	Male, rat	LOAEL	n/a	492	87.9
	Decreased anxiety and/or increased motor function	Male, rat	LOAEL	n/a	492	87.9
Wiaderna et al. (2002)	Altered cognitive function	Male, rat	LOAEL	n/a	123	22.0
Developmental Endpoints						
Saillenfait et al. (2005)	Decreased fetal body weight	Male, rat	BMDL[c]	Exponential 2; 5% RD	1,649	412.0
		Female, rat	NOAEL[c]	n/a	2,952	738.0
Maternal Endpoints						
Saillenfait et al. (2005)	Decreased maternal weight body gain	Female, rat	BMDL	Power, 1 SD	1,303	326.0

[a] Duration adjusted POD_{ADJ} (mg/m³) = POD × (6 hours/24 hours) for developmental/maternal endpoints, or POD × (6 hours/24 hours) × (5 days/week) in accordance with EPA policy (U.S. EPA, 2002)

2.1.6. RfC Derivation for 1,3,5-TMB

Because the selected endpoints for consideration as the critical effect (altered cognitive function, decreased pain sensitivity, decreased anxiety and/or increased motor function, decreased fetal body weight, and maternal body weight gain) are assumed to result primarily from systemic distribution of 1,3,5-TMB, and no available PBPK model exists for 1,3,5-TMB, the human equivalent concentration for 1,3,5-TMB was calculated by the application of the appropriate 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,

1994). 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) (U.S. EPA, 1994). For gases with systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

DAF = (Hb/g)A/(Hb/b)H

DAF = 55.7/43

DAF = 1.3

where:

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

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

In cases where the animal blood:air partition coefficient is higher than the human value (Meulenberg and Vijverberg, 2000; Järnberg and Johanson, 1995), resulting in a DAF > 1, a default value of 1 is substituted (U.S. EPA, 1994). For example, the HEC for altered CNS function (reported in Wiaderna et al. (2002) is calculated as follows:

$POD_{HEC} = POD_{ADJ}$ (mg/m³) × DAF

$POD_{HEC} = POD_{ADJ}$ (mg/m³) × 1.0

POD_{HEC} = 22 mg/m³ × 1.0

POD_{HEC} = 22 mg/m³

Table 2-5 presents the derivation of candidate RfCs from the selected short-term and developmental toxicity studies (Saillenfait et al., 2005; Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001).

Table 2-5: Candidate POD_{ADJ} values, human equivalent concentrations (HECs), and applied uncertainty factors used in the derivation of RfCs for 1,3,5-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}	
Neurotoxicological Endpoints										
Gralewicz and Wiaderna (2001)	Altered cognitive function	87.9	87.9	3	10	10	10	3	10,000	n/a[c]
	Decreased pain sensitivity	87.9	87.9	3	10	10	10	3	10,000	n/a[c]
	Decreased anxiety and/or increased motor function	87.9	87.9	3	10	10	10	3	10,000	n/a[c]
Wiaderna et al. (2002)	Altered cognitive function	22.0	22.0	3	10	10	10	3	10,000	n/a[c]
Developmental Endpoints										
Saillenfait et al. (2005)	Decreased fetal body weight, male	412.0	412.0	3	10	1	1	3	100	4.12
	Decreased fetal body weight, female	738.0	738.0	3	10	1	1	3	100	7.38
Maternal Endpoints										
Saillenfait et al. (2005)	Decreased maternal weight body gain	326.0	326.0	3	10	1	10	3	1,000	3.26×10^{-1}

[a] Human equivalent concentration
[b] As calculated by application of uncertainty factors, not rounded.
[c] Endpoint excluded for further consideration due to a UF_{TOTAL} of 10,000. The 2002 report "A Review of the Reference Dose and Reference Concentration Processes" (U.S. EPA, 2002) recommends a maximum total UF of 3000 for derivation of an RfC.

The magnitude of the total uncertainty factors associated with the neurotoxicological endpoints from Gralewicz and Wiaderna (2001) and Wiaderna et al. (2002) indicate that these endpoints cannot support the derivation of an RfC for 1,3,5-TMB. The composite UF for 1,3,5-TMB for the neurotoxicological endpoints from Gralewicz and Wiaderna (2001) and Wiaderna et al. (2002) would be 10,000. In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) the RfD/RfC Technical Panel concluded that, in cases where maximum uncertainty exists in four or more areas of uncertainty, or when the total uncertainty factor is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Therefore, consistent with the recommendations in U.S. EPA (2002), the available neurotoxicity data following short-term

inhalation exposure to 1,3,5-TMB were considered insufficient to support reference value derivation and an RfC for 1,3,5-TMB was not derived based on these data.

Of the remaining effects considered for derivation of the RfC, decreased maternal weight gain (PODHEC = 326.0 mg/m3) was identified as the most sensitive endpoint. A PODHEC of 326.0 mg/m3 for decreased maternal weight gain by Saillenfait et al. (2005) was used as the PODHEC to derive a candidate chronic RfC for 1,3,5-TMB as shown in Table 2-5. The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (2002), address five areas of uncertainty resulting in a total UF of 1000. This composite uncertainty factor 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 1,3,5-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 1,3,5-TMB than rats due to toxicodynamic differences. A default UF_A of 3 was thus applied to account for this remaining toxicodynamic 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 1,3,5-TMB. No information is currently available to predict potential variability in human susceptibility, including variability in the expression of enzymes involved in 1,3,5-TMB metabolism. Due to this lack of data on variability within the human population, a default 10-fold UF_H is applied.

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 of 1 standard deviation of the model estimated control mean for decreased maternal body weight gain was selected under an assumption that this BMR level represents a minimally, 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.

A database uncertainty factor, UF_D, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for database deficiencies due to the lack of a multi-generation reproductive toxicity

study. The database contains two short-term studies that are well-designed and observe exposure-response effects in the central nervous system of exposed rats. The database additionally contains a well-designed developmental toxicity study that investigated standard measures of maternal and fetal toxicity in a different strain of rat. A limitation of the database is the lack of any information regarding the potential for developmental neurotoxicity. As altered neurobehavioral function is observed in rats exposed to 1,3,5-TMB (manifested as decreased ability to learn passive and active avoidance and decreased pain sensitivity), this raises concern for neurodevelopmental effects of 1,3,5-TMB exposure. However, in the absence of information regarding the magnitude of transfer of 1,3,5-TMB or its metabolites across the placenta, it may be unlikely that neurodevelopmental data would result in a lower RfC.

Application of this 1000-fold composite UF yields the calculation of the chronic RfC for 1,3,5-TMB as follows:

$RfC = POD_{HEC} \div UF = 326\ mg/m^3 \div 1000 = 0.326\ mg/m^3 = 3 \times 10^{-1}\ mg/m^3$ (rounded to one significant digit)

While Saillenfait et al. (2005) is a well-conducted developmental toxicity study that utilizes appropriate study design, group sizes, and statistics, and investigates a wide range of fetal and maternal endpoints resulting from 1,3,5-TMB inhalation exposure, a number of additional factors lessens its suitability with which to derive the RfC for 1,3,5-TMB. First, although maternal and fetal toxicities were observed in this study, it is important to note that the candidate RfC for 1,3,5-TMB derived based on the critical effect of decreased corrected (for gravid uterine weight) maternal body weight gain is 15-fold higher than the RfC derived for 1,2,4-TMB (based on altered CNS function measured as decreased pain sensitivity). As discussed in Section 1.1, the available toxicological database for 1,2,4-TMB and 1,3,5-TMB, across all exposure durations, indicates there are important similarities in the two isomer's toxicity that are supportive of not deriving an RfC for 1,3,5-TMB that is substantially greater than the RfC value derived for 1,2,4-TMB.

In acute studies investigating the respiratory irritative effects of the two isomers, the RD50 of 1,2,4-TMB and 1,3,5-TMB were observed to be very similar: 2,844 and 2,553 mg/m3, respectively (Korsak et al., 1997). This similarity regarding toxicity was also observed in acute neurotoxicity studies: the EC50 for decreased coordination, balance, and neuromuscular function (i.e., performance on the rotarod) was 4,694 mg/m3 for 1,2,4-TMB and 4,738 mg/m3 for 1,3,5-TMB. The EC_{50} for decreased pain sensitivity (i.e., latency to paw-lick measured on the hot plate apparatus) were also similar for both isomers: 5,683

mg/m3 for 1,2,4-TMB and 5,963 mg/m3 for 1,3,5-TMB (Korsak and Rydzyński, 1996). Other neurotoxic endpoints similarly affected by either isomer (albeit from oral exposures or i.p. injections) included increased electrocortical arousal and altered EEG function (Tomas et al., 1999a; Tomas et al., 1999c). However, the doses eliciting these effects were LOAELs, and therefore it is unclear whether this represents true similarity in toxic potency or whether testing at lower doses would reveal differences between the two isomers. Additionally, the magnitude of effect differed between isomers, with 1,2,4-TMB and 1,3,5-TMB inducing greater changes in brain EEGs and electrocortical arousal, respectively.

In short-term neurotoxicity studies, a similar pattern of effects (inability to learn passive or active avoidance, decreased pain sensitivity, increased spontaneous motor activity) indicating altered neurobehavioral function was observed in rats exposed to either isomer (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a). In these studies, 1,3,5-TMB was shown to be more toxic that 1,2,4-TMB, with neurobehavioral effects occurring at lower exposures (123 vs. 492 mg/m3) in animals exposed to 1,3,5-TMB vs. those exposed to 1,2,4-TMB. When comparing the magnitude of TMB isomer-induced neurotoxicity, exposure to 492 mg/m3 1,3,5-TMB induced greater decrements in avoidance acquisition (50% vs. 40%) compared to exposure to the same concentration of 1,2,4-TMB. Lastly, manifestation of neurotoxicity occurred at earlier time points (three vs. seven days) in rats exposed to 1,3,5-TMB compared to those exposed to 1,2,4-TMB.

Finally, the observed developmental effects observed in Saillenfait et al. (2005) were shown to be similar between isomers. Exposure to 1,2,4-TMB and 1,3,5-TMB significantly decreased male fetal body weights to a similar degree (5% and 7%, respectively) at 2952 mg/m3. 1,2,4-TMB and 1,3,5-TMB also decreased female body weights to a similar degree (5% and 6%, respectively) at the same exposure concentration. This body weight decrease was significant in animals exposed to 1,2,4-TMB, but was not significant in those females exposed to 1,3,5-TMB. 1,3,5-TMB was observed to be more toxic with regard to maternal toxicity, inducing a 75% reduction in maternal weight gain at 2952 mg/m3 compared to a 50% reduction in animals exposed to the same concentration of 1,2,4-TMB.

The two isomers are similar to one another in their chemical and toxicokinetic properties, although important differences do also exist. Both isomers have very similar Log KOW values, and blood:air partition coefficients reported for humans and rats in the literature are similar between isomers: 43.0 for 1,2,4-TMB and 59.1 for 1,3,5-TMB. This gives the strong indication that the two isomers would partition into the blood in a similar fashion. Supporting this is the observation that 1,2,4-TMB and 1,3,5-TMB absorb equally

into the bloodstream of exposed humans (6.5 and 6.2 µM, respectively) (Järnberg et al., 1996). Also, the net respiratory uptake of 1,2,4-TMB and 1,3,5-TMB was similar in humans, and the respiratory uptake for 1,2,4-TMB was similar between humans and rats (Järnberg et al., 1996; Dahl et al., 1988). Distribution of the two isomers throughout the body is qualitatively similar, although it appears that liver and kidney concentrations for 1,2,4-TMB were greater than those for 1,3,5-TMB in both acute and short-term exposures (Swiercz et al., 2006; Swiercz et al., 2003; Swiercz et al., 2002). Although 1,2,4-TMB was observed to distribute to the brain (Swiercz et al., 2003; Eide and Zahlsen, 1996), distribution of 1,3,5-TMB to the brain was not experimentally measured in any study. However, the predicted brain:air partition coefficient was similar between 1,2,4-TMB and 1,3,5-TMB for both humans (206 vs. 199) and rats (552 vs. 535) (Meulenberg and Vijverberg, 2000). This strongly suggests that 1,2,4-TMB and 1,3,5-TMB can be expected to distribute similarly to the brain in both humans and rats. Both isomers were observed to primarily metabolize to benzoic and hippuric acids in humans and rats (Järnberg et al., 1996; Huo et al., 1989; Mikulski and Wiglusz, 1975), although the amount of inhaled TMB recovered as hippuric acid metabolites following exposure to 1,2,4-TMB or 1,3,5-TMB was somewhat dissimilar in humans (22% vs. 3%, respectively) and rats (24-38% vs. 59%, respectively) (Järnberg et al., 1996; Mikulski and Wiglusz, 1975). Other terminal metabolites included mercapturic acids (~14-19% total dose), phenols (~12% total dose), and glucuronides and sulphuric acid conjugates (4-9% total dose) for 1,2,4-TMB and phenols (~4-8% total dose) and glucuronides and sulphuric acid conjugates (~5-9% total dose) for 1,3,5-TMB (Tsujimoto et al., 2005; Tsujimoto et al., 2000; Huo et al., 1989; Wiglusz, 1979; Mikulski and Wiglusz, 1975). In humans, the half-lives of elimination from blood were observed to be greater for 1,3,5-TMB (1.7 minutes, 29 minutes, 4.9 hours, and 120 hours) than for 1,2,4-TMB (1.3 minutes, 21 minutes, 3.6 hours, and 87 hours) (Järnberg et al., 1997a; Järnberg et al., 1997b; Järnberg et al., 1996), although this difference may be due to small sample sizes and difficulties in measuring slow elimination phases rather than a true difference in half-lives. At low exposure concentrations, half-lives in elimination from the blood were somewhat similar for 1,2,4-TMB and 1,3,5-TMB (3.6 vs. 2.7 hours), but this difference became much greater with increasing doses (17.3 hours for 1,2,4-TMB and 4 hours for 1,3,5-TMB following exposure to 1230 mg/m3 for six hours) (Swiercz et al., 2003; Swiercz et al., 2002).

Given the above information regarding the observed toxicity following 1,2,4-TMB and 1,3,5-TMB exposures across acute, short-term, and developmental studies, the use of 1,3,5-TMB-specific data for derivation of an RfC was not considered by EPA to be scientifically supported. Derivation of an RfC for 1,3,5-TMB using the only adequate

toxicity data available (i.e., Saillenfait et al. (2005)) would result in an RfC 15-fold higher than the RfC derived for 1,2,4-TMB based on altered CNS function (i.e., decreased pain sensitivity). The available toxicity data indicates that 1,2,4-TMB and 1,3,5-TMB are similar in acute respiratory and neurological toxicity and developmental toxicity, but that 1,3,5-TMB appears to be more potent in eliciting neurotoxicity and maternal toxicity following short-term exposures. 1,3,5-TMB is observed to elicit neurotoxic effects in rats in acute and short-term studies, and therefore the selected critical effect for 1,2,4-TMB, altered CNS function, is relevant to observed 1,3,5-TMB-induced toxicity. Similarities in blood:air partition coefficients, respiratory uptake, and absorption into the bloodstream between the two isomers support the conclusion that internal blood dose metrics for 1,3,5-TMB would be similar as those calculated for 1,2,4-TMB using the available PBPK model.

Thus, the chronic RfC of 2 × 10-2 mg/m3 derived for 1,2,4-TMB was adopted as the RfC for 1,3,5-TMB based on the conclusion that the two isomers were sufficiently similar regarding chemical properties, kinetics, and toxicity.

As noted previously, 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 EPA (1994), Section 4.3.9.2. The chronic RfC of 2 × 10-2 mg/m3 derived for 1,2,4-TMB was adopted as the RfC for 1,3,5-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 developmental toxicity studies in rats and mice. The database lacks a chronic, subchronic, and multigenerational reproductive study. Additionally, the studies supporting the critical effect predominately come from the same research institute. Overall confidence in the RfC for 1,3,5-TMB is low due to uncertainties surrounding the adoption of the RfC derived for 1,2,4-TMB as the RfC for 1,3,5-TMB.

2.1.7. *Uncertainties in the Derivation of the RfC for 1,3,5-TMB*

Uncertainties exist in adopting the RfC derived for 1,2,4-TMB based on altered CNS function (i.e., decreased pain sensitivity) as the RfC for 1,3,5-TMB. The available database for 1,3,5-TMB was considered insufficient with which to derive an RfC. If the most sensitive endpoint from the only adequate study in the 1,3,5-TMB database (i.e., decreased maternal weight gain; Saillenfait et al. (2005)), was used for the RfC derivation, an RfC 15-fold higher would be derived for 1,3,5-TMB vs. that derived for 1,2,4-TMB (3 × 10-1 vs. 2 × 10-2 mg/m3, respectively). Although uncertainty exists in adopting the 1,2,4-TMB RfC

value for 1,3,5-TMB, both isomers share multiple commonalities and similarities regarding their toxicokinetic and toxicological properties that support the adoption of the value of one isomer for the other. The majority of uncertainty regarding 1,3,5-TMB's database involves the lack of a chronic , subchronic, or multi-generational reproductive study for this isomer. Given the similarities in toxicity from the available developmental toxicity study, and neurotoxicity and respiratory toxicity observed in the available acute and short-term studies, there is strong evidence that the two isomer's toxicity resulting from subchronic exposure can be expected to be similar. More so, 1,3,5-TMB may actually be expected to be slightly more toxic than 1,2,4-TMB following subchronic exposures than 1,2,4-TMB given the observation of greater magnitude and earlier onset of effect following 1,3,5-TMB exposures in short-term studies. Therefore, while uncertainty does exist in the derivation of 1,3,5-TMB's RfC, the available information regarding sufficient toxicokinetic and toxicological similarity between the two isomers indicates this uncertainty does not preclude adopting the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB.

2.2. Oral Reference Dose for Effects other than Cancer

2.2.1. Methods of analysis for RfD derivation for 1,2,4-TMB

No chronic or subchronic studies were identified for 1,2,4-TMB that utilized the oral route of exposure. Therefore, the available oral database for 1,2,4-TMB is minimal as defined by EPA guidance (i.e., there is no human data available nor any adequate oral animal data) (U.S. EPA, 2002), and this database is inadequate for the derivation of an RfD.

Even though the available oral database for 1,2,4-TMB is inadequate to derive an RfD, a route-to-route extrapolation from inhalation to oral for the purposes of deriving an RfD is possible using the existing inhalation data and the available 1,2,4-TMB PBPK model (Hissink et al., 2007). Using route-to-route extrapolation via application of PBPK models is supported by EPA guidance (U.S. EPA, 2002, 1994) given enough data and ability to interpret that data regarding differential metabolism and toxicity between different routes of exposure. 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 (Appendix A (U.S. EPA, 2011d)). Further, no evidence exists that would suggest toxicity profiles would differ to a substantial degree between oral and inhalation exposures.

Therefore, assuming oral exposure would result in the same systemic effect as inhalation exposure (altered CNS function, measured as decreased pain sensitivity (Korsak and Rydzyński, 1996), an oral exposure component was added to the Hissink et al. (2007) PBPK model by EPA (Appendix A (U.S. EPA, 2011d); Section A.1.4), assuming continuous oral ingestion and 100% of the ingested 1,2,4-TMB is absorbed by constant infusion of the oral dose into the liver. This is a common assumption when information about the oral absorption of the compound is unknown. The contribution of the first-pass metabolism in the liver for oral dosing was evaluated by simulating steady state venous blood levels (at the end of 50 days continuous exposure) for a standard human at rest (70 kg) for a range of concentrations and doses; at low daily doses (0.1-10 mg/kg-day), equivalent inhalation concentrations result in steady state blood concentrations 4-fold higher than those resulting from oral doses, indicating the presence of first-pass metabolism following oral exposure. This difference became insignificant for daily doses exceeding 50 mg/kg-day (Appendix A (U.S. EPA, 2011d); Section A.1.4).

The human PBPK model inhalation dose metric (weekly average blood concentration, mg/L) for the PODADJ (0.085 mg/L) was used as the target for the oral dose metric. The human PBPK model was run to determine what oral exposure would yield an equivalent weekly average blood concentration and the resulting value of 6.2 mg/kg-day was used as the human equivalent dose POD (PODHED) for the RfD derivation.

2.2.2. RfD Derivation for 1,2,4-TMB

A PODHED of 6.2 mg/kg-day was derived for the oral database using route-to-route extrapolation based on the neurotoxic effects observed by Korsak and Rydzyński (1996) following inhalation exposure (decreased pain sensitivity). Thus, the same uncertainty factors applied to derive the RfC (see Section 2.1.2) were also applied to derive the RfD. The uncertainty factors, selected based on EPA's report, *A Review of the Reference Dose and Reference Concentration Processes* (2002), address five areas of uncertainty resulting in a composite UF of 1,000.

Application of this 1,000-fold composite UF yields the calculation of the chronic RfD for 1,2,4-TMB as follows:

$RfD = POD_{HED} \div UF = 6.2$ mg/kg-day $\div$ 1,000 = 0.006 mg/kg-day = 6×10^{-3} mg/kg-day (rounded to one significant digit)

A PBPK model was utilized to perform a route-to-route extrapolation to determine a POD for the derivation of the RfD from the Korsak and Rydzyński (1996) inhalation study

and corresponding critical effect. The confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996) is medium (see above). Confidence in the database for 1,2,4-TMB is low to medium as the database includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. The database lacks a multigenerational reproductive study, and the studies supporting the critical effect predominately come from the same research institute. Overall confidence in the RfD for 1,2,4-TMB is low due to uncertainties surrounding the application of the available PBPK model for the purposes of a route-to-route extrapolation.

2.2.3. Uncertainties in the Derivation of the RfD for 1,2,4-TMB

As the oral RfD for 1,2,4-TMB was based on a route-to-route extrapolation in order to determine the oral dose that would result in the same effect as inhalation exposure (decreased pain sensitivity; Korsak and Rydzyński (1996)), the uncertainties regarding this derivation are the same as for the RfC for 1,2,4-TMB (see Section 2.1.4), with the exception of the uncertainty surrounding the route-to-route extrapolation. The model used to perform this route-to-route extrapolation is a well-characterized model deemed appropriate for the purposes of the Toxicological Review. One source of uncertainty regarding the route-to-route extrapolation is the assumption of 100% bioavailability, that is,100% of the ingested 1,2,4-TMB would be absorbed and pass through the liver. If not all of the compound is bioavailable, a lower blood concentration would be expected compared to the current estimate, and thus, a higher RfD would be calculated.

2.2.4. Methods of analysis for RfD derivation for 1,3,5-TMB

The available oral database is inadequate to derive an RfD for 1,3,5-TMB. No chronic, subchronic, or short-term oral exposure studies were found in the literature. However, as outlined in RfC Derivation for 1,3,5-TMB, the toxicokinetic and toxicological similarities between 1,3,5-TMB and 1,2,4-TMB support adopting the RfC for 1,2,4-TMB as the RfC 1,3,5-TMB. These considerations also apply to the oral reference value, thus the RfD for 1,2,4-TMB was adopted for 1,3,5-TMB. 1,3,5-TMB is observed to elicit neurotoxic effects in rats in acute and short-term studies, and therefore the selected critical effect for 1,2,4-TMB, altered CNS function, is relevant to observed 1,3,5-TMB-induced toxicity. Similarities in blood:air and tissue:air partition coefficients and absorption into the bloodstream between the two isomers support the conclusion that internal blood dose metrics for 1,3,5-TMB would be similar as those calculated for 1,2,4-TMB using the 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, so that first-pass metabolism through the liver is not expected to differ greatly between 1,2,4-TMB and 1,3,5-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 1,3,5-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 EPA (1994), 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 1,3,5-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 developmental toxicity studies in rats and mice. The database lacks a multigenerational reproductive study, and the studies supporting the critical effect predominately come from the same research institute. Overall confidence in the RfD for 1,3,5-TMB is low due to uncertainties surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,3,5-TMB.

2.2.5. Uncertainties in the Derivation of the RfD for 1,3,5-TMB

The uncertainties regarding adopting the RfD for 1,2,4-TMB as the RfD for 1,3,5-TMB encompass previous areas of uncertainty involved in the derivation of the RfC for 1,3,5-TMB and the RfD for 1,2,4-TMB (see Sections 2.1.7 and 2.2.4). There does exist uncertainty regarding this adoption. However, as discussed above in Section 2.1.7, both isomers share multiple commonalities and similarities regarding their toxicokinetic and toxicological properties that support adopting one isomer's value for the other. Additionally, as the RfD derivation for 1,2,4-TMB was based on a route-to-route extrapolation, the uncertainties in that toxicity value's derivation (see Section 2.2.4) apply to the derivation of the RfD for 1,3,5-TMB.

2.3. Cancer Assessment

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005a), the database for 1,2,4-TMB and 1,3,5-TMB provides "inadequate information to assess 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 value is derived.

3. REFERENCES

ACGIH. (American Conference of Governmental Industrial Hygienists). (2002). Trimethyl benzene isomers. In Documentation of the threshold limit values and biological exposure indices (7 ed.). Cincinnati, OH.

Andersson, K; Fuxe, K; Toftgard, R; Nilsen, OG; Eneroth, P; Gustafsson, JA. (1980). Toluene-induced activation of certain hypothalamic and median eminence catecholamine nerve terminal systems of the male rat and its effects on anterior pituitary hormone secretion. Toxicol Lett 5: 393-398. http://dx.doi.org/10.1016/0378-4274(80)90021-1.

Andersson, K; Fuxe, K; Nilsen, OG; Toftgard, R; Eneroth, P; Gustafsson, JA. (1981). Production of discrete changes in dopamine and noradrenaline levels and turnover in various parts of the rat brain following exposure to xylene, ortho-, meta-, and para-xylene, and ethylbenzene. Toxicol Appl Pharmacol 60: 535-548.

Andersson, K; Nilsen, OG; Toftgard, R; Eneroth, P; Gustafsson, JA; Battistini, N; Agnati, LF. (1983). Increased amine turnover in several hypothalamic noradrenaline nerve exposure to various concentrations of toluene. Neurotoxicology 4: 43-55.

Balster, RL. (1998). Neural basis of inhalant abuse. Drug Alcohol Depend 51: 207-214.

Battig, K; Grandjean, E; Turrian, V. (1956). Health damage after continuous exposure to trimethyl benzene in a painting workshop. Soz Praventivmed 1: 389-403. http://dx.doi.org/10.1007/BF02031676.

Billionnet, C; Gay, E; Kirchner, S; Leynaert, B; Annesi-Maesano, I. (2011). Quantitative assessments of indoor air pollution and respiratory health in a population-based sample of French dwellings. Environ Res 111: 425-434. http://dx.doi.org/10.1016/j.envres.2011.02.008.

Bowen, SE; Batis, JC; Paez-Martinez, N; Cruz, SL. (2006). The last decade of solvent research in animal models of abuse: Mechanistic and behavioral studies. Neurotoxicol Teratol 28: 636-647. http://dx.doi.org/10.1016/j.ntt.2006.09.005.

Bracs, PU; Gregory, P; Jackson, DM. (1984). Passive avoidance in rats: Disruption by dopamine applied to the nucleus accumbens. Psychopharmacology 83: 70-75.

CDC. (Centers for Disease Control and Prevention). (2004). The health consequences of smoking: A report of the Surgeon General. Washington, DC: U.S. Department of Health and Human Services. http://www.surgeongeneral.gov/library/smokingconsequences/.

Chen, R; Dick, F; Seaton, A. (1999). Health effects of solvent exposure among dockyard painters: Mortality and neuropsychological symptoms. Occup Environ Med 56: 383-387. http://dx.doi.org/10.1136/oem.56.6.383.

Chou, CC; Riviere, JE; Monteiro-Riviere, NA. (2003). The cytotoxicity of jet fuel aromatic hydrocarbons and dose-related interleukin-8 release from human epidermal keratinocytes. Arch Toxicol 77: 384-391. http://dx.doi.org/10.1007/s00204-003-0461-z.

Dahl, AR; Damon, EG; Mauderly, JL; Rothenberg, SJ; Seiler, FA; McClellan, RO. (1988). Uptake of 19 hydrocarbon vapors inhaled by F344 rats. Fundam Appl Toxicol 10: 262-269. http://dx.doi.org/10.1016/0272-0590(88)90310-7.

Eide, I; Zahlsen, K. (1996). Inhalation experiments with mixtures of hydrocarbons. Experimental design, statistics and interpretation of kinetics and possible interactions. Arch Toxicol 70: 397-404. http://dx.doi.org/10.1007/s002040050291.

Gage, JC. (1970). The subacute inhalation toxicity of 109 industrial chemicals. Br J Ind Med 27: 1-18. http://dx.doi.org/10.1136/oem.27.1.1.

Gaschen, A; Lang, D; Kalberer, M; Savi, M; Geiser, T; Gazdhar, A; Lehr, CM; Bur, M; Dommen, J; Baltensperger, U; Geiser, M. (2010). Cellular responses after exposure of lung cell cultures to secondary organic aerosol particles. Environ Sci Technol 44: 1424-1430. http://dx.doi.org/10.1021/es902261m.

Ginsberg, G; W Jr, S; Bruckner, J; Sonawane, B. (2004). Incorporating children's toxicokinetics into a risk framework. Environ Health Perspect 112: 272-283. http://dx.doi.org/10.1289/ehp.6013.

Gralewicz, S; Wiaderna, D; Tomas, T; Rydzyński, K. (1997a). Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4-trimethylbenzene) in the rat. Neurotoxicol Teratol 19: 327-333. http://dx.doi.org/10.1016/S0892-0362(97)00001-9.

Gralewicz, S; Wiaderna, D; Tomas, T. (1997b). Retardation of the age-related increase in spontaneous cortical spike-wave discharges (SWD) in rats after a 28-day inhalation (SWD) in rats after a 28-day inhalation exposure to an industrial solvent, pseudocumene (1,2,4-trimethylbenzene). Int J Occup Med Environ Health 10: 213-222.

Gralewicz, S; Wiaderna, D. (2001). Behavioral effects following subacute inhalation exposure to m-xylene or trimethylbenzene in the rat: A comparative study. Neurotoxicology 22: 79-89. http://dx.doi.org/10.1016/S0161-813X(00)00003-6.

Guyatt, GH; Oxman, AD; Vist, GE; Kunz, R; Falck-Ytter, Y; Alonso-Coello, P; Schünemann, HJ. (2008a). GRADE: An emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336: 924-926. http://dx.doi.org/10.1136/bmj.39489.470347.AD.

Guyatt, GH; Oxman, AD; Kunz, R; Vist, GE; Falck-Ytter, Y; Schünemann, HJ. (2008b). GRADE: What is "quality of evidence" and why is it important to clinicians. BMJ 336: 995-998. http://dx.doi.org/10.1136/bmj.39490.551019.BE.

Henderson, RF. (2001). Aromatic hydrocarbons: Benzene and other alkylbenzenes. 4: 231-301.

Hill, AB. (1965). The environment and disease: Association or causation? Proc R Soc Med 58: 295-300.

Hillefors-Berglund, M; Liu, Y; von Euler, G. (1995). Persistent, specific and dose-dependent effects of toluene exposure on dopamine D2 agonist binding in the rat caudate-putamen. Toxicology 100: 185-194. http://dx.doi.org/10.1016/0300-483X(95)03084-S.

Hissink, AM; Krüse, J; Kulig, BM; Verwei, M; Muijser, H; Salmon, F; Leenheers, LH; Owen, DE; Lammers, JH; Freidig, AP; McKee, RH. (2007). Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents III. PBPK modeling of white spirit constituents as a tool for integrating animal and human test data. Neurotoxicology 28: 751-760. http://dx.doi.org/10.1016/j.neuro.2007.03.005.

Huo, JZ; Aldous, S; Campbell, K; Davies, N. (1989). Distribution and metabolism of 1,2,4-trimethylbenzene (pseudocumene) in the rat. Xenobiotica 19: 161-170. http://dx.doi.org/10.3109/00498258909034688.

IARC. (International Agency for Research on Cancer). (2006). Preamble to the IARC monographs. Lyon, France. http://monographs.iarc.fr/ENG/Preamble/.

Jackson, DM; Westlind-Danielsson, A. (1994). Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 64: 291-370. http://dx.doi.org/10.1016/0163-7258(94)90041-8.

Janik-Spiechowicz, E; Wyszyńska, K; Dziubałtowska, E. (1998). Genotoxicity evaluation of trimethylbenzenes. Mutat Res Genet Toxicol Environ Mutagen 412: 299-305. http://dx.doi.org/10.1016/S1383-5718(97)00202-7.

Järnberg, J; Johanson, G. (1995). Liquid/air partition coefficients of the trimethylbenzenes. Toxicol Ind Health 11: 81-88. http://dx.doi.org/10.1177/074823379501100107.

Järnberg, J; Johanson, G; Löf, A. (1996). Toxicokinetics of inhaled trimethylbenzenes in man. Toxicol Appl Pharmacol 140: 281-288. http://dx.doi.org/10.1006/taap.1996.0223.

Järnberg, J; Johanson, G; Löf, A; Stahlbom, B. (1997a). Inhalation toxicokinetics of 1,2,4-trimethylbenzene in volunteers: Comparison between exposure to white spirit and 1,2,4-trimethylbenzene alone. Sci Total Environ 199: 65-71. http://dx.doi.org/10.1016/S0048-9697(97)05482-X.

Järnberg, J; Stahlbon, B; Johanson, G; Löf, A. (1997b). Urinary excretion of dimethylhippuric acids in humans after exposure to trimethylbenzenes. Int Arch Occup Environ Health 69: 491-497. http://dx.doi.org/10.1007/s004200050179.

Jones, K; Meldrum, M; Baird, E; Cottrell, S; Kaur, P; Plant, N; Dyne, D; Cocker, J. (2006). Biological monitoring for trimethylbenzene exposure: A human volunteer study and a practical example in the workplace. Ann Occup Hyg 50: 593-598. http://dx.doi.org/10.1093/annhyg/mel016.

Korsak, Z; Swiercz, R; Rydzyński, K. (1995). Toxic effects of acute inhalation exposure to 1,2,4-trimethylbenzene (pseudocumene) in experimental animals. Int J Occup Med Environ Health 8: 331-337.

Korsak, Z; Rydzyński, 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; Rydzyński, K; Jajte, J. (1997). Respiratory irritative effects of trimethylbenzenes: An experimental animal study. Int J Occup Med Environ Health 10: 303-311.

Korsak, Z; Stetkiewicz, J; Majcherek, W; Stetkiewicz, I; Jajte, J; Rydzyński, K. (2000). Sub-chronic inhalation toxicity of 1,2,4-trimethylbenzene (pseudocumene) in rats. Int J Occup Med Environ Health 13: 155-164.

Kyrklund, T. (1992). The use of experimental studies to reveal suspected neurotoxic chemicals as occupational hazards: Acute and chronic exposures to organic solvents. Am J Ind Med 21: 15-24. http://dx.doi.org/10.1002/ajim.4700210105.

Lammers, JH; Emmen, HH; Muijser, H; Hoogendijk, EM; McKee, RH; Owen, DE; Kulig, BM. (2007). Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents II. Neurobehavioral effects of white spirit in rat and human. Neurotoxicology 28: 736-750. http://dx.doi.org/10.1016/j.neuro.2007.03.003.

Maltoni, C; Ciliberti, A; Pinto, C; Soffritti, M; Belpoggi, F; Menarini, L. (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. http://dx.doi.org/10.1111/j.1749-6632.1997.tb56863.x.

McKee, RH; Lammers, JH; Muijser, H; Owen, DE; Kulig, BM. (2010). Neurobehavioral effects of acute exposure to aromatic hydrocarbons. Int J Toxicol 29: 277-290. http://dx.doi.org/10.1177/1091581810365089.

Meulenberg, C; Vijverberg, H. (2000). Empirical relations predicting human and rat tissue: Air partition coefficients of volatile organic compounds. Toxicol Appl Pharmacol 165: 206-216. http://dx.doi.org/10.1006/taap.2000.8929.

Mikulski, PI; Wiglusz, R. (1975). The comparative metabolism of mesitylene, pseudocumene, and hemimellitene in rats. Toxicol Appl Pharmacol 31: 21-31. http://dx.doi.org/10.1016/0041-008X(75)90048-4.

MOE. (Ontario Ministry of the Environment). (2006). Rationale for the development of Ontario air standards for trimethylbenzenes: 1,2,3-Trimethylbenzene. Ontario, Canada.

Mögel, I; Baumann, S; Böhme, A; Kohajda, T; von Bergen, M; Simon, JC; Lehmann, I. (2011). The aromatic volatile organic compounds toluene, benzene and styrene induce COX-2 and prostaglandins in human lung epithelial cells via oxidative stress and p38 MAPK activation. Toxicology 289: 28-37. http://dx.doi.org/10.1016/j.tox.2011.07.006.

Myhre, O; Vestad, TA; Sagstuen, E; Aarnes, H; Fonnum, F. (2000). The effects of aliphatic (n-nonane), naphtenic (1,2,4-trimethylcyclohexane), and aromatic (1,2,4-trimethylbenzene) hydrocarbons on respiratory burst in human neutrophil granulocytes. Toxicol Appl Pharmacol 167: 222-230. http://dx.doi.org/10.1006/taap.2000.9008.

Myhre, O; Fonnum, F. (2001). The effect of aliphatic, naphthenic, and aromatic hydrocarbons on production of reactive oxygen species and reactive nitrogen species in rat brain synaptosome fraction: the involvement of calcium, nitric oxide synthase, mitochondria, and phospholipase A. Biochem Pharmacol 62: 119-128. http://dx.doi.org/10.1016/S0006-2952(01)00652-9.

NIOSH. (National Institute for Occupational Safety and Health). (1992). NIOSH recommendations for occupational safety and health: Compendium of policy documents and statements. (92-100). Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. http://www.cdc.gov/Niosh/92-100.html.

Norseth, T; Waage, J; Dale, I. (1991). Acute effects and exposure to organic compounds in road maintenance workers exposed to asphalt. Am J Ind Med 20: 737-744. http://dx.doi.org/10.1002/ajim.4700200604.

Ramaiah, SK. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol 45: 1551-1557. http://dx.doi.org/10.1016/j.fct.2007.06.007.

Rea, TM; Nash, JF; Zabik, JE; Born, GS; Kessler, WV. (1984). Effects of toluene inhalation on brain biogenic amines in the rat. Toxicology 31: 143-150. http://dx.doi.org/10.1016/0300-483X(84)90006-4.

Rothman, KJ; Greenland, S. (1998). Modern epidemiology (2nd ed.). Philadelphia, PA: Lippincott, Williams, & Wilkins.

Saillenfait, AM; Gallissot, F; Sabate, JP; Morel, G. (2005). Developmental toxicity of two trimethylbenzene isomers, mesitylene and pseudocumene, in rats following inhalation exposure. Food Chem Toxicol 43: 1055-1063. http://dx.doi.org/10.1016/j.fct.2005.02.008.

Snead, OC, III. (1995). Basic mechanisms of generalized absence seizures. Ann Neurol 37: 146-157. http://dx.doi.org/10.1002/ana.410370204.

Swiercz, R; Rydzyński, K; Wasowicz, W; Majcherek, W; Wesolowski, W. (2002). Toxicokinetics and metabolism of pseudocumene (1,2,4-trimethylbenzene) after inhalation exposure in rats. Int J Occup Med Environ Health 15: 37-42.

Swiercz, R; Wiaderna, D; Wasowicz, W; Rydzyński, K. (2003). Pseudocumene in brain, liver, lung and blood of rats after single and repeated inhalation exposure. Int J Occup Med Environ Health 16: 61-66.

Swiercz, R; Wasowicz, W; Majcherek, W. (2006). Mesitylene (1,3,5-trimethylbenzene) in the liver, lung, kidney, and blood and 3,5-dimethylbenzoic acid in the liver, lung, kidney and urine of rats after single and repeated inhalation exposure to mesitylene. Pol J Environ Stud 15: 485-492.

Tomas, T; Lutz, P; Wiaderna, D. (1999a). Changes in electrocortical arousal following acute trimethylbenzene administration in rats. Int J Occup Med Environ Health 12: 67-78.

Tomas, T; Swiercz, R; Wiaderna, D; Gralewicz, S. (1999b). Effects of acute exposure to aromatic hydrocarbons C 9 on locomotor activity in rats. Trimethylbenzene isomers. Int J Occup Med Environ Health 12: 331-343.

Tomas, T; Wiaderna, D; Swiercz, R. (1999c). Neurotoxicity assessment of selected organic solvents based on spontaneous and evoked cortical and hippocampal activity in rats. Int J Occup Med Environ Health 12: 73-84.

Tsujimoto, Y; Noda, T; Shimizu, M; Moriwaki, H; Tanaka, M. (2000). Identification of the dimethylbenzyl mercapturic acid in urine of rats administered with 1,2,4-trimethylbenzene. Chemosphere 40: 893-896. http://dx.doi.org/10.1016/S0045-6535(99)00467-1.

Tsujimoto, Y; Warashina, M; Nam, VD; Noda, T; Shimizu, M; Yamaguchi, Y; Moriwaki, H; Morimoto, T; Kakiuchi, K; Maeda, Y; Tanaka, M. (2005). Determination of urinary phenolic metabolites from rats treated with 1,2,3-and 1,3,5-trimethylbenzenes. J Occup Health 47: 337-339.

U.S. EPA. (U.S. Environmental Protection Agency). (1986a). Guidelines for mutagenicity risk assessment [EPA Report]. (EPA/630/R-98/003). Washington, DC. http://www.epa.gov/iris/backgrd.html.

U.S. EPA. (U.S. Environmental Protection Agency). (1986b). Guidelines for the health risk assessment of chemical mixtures [EPA Report]. (EPA/630/R-98/002). Washington, DC.

U.S. EPA. (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment [EPA Report]. (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/iris/backgrd.html.

U.S. EPA. (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993.

U.S. EPA. (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment [EPA Report]. (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/REPRO51.PDF.

U.S. EPA. (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment. (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF.

U.S. EPA. (U.S. Environmental Protection Agency). (2000a). Benchmark dose technical guidance document [external review draft]. (EPA/630/R-00/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/benchmark-dose-doc-draft.htm.

U.S. EPA. (U.S. Environmental Protection Agency). (2000b). Supplementary guidance for conducting health risk assessment of chemical mixtures. (EPA/630/R-00/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533.

U.S. EPA. (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes. (EPA/630/P-02/002F). Washington, DC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=51717.

U.S. EPA. (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk assessment. (EPA/630/P-03/001F). Washington, DC. http://www.epa.gov/cancerguidelines/.

U.S. EPA. (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/cancerguidelines/guidelines-carcinogen-supplement.htm.

U.S. EPA. (U.S. Environmental Protection Agency). (2006). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (final report). (EPA/600/R-05/043F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development.

U.S. EPA. (U.S. Environmental Protection Agency). (2007). Acute exposure guideline levels (AEGLs) for 1,3,5-trimethylbenzene (CAS reg. no. 108-67-8), 1,2,4-trimethylbenzene (CAS reg. no. 95-63-6), 1,2,3-trimethylbenzene (CAS reg. no. 526-73-8). Washington, DC. http://www.epa.gov/opptintr/aegl/pubs/123_%20124_%20135_trimethylbenzenes_%20interim_11_2007.v1.pdf.

U.S. EPA. (U.S. Environmental Protection Agency). (2011a). IRIS Process Retrieved August 24, 2011, from http://epa.gov/iris/process.htm

U.S. EPA. (U.S. Environmental Protection Agency). (2011b). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC. http://www.epa.gov/raf/publications/interspecies-extrapolation.htm.

U.S. EPA. (U.S. Environmental Protection Agency). (2011c). Supplemental Material: Benchmark Dose Modeling Results for the Derivation of Reference Values for 1,2,4- and 1,3,5-Trimethylbenzene (Science Consultation Draft [Appendix C]). Washington, DC.

U.S. EPA. (U.S. Environmental Protection Agency). (2011d). Supplemental Material: Toxicological Information (PBPK and TK) in Support of Hazard Identification and Dose-Response Analysis for 1,2,4- and 1,3,5-Trimethylbenzene (Science Consulation Draft [Appendix A]). Washington, DC.

von Euler, G; Ögren, SO; Li, XM; Fuxe, K; Gustafsson, JA. (1993). Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. Toxicology 77: 223-232. http://dx.doi.org/10.1016/0300-483X(93)90162-L.

von Euler, G; Ögren, SO; Eneroth, P; Fuxe, K; Gustafsson, JA. (1994). Persistent effects of 80 ppm toluene on dopamine-regulated locomotor activity and prolactin secretion in the male rat. Neurotoxicology 15: 621-624.

Warter, JM; Vergnes, M; Depaulis, A; Tranchant, C; Rumbach, L; Micheletti, G; Marescaux, C. (1988). Effects of drugs affecting dopaminergic neurotransmission in rats with spontaneous petit mal-like seizures. Neuropharmacology 27: 269-274. http://dx.doi.org/10.1016/0028-3908(88)90043-3.

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.

Wiglusz, R; Kienitz, M; Delag, G; Galuszko, E; Mikulski, P. (1975a). Peripheral blood of mesitylene vapour treated rats. Bull Inst Marit Trop Med Gdynia 26: 315-321.

Wiglusz, R; Delag, G; Mikulski, P. (1975b). Serum enzymes activity of mesitylene vapour treated rats. Bull Inst Marit Trop Med Gdynia 26: 303-313.

Wiglusz, R. (1979). The effect of 1, 3, 5-trimethylbenzene inhalation exposure on the glucuronic acid pathway and activity of some xenobiotic-metabolizing enzymes. Bull Inst Marit Trop Med Gdynia 30: 189-196.

APPENDICES

Appendix A: Toxicological Information in Support of Hazard Identification and Dose-Response Analysis for 1,2,4- and 1,3,5-Trimethylbenzene

Appendix B: Benchmark Dose Modeling Results for the Derivation of Reference Values for 1,2,4- and 1,3,5-Trimethylbenzene

Appendix C: Summary of External Peer Review and Public Comments and Disposition