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Toxicological Review of Trimethylbenzenes

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

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

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ABBREVIATIONS AND ACRONYMS

AAQC	Ambient air quality criterion
ACGIH	American Conference of Governmental Industrial Hygienists
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Levels
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
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
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 and Environmental Research Online
HEV	human epithelial keratinocytes
HSDB	Hazardous Substances Data Bank
IL-8	interleukin-8
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
JP-8	jet propulsion fuel 8
K_m	Michaelis-Menten constant
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
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
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
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
UV	ultraviolet
VOC	volatile organic compound
WBC	white blood cell
WS	white spirit
χ^2	chi-squared

AUTHORS | CONTRIBUTORS | REVIEWERS

Assessment Team

J. Allen Davis, M.S.P.H. (Chemical Manager) Eva McLanahan, Ph.D. (LCDR, USPHS) Paul Schlosser, Ph.D. John Cowden[1], 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[1], 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

Scientific Support Team

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
Martin Gehlhaus[1], MHS	U.S. Environmental Protection Agency Office of Research and Development National Center for Environmental Assessment Washington, DC
Nina Wang[1], Ph.D.	U.S. Environmental Protection Agency Office of Research and Development National Center for Environmental Assessment Cincinnati, OH

[1] Chemical Assessment Support Team (CAST) Member

Production Team

Ellen Lorang, M.S.	U.S. Environmental Protection Agency
Deborah Wales	Office of Research and Development
Gerald Gurevich	National Center for Environmental Assessment
J. Sawyer Lucy	Research Triangle Park, NC
Maureen Johnson	U.S. Environmental Protection Agency
Vicki Soto	Office of Research and Development
	National Center for Environmental Assessment
	Washington, DC

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

Kenneth Olden, Ph.D., Sc.D., L.H.D.	U.S. Environmental Protection Agency
(Center Director)	Office of Research and Development
Lynn Flowers, Ph.D., DABT	National Center for Environmental Assessment
(Associate Director for Health)	Washington, DC
Vincent Cogliano, Ph.D.[2]	
(IRIS Program Director—Acting)	
Samantha Jones, Ph.D.	
(IRIS Associate Director for Science)	
Jamie Strong, Ph.D.	
Ted Berner, MS	
Reeder Sams, Ph.D.	U.S. Environmental Protection Agency
Lyle Burgoon, Ph.D.	Office of Research and Development
John Vandenberg, Ph.D.	National Center for Environmental Assessment
Debra Walsh, M.S.	Research Triangle Park, NC

[2] Chemical Assessment Support Team (CAST) Lead

Reviewers

This assessment was provided for review to scientists in EPA's Program and Regional Offices. Comments were submitted by:

Region/Office

Region 8, Denver, CO

Region 2, New York City, NY

Office of Children's Health Protection, Washington, DC

Office of Air and Radiation, Washington, DC

Office of Environmental Information, Washington, DC

Office of Solid Waste and Emergency Response, Washington, DC

Office of Pesticides, Washington, DC

Office of Water, Washington, DC

This assessment was provided for review to other federal agencies and the Executive Offices of the President. Comments were submitted by:

Agency/ Office / Program

Chemical Material Risk Management Program, Department of Defense

Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention, Department of Health & Human Services

The National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Department of Health & Human Services

National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health & Human Services

Council on Environmental Quality, Executive Office of the President

This assessment was released for public comment on June 26th, 2012 and comments were due on August 28th, 2012. Comments were received from the following entities:

Non-Government

Leslie Berry Allison Starmann	American Chemistry Council

A public listening session was held by EPA on August 1st, 2012. Attendees external to the EPA are listed below.

Listening Session Attendees (Non-EPA)

Leslia Berry Richard Becker	American Chemistry Council

PREFACE

This Toxicological Review critically reviews the publicly available studies on the three isomers of trimethylbenzene (i.e., 1,2,3-trimethylbenzene [1,2,3-TMB], 1,2,4-trimethylbenzene [1,2,4-TMB], and 1,3,5-trimethylbenzene [1,3,5-TMB]) in order to identify their adverse health effects and to characterize exposure-response relationships. Because more types of studies are available for the 1,2,4-TMB isomer, it generally appears first when the individual isomers are listed. This assessment was prepared under the auspices of EPA's Integrated Risk Information System (IRIS) program.

This assessment was prepared because of the presence of trimethylbenzenes (TMB) at Superfund sites. Of sites on EPA's National Priorities List that report TMB isomer contamination (38 sites), 93% report 1,3,5-TMB contamination, 85% report 1,2,4-TMB contamination, 12% report 1,2,3-TMB contamination, and 17% report contamination by unspecified TMB isomers.

The *Toxicological Review of Trimethylbenzenes* is a new assessment; there is no previous entry on the IRIS Database for 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. This assessment reviews information on all health effects by all exposure routes.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. The findings of this assessment and related documents produced during its development are available on the IRIS website (http://www.epa.gov/iris). Appendices for chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental Information* (See Appendix A to C).

Implementation of the 2011 National Research Council Recommendations

On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law (U.S. Congress, 2011). The report language included direction to EPA for the IRIS Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde (NRC, 2011). The NRC's recommendations, provided in Chapter 7 of the review report, offered suggestions to EPA for improving the development of IRIS assessments. The report language included the following:

> The Agency shall incorporate, as appropriate, based on chemical-specific datasets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process …. For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS) have been implemented or addressed, including an explanation for why certain recommendations were not incorporated.

Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in Appendix D. This documentation also includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that, "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. The IRIS TMBs assessment is one of the first assessments in Phase 2 of implementation, which addresses all of the short-term NRC recommendations (see Appendix D, Table D-1). The IRIS Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined in Table D-2, including the development of a standardized approach to describe the strength of evidence for noncancer effects. On May 16, 2012, EPA announced (U.S. EPA, 2012c) that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

Assessments by Other National and International Health Agencies

Toxicity information on 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB has been evaluated by the National Institute for Occupational Safety and Health (NIOSH), the American Conference of

Governmental Industrial Hygienists (ACGIH), the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances, and the Ontario Ministry of the Environment (MOE). The results of these assessments are summarized in Appendix A (Table A-1). It is important to recognize that these assessments may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

Chemical Properties and Uses

TMBs are aromatic hydrocarbons with three methyl groups attached to a benzene ring and the chemical formula C_9H_{12}. The chemical and physical properties of the TMB isomers are similar to one another. TMBs are colorless, flammable liquids with a strong aromatic odor; an odor threshold of 0.4 parts per million (ppm) of air has been reported (U.S. EPA, 1994a). They are insoluble in water but miscible with organic solvents such as ethyl alcohol, benzene, and ethyl ether (OSHA, 1996). Production and use of TMBs may result in their release to the environment through various waste streams. If released to the atmosphere, 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB will exist solely in the vapor phase in the atmosphere under ambient conditions, based on measured vapor pressures of 1.69, 2.10, and 2.48 mm Hg at 25°C, respectively (HSDB, 2011a, b, c). All three isomers are expected to have limited mobility through soil based on their Log K_{oc} 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 Appendix B, Table B-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, c). Non-volatilized TMBs may be subject to biodegradation under aerobic conditions (HSDB, 2011a, b, c). The estimated bio-concentration factors (133–439) and high volatility of TMBs suggest that bioaccumulation of these chemicals will not be significant (U.S. EPA, 1987). Additional information on the chemical identities and physicochemical properties of TMBs are listed in Table B-1 in Appendix B.

The commercially available substance known as trimethylbenzene, CAS No. 25551-13-7, is a mixture of three isomers in various proportions, namely CAS No. 526-73-8 (1,2,3-TMB or hemimellitene), CAS No. 95-63-6 (1,2,4-TMB or pseudocumene), and CAS No. 108-67-8 (1,3,5-TMB or mesitylene). Production of TMB isomers occurs during petroleum refining, and 1,2,4-TMB individually makes up approximately 40% of the C9 aromatic fraction (i.e., aromatic hydrocarbons with nine carbons) (U.S. EPA, 1994a). The domestic production of the C9 fraction in 1991 was estimated to be approximately 80 billion pounds (40 million tons) (U.S. EPA, 1994a). Vehicle emissions are a major anthropogenic source of TMBs, due to the widespread use of the C9 fraction as a component of gasoline (U.S. EPA, 1994a). Other uses of TMBs include solvents in research and industry, dyestuff intermediate, paint thinner, and as a UV oxidation stabilizer for plastics (HSDB, 2011b, c).

Occupational levels of exposure for TMBs have been measured between 20–8,540 µg/m^3 (HSDB, 2011a, b, c; Jiun-Horng et al., 2008), whereas residential exposures are generally much lower: 0.29-7.8 µg/m^3 (Martins et al., 2010; Choi et al., 2009; Guo et al., 2009). Total atmospheric releases of 1,2,4-TMB to the environment in 2008 equaled 5.8 million pounds (2,900 tons), 265,000 pounds (132.5 tons) were released to surface waters, underground injection sites, or land (TRI, 2008). No information is currently available regarding 1,2,3-TMB or 1,3,5-TMB releases.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

Soon after the 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 the 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, the EPA uses information on the adverse effects of chemicals and on exposure levels below which these effects are not anticipated to occur.

IRIS assessments critically review the publicly available studies to identify adverse health effects from exposure to chemicals and to characterize exposure-response relationships. In terms set forth by the National Research Council (NRC, 1983), IRIS assessments cover the hazard identification and dose-response assessment steps of risk assessment, not the exposure assessment or risk characterization steps that are conducted by the EPA's program and regional offices and by other federal, state, and local health agencies that evaluate risk in specific populations and exposure scenarios. IRIS assessments are distinct from and do not address political, economic, and technical considerations that influence the design and selection of risk management alternatives.

An IRIS assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. These agents may be found in air, water, soil, or sediment. 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).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state health agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. Agents may be considered for reassessment as significant new studies are published. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. Other agents may also be assessed in response to an urgent public health need.

2. Process for developing and peer-reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009 and enhanced in July 2013) involves critical analysis of the pertinent studies, opportunities for public input, and multiple levels of scientific review. The EPA revises draft assessments after each review, and external drafts and comments become part of the public record (U.S. EPA, 2009).

Before beginning an assessment, the IRIS Program discusses the scope with other EPA programs and regions to ensure that the assessment will meet their needs. Then a public meeting on problem formulation invites discussion of the key issues and the

studies and analytical approaches that might contribute to their resolution.

Step 1. Development of a draft Toxicological Review. The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate study quality, identify health effects, identify mechanistic events and pathways, integrate the evidence of causation for each effect, and derive toxicity values. A public meeting prior to the integration of evidence and derivation of toxicity values promotes public discussion of the literature search, evidence, and key issues.

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

Step 3. Interagency science consultation with other federal agencies and the Executive Offices of the President. The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and the EPA's response to major comments become part of the public record.

Step 4. Public review and comment, followed by external peer review. The EPA releases the draft assessment for public review and comment. A public meeting provides an opportunity to discuss the assessment prior to peer review. Then the EPA releases a draft for external peer review. The peer review meeting is open to the public and includes time for oral 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, written public comments, and peer review report become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary. The draft assessment is revised to reflect the peer review comments, public comments, and newly published studies that are critical to the conclusions of the assessment. 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 the Executive Offices of the President The draft assessment and summary are revised to address the 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. 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 (U.S. EPA, 2005a, b, 2000, 1998, 1996, 1991, 1986a, b) and other methods (U.S. EPA, 2012a, b, 2011, 2006a, b, 2002, 1994b). 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, the 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, Web of Science, and other databases listed in the EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Searches for information on mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms.

Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. The EPA posts the results of the literature search on the IRIS web site and requests information from the public on additional studies and ongoing research.

The EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act. Material submitted as Confidential Business Information is considered only if it includes health and safety data that can be publicly released. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, the EPA will have it peer-reviewed.

The 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.

The literature search seeks, in decreasing order of preference (U.S. EPA, 2000, §2.2; 1986b, §2.1)]:

- 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, case-control studies, and some population-based surveys (for example, NHANES) provide the strongest epidemiologic evidence, especially if they collect information about individual exposures and effects.
- Ecological studies (geographic correlation studies) relate exposures and effects 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 effect or about the relevance of analogous results in animals.

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

3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental animal studies or human clinical studies.

- 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 animal studies.

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.
- Studies of effects from less-than-chronic exposure are pertinent but less preferred for identifying effects from lifetime human exposure. Such studies may be indicative of effects from less-than-lifetime human exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information.

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 (U.S. EPA, 2006b, 1998, 1996, 1991).

4. Evaluating the quality of individual studies

After the subsets of pertinent epidemiologic and experimental studies have been selected from the literature searches, the assessment evaluates the quality of each individual study. This evaluation considers the design, methods, conduct, and documentation of each study, but not whether the results are positive, negative, or null. The objective is to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

4.1. Evaluating the quality of epidemiologic studies

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

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study group and comparison group.
- Ascertainment of exposure to the chemical or mixture.
- Ascertainment of disease or health effect.

- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during critical periods.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and potential for selection bias as a result of the achieved participation rates.
- Measurement error (can lead to misclassification of exposure, health outcomes, and other factors) and other types of information bias.
- Potential confounding and other sources of bias 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 related to both exposure and outcome and is sufficiently prevalent to result in bias.

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

4.2. Evaluating the quality of experimental studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each experimental animal study, in-vitro study, or human clinical study (U.S. EPA, 2005a, 1998, 1996, 1991). Research involving human subjects is considered only if conducted according to ethical principles.

- Documentation of study design, animals or study population, methods, basic data, and results.
- Nature of the assay and validity for its intended purpose.
- 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 (U.S. EPA, 2005a, §2.2.2.1.3).

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991). 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 (U.S. EPA, 1998, §3.1.2.4.5.4; 1991, §3.1.1.4),.

4.3. Reporting study results

The assessment uses evidence tables to present 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 quality characteristics in this section to identify the strongest studies or types of study. The tables present 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 information 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, the EPA asks peer reviewers to identify studies that were not adequately considered.

5. Evaluating the overall evidence of each effect

5.1. Concepts of causal inference

For each health effect, the assessment evaluates the evidence as a whole to determine whether it is reasonable to infer a causal association between exposure to the agent and the occurrence of the effect. This inference is based on information from pertinent human studies, animal studies, and mechanistic studies of adequate quality. Positive, negative, and null results are given weight according to study quality.

Causal inference involves scientific judgment, and the considerations are nuanced and complex. Several health agencies have developed frameworks for causal inference, among them the U.S. Surgeon General (CDC, 2004; HEW, 1964), the International Agency for Research on Cancer (IARC, 2006), the Institute of Medicine (IOM, 2008), and the EPA (2010, §1.6; 2005a, §2.5). Although developed for different purposes, the frameworks are similar in nature and provide an established structure and language for causal inference. Each considers aspects of an association that suggest causation, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998), and U.S. EPA (2005a, §2.2.1.7; 1994b, Appendix C).

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 an effect that is common, exposure misclassification, or other sources of bias.

Consistency of association: An inference of causation 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 causation. Discordant results sometimes reflect differences in study design, exposure, or confounding factors.

Specificity of association: As originally intended, this refers to one cause associated with one effect. Current understanding that many agents cause multiple effects and many effects have multiple causes make this a less informative aspect of causation, unless

the effect is rare or unlikely to have multiple causes.

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

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

Biologic plausibility: An inference of causation is strengthened by data demonstrating plausible biologic mechanisms, if available. Plausibility may reflect subjective prior beliefs if there is insufficient understanding of the biologic process involved.

Coherence: An inference of causation 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, as it tests the hypothesis of causation. An example would be an intervention to reduce exposure in the workplace or environment that is followed by a reduction of an adverse effect.

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

These considerations are consistent with guidelines for systematic reviews 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., 2008b; Guyatt et al., 2008a).

5.2. Evaluating evidence in humans

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole. The objective is to determine whether a credible association has been observed and, if so, whether that association is consistent with causation. In doing this, the assessment explores alternative explanations (such as chance, bias, and confounding) and draws a conclusion about whether these alternatives can satisfactorily explain any observed association.

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may select a standard descriptor to characterize the epidemiologic evidence of association between exposure to the agent and occurrence of a health effect.

Sufficient epidemiologic evidence of an association consistent with causation: The evidence establishes a causal association for which alternative explanations such as chance, bias, and confounding can be ruled out with reasonable confidence.

Suggestive epidemiologic evidence of an association consistent with causation: The evidence suggests a causal association but chance, bias, or confounding cannot be ruled out as explaining the association.

Inadequate epidemiologic evidence to infer a causal association: The available studies do not permit a conclusion

regarding the presence or absence of an association.

Epidemiologic evidence consistent with no causal association: Several adequate studies covering the full range of human exposures and considering susceptible populations, and for which alternative explanations such as bias and confounding can be ruled out, are mutually consistent in not finding an association.

5.3. Evaluating evidence in animals

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. Several concepts discussed by Hill (1965) are pertinent to the weight of experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (U.S. EPA, 2005a, §2.2.1.7; 1994b, Appendix C).

In weighing evidence from multiple experiments, U.S. EPA (2005a, §2.5) 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. The EPA regards all as valid observations and looks to explain differing results using mechanistic information (for example, physiologic or metabolic differences across test systems) or methodological differences (for example, relative sensitivity of the tests, differences in dose levels, insufficient sample size, or timing of dosing or data collection).

It is well established that there are critical periods for some developmental and reproductive effects (U.S. EPA, 2006b, 2005a, b, 1998, 1996, 1991). Accordingly, the assessment determines whether critical periods have been adequately investigated. Similarly, the assessment determines whether the database is adequate to evaluate other critical sites and effects.

In evaluating evidence of genetic toxicity:

- 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, The EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (U.S. EPA, 1986a, §2.3).

5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

- The biologic plausibility of a causal interpretation of human studies.
- The generalizability of animal studies to humans.
- The susceptibility of particular populations or lifestages.

The focus of the analysis is to describe, if possible, mechanistic pathways that lead to a health effect. These pathways encompass:

- *Toxicokinetic processes* of absorption, distribution, metabolism, and elimination that lead to the formation of an active agent and its presence at the site of initial biologic interaction.
- *Toxicodynamic processes* that lead to a health effect at this or another site (also known as a *mode 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. Information on mode of action is not required for a conclusion that the agent is causally related to an effect (U.S. EPA, 2005a, §2.5).

The assessment addresses several questions about each hypothesized mode of action(U.S. EPA, 2005a, §2.4.3.4).

1) **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, in which studies that suppress a key event observe suppression of the effect. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, much more so than by replicate experiments in the same model. The assessment may consider various aspects of causation in addressing this question.

2) **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 effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

3) **Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?** The assessment reviews the key events to identify populations and lifestages that might be susceptible to their occurrence. Quantitative differences may result in separate toxicity values for susceptible populations or lifestages.

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 (U.S. EPA, 2005a, §2.4.3.3). It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 2008a).

For cancer, the assessment evaluates evidence of a mutagenic mode of action to guide extrapolation to lower doses and consideration of susceptible lifestages. Key data include the ability of the agent or a metabolite to react with or bind to DNA, positive results in multiple test systems, or similar properties and structure-activity relationships to mutagenic carcinogens (U.S. EPA, 2005a ,§2.3.5).

5.5. Characterizing the overall weight of the evidence

After evaluating the human, animal, and mechanistic evidence pertinent to an effect, the assessment answers the question: Does the agent cause the adverse effect? (NRC, 2009, 1983). In doing this, the assessment develops a narrative that integrates the evidence pertinent to causation. To provide clarity and consistency, the narrative includes a standard hazard descriptor. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a, §2.5).

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 evidence.

Suggestive evidence of carcinogenic potential: The evidence raises concern for effects in humans but is 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 is robust evidence 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.

Multiple descriptors may be used if there is evidence that carcinogenic effects differ by dose range or exposure route (U.S. EPA, 2005a, §2.5).

Another example of standard descriptors comes from the EPA's Integrated Science Assessments, which evaluate causation for the effects of the criteria pollutants in ambient air (U.S. EPA, 2010, §1.6).

Causal relationship: Sufficient evidence to conclude that there is a causal relationship. Observational studies cannot be explained by plausible alternatives, or they are supported by other lines of evidence, for example, animal studies or mechanistic information.

Likely to be a causal relationship: Sufficient evidence that a causal relationship is likely, but important uncertainties remain. For example, observational studies show an association but co-exposures are difficult to address or other lines of evidence are limited or inconsistent; or multiple animal studies from different laboratories demonstrate effects and there are limited or no human data.

Suggestive of a causal relationship: At least one high-quality epidemiologic study shows an association but other studies are inconsistent.

Inadequate to infer a causal relationship: The studies do not permit a conclusion regarding the presence or absence of an association.

Not likely to be a causal relationship: Several adequate studies, covering the full range of human exposure and considering susceptible populations, are mutually consistent in not showing an effect at any level of exposure.

The EPA is investigating and may on a trial basis use these or other standard descriptors to characterize the overall weight of the evidence for effects other than cancer.

6. Selecting studies for derivation of toxicity values

For each effect where there is credible evidence of an association with the agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The decision to derive toxicity values may be linked to the hazard descriptor.

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal:

- 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 with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

Studies with non-monotonic exposure-response relationships are not necessarily excluded from the analysis. A diminished effect at higher exposure levels may be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, exposure misclassification, or selection bias.

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, the EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1. General framework for dose-response analysis

The EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a, §3).

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). If response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors to the point of departure in order to account for sources of uncertainty and variability (Section 7.6).

For a group of agents that induce an effect through a common mode of action, the dose-response analysis may derive a *relative potency factor* for each agent. A full dose-response analysis is conducted for one well-studied *index chemical* in the group, then the potencies of other members are expressed in relative terms based on relative toxic effects, relative absorption or metabolic rates, quantitative structure-activity relationships, or receptor binding characteristics (U.S. EPA, 2005a, §3.2.6; 2000, §4.4).

Increasingly, the EPA is basing toxicity values on combined analyses of multiple data sets or multiple responses. The EPA also considers multiple dose-response approaches if they can be supported by robust data.

7.2. Modeling dose to sites of biologic effects

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 (U.S. EPA, 2006a; 2005a, §3.1; 1994b, §4.3).

Because toxicokinetic modeling can require many parameters and more data than are typically available, the 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 (U.S. EPA, 2005a, §3.1.1; 1991, §3.2).
- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.
- Oral doses are scaled allometrically using $mg/kg^{3/4}$-day as the equivalent dose metric across species. Allometric scaling pertains to equivalence across species, not across lifestages, and is not used to scale doses from adult humans or mature animals to infants or children (U.S. EPA, 2011; 2005a, §3.1.3).
- 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 (U.S. EPA, 2012a; 1994b, §3).

It can be informative to convert doses across exposure routes. If this is done, the assessment describes the underlying data, algorithms, and assumptions (U.S. EPA, 2005a, §3.1.4).

In the absence of study-specific data on, for example, intake rates or body weight, the EPA has developed recommended values for use in dose-response analysis (U.S. EPA, 1988).

7.3. Modeling response in the range of observation

Toxicodynamic ("biologically based") modeling can incorporate data on biologic processes leading to an effect. 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 (U.S. EPA, 2005a, §3.2.2).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, the 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. The EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2012b). Additional judgment or alternative analyses are used if 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 (U.S. EPA, 2005a, §3.2.3).

Modeling is used to derive a point of departure (U.S. EPA, 2012b; 2005a, §3.2.4). (See Section 7.6 for alternatives if a point of departure cannot be derived by modeling.):

- If linear extrapolation is used, selection of a response level corresponding to the point of departure is not highly influential, so standard values near the low end of the observable range are generally used (for example, 10% extra risk for cancer bioassay data, 1% for epidemiologic data, lower for rare cancers).
- For nonlinear approaches, both statistical and biologic considerations are taken into account.
- For dichotomous data, a response level of 10% extra risk is generally used for minimally adverse effects, 5% or lower for more severe effects.
- For continuous data, a response level is ideally based on an established definition of biologic significance. In the absence of such definition, one control standard deviation from the control mean is often used for minimally adverse effects, one-half standard deviation for more severe effects.

The point of departure is the 95% lower bound on the dose associated with the selected response level.

7.4. Extrapolating to lower doses and response levels

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation, typically used for cancer data, considers what is known about modes of action (U.S. EPA, 2005a, §3.3.1 and §3.3.2).

1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model below the observed range if significant model uncertainty can be ruled out with reasonable confidence.

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 when data are insufficient to establish mode of action and when scientifically plausible.

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 or concentrations, respectively.

3) Nonlinear models are used for extrapolation 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. Nonlinear approaches generally should not be used in cases where mode of action has not ascertained. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

4) Both linear and nonlinear approaches may be used if there a multiple modes of action. For example, modeling to a low response level can be useful for estimating the response at doses where a high-dose mode of action would be less important.

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 or selects 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 lifestages

The assessment analyzes the available information on populations and lifestages that may be particularly susceptible to each effect. A tiered approach is used (U.S. EPA, 2005a, §3.5).

1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or lifestage, 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, the EPA has developed *age-dependent adjustment factors* for early-life exposure to potential carcinogens that have a mutagenic mode of action. 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, the EPA recommends (U.S. EPA, 2005b, §5):

- 10-fold adjustment for exposures before age 2 years.
- 3-fold adjustment for exposures between ages 2 and 16 years.

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 (U.S. EPA, 2002, §4.2). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a necessary key event does not occur below a specific dose. Reference values provide no information about risks at higher exposure levels.

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 toxicity, reproductive toxicity, and neurotoxicity there is guidance on adverse effects and their biologic markers (U.S. EPA, 1998, 1996, 1991).

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

Human variation. The assessment accounts 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. A factor of 10 is generally used to account for this variation. This factor is reduced only if the point of departure is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994b, §4.3.9.1; 1991, §3.4).

Animal-to-human extrapolation. If animal results are used to make inferences about humans, the assessment adjusts for cross-species differences. These may arise from differences in toxicokinetics or toxicodynamics. Accordingly, if the point of departure is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. In most other cases, a factor of 10 is applied (U.S. EPA, 2011; 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994b, §4.3.9.1; 1991, §3.4).

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 (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994b, §4.3.9.1; 1991, §3.4).

Subchronic-to-chronic exposure. If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. 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 (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1994b, §4.3.9.1).

Incomplete database. If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994b, §4.3.9.1; 1991, §3.4). The size of the factor depends on the nature of the database deficiency. For example, the 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 (U.S. EPA, 2002, §4.4.5).

In this way, the assessment derives candidate values for each suitable data set and effect that is credibly associated with the agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures (U.S. EPA, 1994b, §4.3.9).

The assessment derives or selects an *organ- or system-specific reference value* for each organ or system affected by the agent. The assessment explains the rationale for each organ/system-specific reference value (based on, for example, the highest quality studies, the most sensitive outcome, or a clustering of values). By providing these organ/system-specific reference values, IRIS assessments facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (NRC, 2009).

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. This is generally the most sensitive organ/system-specific reference value, though consideration of study quality and confidence in each value may lead to a different selection.

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 (U.S. EPA, 1994b, §4.3.9.2).

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 guidelines for systematic reviews 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., 2008b).

All assessments discuss the significant uncertainties encountered in the analysis. The EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a, §3.6). For example, the discussion distinguishes model uncertainty (lack of knowledge about the most appropriate

experimental or analytic model) and parameter uncertainty (lack of knowledge about the parameters of a model). Assessments also discuss human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

Note: *The Preamble reflects methods that will be employed once all the 2011 NAS recommendations have been fully implemented. As this Toxicological Review was created during a period in which the NAS recommendations were being incorporated into the IRIS process, the methods utilized in the assessment may not completely reflect those detailed in the Preamble. For further information on which specific NAS recommendations have been implemented in this document, please refer to Appendix D (Documentation of Implementation of the 2011 National Research Council Recommendations) in the Supplemental Information.*

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EXECUTIVE SUMMARY

Occurrence and Health Effects

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

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

1. Effects Other Than Cancer Following Inhalation Exposure

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

Human studies included occupational exposure to various solvent mixtures containing TMBs. Health effects noted in these studies were eye irritation, neurological (hand tremble,

abnormal fatigue, lack of coordination), and hematological effects (Chen et al., 1999; Norseth et al., 1991; Bättig et al., 1958; Battig et al., 1956). Also, residential exposure to mixtures containing 1,2,4-TMB were observed to result in asthma (Billionnet et al., 2011). However, as these studies involved exposures to mixtures containing multiple TMB isomers and other volatile organic compounds (VOCs), it is difficult to ascertain the specific contribution of each TMB isomer to the specific health effects reported. Controlled human exposures to individual isomers also exist, although these studies generally report little or no effect on respiratory or sensory irritation (Jones et al., 2006; Järnberg et al., 1997a; Järnberg et al., 1997b; Kostrzewski et al., 1997; Järnberg et al., 1996; Kostrewski and Wiaderna-Brycht, 1995). One controlled human exposure study reported some deficits in attention following exposure to white spirit (WS), a complex mixture containing 1,2,4-TMB (Lammers et al., 2007).

Animal inhalation studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak et al., 1995) included acute and short-term studies of TMBs that reported respiratory irritation (decreased respiration rates) and neurological effects (decreased pain sensitivity, altered cognitive function, and decreased anxiety and/or increased motor function) that are consistent with effects seen in human studies. Four subchronic inhalation studies for 1,2,3-TMB and 1,2,4-TMB observed exposure-response effects in multiple organ systems, including the nervous, hematological, and respiratory systems (Korsak et al., 2000a, b; Korsak et al., 1997; Korsak and Rydzyński, 1996). In these studies, disturbances in central nervous system (CNS) function, including decreased pain sensitivity and decreased neuromuscular function and coordination, appear to be the most sensitive endpoints following exposure to 1,2,3-TMB or 1,2,4-TMB. No subchronic studies were found that investigated exposure to 1,3,5-TMB. One developmental toxicity study (Saillenfait et al., 2005) observed similar levels of maternal and fetal toxicity (i.e., decreased maternal weight gain and fetal weight) following exposure to either 1,2,4-TMB or 1,3,5-TMB; other indices of fetal toxicity (i.e., fetal death and malformations) were not affected by exposure.

Table ES-1 summarizes the RfCs derived for all three TMB isomers, and the sections that follow provide details on the RfC derivation for each isomer.

Table ES-1. Summary of inhalation reference concentrations (RfCs)

Isomer	Source	Reference value (mg/m^3)	Confidence
1,2,4-TMB	Decreased pain sensitivity	5×10^{-2}	Low-to-medium
1,2,3-TMB	Decreased pain sensitivity	5×10^{-2}	Low-to-medium
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of these isomers	5×10^{-2}	Low

2. Inhalation Reference Concentration (RfC) for 1,2,4-TMB for Effects Other Than Cancer

Table ES-2. Summary of reference concentration (RfC) derivation for 1,2,4-TMB

Critical effect	Point of departure	Uncertainty factor	Chronic RfC (mg/m^3)
Decreased pain sensitivity 90 day male rat study Korsak and Rydzyński (1996)	POD_{HEC} (mg/m^3) = 15.8	300	5×10^{-2}

Decreased pain sensitivity was observed in multiple studies of acute, short-term, and subchronic durations (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b; Korsak and Rydzyński, 1996; Korsak et al., 1995). Given the consistency of this effect and the determination that decreased pain sensitivity is an appropriate adverse effect with which to derive reference values (see Section 2.1.5 of this Toxicological Review), in accordance with the U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (1998), decreased pain sensitivity was selected as the critical effect and Korsak and Rydzyński (1996) was selected as the principal study for derivation of the RfC for 1,2,4-TMB.

The RfC calculation is summarized in Table ES-2. The available rat PBPK model (Hissink et al., 2007) was used to convert the external concentrations (in mg/m^3) from the animal study to the internal blood metric of weekly average venous 1,2,4-TMB concentration (in mg/L). These internal blood metrics were then used as the dose inputs for benchmark dose (BMD) modeling.
A benchmark response (BMR) equal to a 1 standard deviation change in the control mean for decreased pain sensitivity was used. A $BMDL_{1SD}$ of 0.086 mg/L was estimated for decreased pain sensitivity in male rats exposed to 1,2,4-TMB via inhalation for 90 days (6 hours/day, 5 days/week) [data used in model; (Korsak and Rydzyński, 1996)].

The available human PBPK model (Hissink et al., 2007) was then used to estimate a human equivalent concentration (HEC) of 15.8 mg/m^3 from the $BMDL_{1SD}$ of 0.086 mg/L. This HEC was used as the POD_{HEC} with which to derive the RfC. A composite uncertainty factor (UF) of 300 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), 3 to account for subchronic-to-chronic extrapolation due to the use of a subchronic study with effects observed to recover within weeks of exposure termination, and 3 to account for deficiencies in the database (no two-generation reproductive/developmental toxicity or

developmental neurotoxicity studies were available). Dividing the POD_{HEC} by the composite UF of 300 yielded **a chronic RfC of 5×10^{-2} mg/m^3 for 1,2,4-TMB.**

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

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

Confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium. This peer-reviewed study was well designed, using three dose groups plus untreated controls and a typical number of animals per dose group for evaluating neurotoxicity following subchronic exposure.

One area of uncertainty regarding this study is the lack of reported actual concentrations. However, as the methods by which the test atmosphere was generated and analyzed were reported in sufficient detail, and given the fact that this laboratory has used this methodology in subsequent studies (Korsak et al., 2000a, b) and achieved appropriate actual concentrations (i.e., within 10% of target concentrations), the concern regarding the lack of reported actual concentrations is minimal. Another source of uncertainty is the fact that Korsak and Rydzyński (1996) does not explicitly state that the reported measures of variance in Table 1 of that reference are standard deviations. However, careful analysis of the reported levels of variance and magnitude of statistical significance reported indicate that the measures of variance are standard deviations. Supporting this conclusions is the observation that all other papers by Korsak et al. (2000a, b; 1997; 1995) report variance as standard deviations. The critical effect on which the RfC is based is well-supported as the 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 subchronic) (Gralewicz and Wiaderna, 2001; Chen et al., 1999; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996; Norseth et al., 1991).

The database for 1,2,4-TMB includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. However, confidence in the database is low to medium because it lacks chronic, multi-generation reproductive/developmental, and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Consequently, the overall confidence in the RfC for 1,2,4-TMB is low to medium.

4. Inhalation Reference Concentration (RfC) for 1,2,3-TMB for Effects Other Than Cancer

Table ES-3. Summary of reference concentration (RfC) derivation for 1,2,3-TMB

Critical effect	Point of departure	Uncertainty factor	Chronic RfC (mg/m^3)
Decreased pain sensitivity 90 day male rat study Korsak and Rydzyński (1996)	POD_{HEC} (mg/m^3) = 16.3	300	5×10^{-2}

Decreased pain sensitivity was observed in multiple studies of acute, short-term, and subchronic durations (Lutz et al., 2010; Wiaderna et al., 1998; Korsak and Rydzyński, 1996). Given the consistency of this effect and the determination that decreased pain sensitivity is an adverse effect, in accordance with the U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), decreased pain sensitivity was selected as the critical effect and Korsak and Rydzyński (1996) was selected as the principal study for derivation of the RfC for 1,2,3-TMB.

The RfC calculation is summarized in Table ES-3. BMD modeling was used in order to identify the POD for decreased pain sensitivity. A BMR equal to a 1 standard deviation change in the control mean was used. A $BMDL_{1SD}$ of 17.36 mg/m^3 was estimated for decreased pain sensitivity in male rats exposed to 1,2,3-TMB via inhalation for 90 days (6 hours/day, 5 days/week) (Korsak and Rydzyński, 1996).

As no PBPK model was available for 1,2,3-TMB, default dosimetry methodologies were used to estimate the HEC of 16.3 mg/m^3, based on the ratio of the human and animal blood:air partition coefficients (U.S. EPA, 1994b). This POD_{HEC} was used to derive the RfC. A composite uncertainty factor (UF) of 300 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), 3 to account for subchronic-to-chronic extrapolation due to the use of a subchronic study, and 3 to account for deficiencies in the database (no two-generation reproductive/developmental toxicity, developmental toxicity, or developmental neurotoxicity studies were available). Dividing the POD_{HEC} by the composite UF of 300 yielded **a chronic RfC of 5×10^{-2} mg/m^3 for 1,2,3-TMB.**

5. Confidence in the Chronic Inhalation RfC for 1,2,3-TMB

Confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996) is low to medium. This peer-reviewed study was well designed, using three dose groups plus untreated controls and a typical number of animals per dose group for evaluating

neurotoxicity following subchronic exposure. One area of uncertainty regarding this study is the lack of reported actual concentrations. However, as the methods by which the test atmosphere was generated and analyzed were reported in sufficient detail, and given the fact that this laboratory has used this methodology in subsequent studies (Korsak et al., 2000a, b) and achieved appropriate actual concentrations (i.e., within 10% of target concentrations), the concern regarding the lack of reported actual concentrations is minimal. Another source of uncertainty is the fact that Korsak and Rydzyński (1996) does not explicitly state that the reported measures of variance in Table 1 of that reference are standard deviations. However, careful analysis of the reported levels of variance and magnitude of statistical significance reported indicate that the measures of variance are standard deviations. Supporting this conclusions is the observation that all other papers by Korsak et al. (2000a, b; 1997; 1995) report variance as standard deviations.The critical effect on which the RfC is based is well-supported as the evidence for 1,2,3-TMB-induced neurotoxicity is coherent across multiple animals species (i.e., mouse, and rat) and consistent across multiple exposure durations (i.e., acute, short-term, and subchronic) (Lutz et al., 2010; Wiaderna et al., 1998; Korsak and Rydzyński, 1996).

The database for 1,2,3-TMB includes acute, short-term, and subchronic toxicity studies in rats and mice. However, confidence in the database is low to medium because it lacks chronic, multi-generation reproductive/developmental, developmental toxicity, or developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Consequently, the overall confidence in the RfC for 1,2,3-TMB is low to medium.

6. Inhalation Reference Concentration (RfC) for 1,3,5-TMB for Effects Other Than Cancer

No chronic or subchronic studies exist that would support the derivation of an RfC for 1,3,5-TMB, however one developmental toxicity study (Saillenfait et al., 2005) was identified as a potential study from which to identify a critical effect for RfC derivation.

The use of decreased maternal weight gain observed in Saillenfait et al. (2005) as the critical effect for RfC derivation would result in an RfC 20-fold greater than that derived for 1,2,4-TMB (1 mg/m^3 vs. 5×10^{-2} mg/m^3). This large difference is not consistent with the rest of the toxicological database for 1,2,4-TMB and 1,3,5-TMB, which demonstrates that the two isomers are similar to one another with regard to respiratory and developmental toxicity in acute and developmental studies (Saillenfait et al., 2005; Korsak and Rydzyński, 1996; Korsak et al., 1995). The 1,3,5-TMB isomer was observed to induce some measures of neurotoxicity (e.g., passive and active avoidance) at lower doses than 1,2,4-TMB, in short-term studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b). 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, **the chronic RfC of 5 × 10^{-2} mg/m^3 derived for 1,2,4-TMB was adopted as the RfC for 1,3,5-TMB.** This is based on the determination of sufficient similarity with regard to chemical properties, kinetics, and toxicity between the two isomers (see Section 2.3.5).

7. Confidence in the Chronic Inhalation RfC for 1,3,5-TMB

The chronic RfC for 1,2,4-TMB was adopted as the RfC for 1,3,5-TMB; thus, confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium (see above). The database for 1,3,5-TMB includes acute, short-term, and developmental toxicity studies in rats and mice. However, confidence in the database is low to medium because it lacks chronic, subchronic, multi-generation reproductive/developmental toxicity, and developmental neurotoxicity studies and most of the studies supporting the critical effect come from the same research institute.

Reflecting the confidence in the study and the database and the uncertainty surrounding the adoption of the RfC derived for 1,2,4-TMB as the RfC for 1,3,5-TMB, the overall confidence in the RfC for 1,3,5-TMB is low.

8. Effects Other Than Cancer Observed Following Oral Exposure

Only one subchronic study was identified that examined the effects of oral exposure to 1,3,5-TMB. Effects in the hematological system, including changes in clinical chemistry parameters and differential white blood cell numbers, were observed following exposure to 1,3,5-TMB via oral gavage. Ultimately, the Koch Industries (1995b) study was determined to not be suitable for RfD derivation following an external peer review of the study (see Appendix F). No other subchronic studies were found that investigated the effects of oral exposure to 1,2,4-TMB or 1,2,3-TMB, and no chronic oral studies were found that investigated noncancer effects of any of the TMB isomers.

A series of studies utilizing single exposures (oral gavage or i.p. injection) were identified that investigated the acute neurotoxic effects of TMBs (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 altered locomotor activity in open field tests. As these effects were only observed in studies investigating acute exposures, they were considered insufficient for derivation of oral toxicity reference values.

Therefore, given that Koch Industries study was not suitable for RfD derivation and effects from acute studies generally are not suitable for derivation of chronic health values, RfDs were derived for 1,2,4-TMB using route-to-route extrapolation and for 1,2,3-TMB and 1,3,5-TMB based on sufficient similarity.

Table ES-4 below summarizes the RfDs derived for all three TMB isomers, and the sections that follow provide details on the derivation of the RfD for each isomer.

Table ES-4. Summary of reference doses (RfDs) for TMB isomers

Isomer	Source	Reference value	Confidence
1,2,4-TMB	Route-to-route extrapolation from RfC for 1,2,4-TMB	2×10^{-2}	Low
1,2,3-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of these isomers	2×10^{-2}	Low
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of these isomers	2×10^{-2}	Low

9. Oral Reference Dose (RfD) for 1,2,4-TMB for Effects Other Than Cancer

Table ES-5. Summary of reference dose (RfD) derivation for 1,2,4-TMB

Critical effect	Point of departure	Uncertainty factor	Chronic RfD (mg/kg-day)
Decreased pain sensitivity 90 day male rat study Korsak and Rydzyński (1996)	Route-to-route extrapolation using Korsak and Rydzyński (1996) subchronic inhalation study in Wistar rats POD_{HED} (mg/kg-day) = 6.3	300	2×10^{-2}

A human 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 (Section B.3.3.5, Appendix B). The RfD calculation is summarized in Table ES-5. Under the assumption of constant oral ingestion and 100% absorption of 1,2,4-TMB via constant infusion rate into the liver, a POD_{HED} of 6.3 mg/kg-day was derived. Hepatic first-pass metabolism was also evaluated in humans using the modified PBPK model: following 50 days of 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 (see Figure B-18, Appendix B). The same composite UF of 300 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), 3 to account for subchronic-to-chronic extrapolation due to the use of a subchronic study, and 3 to account for deficiencies in the database (no multi-generation reproductive/developmental toxicity or developmental neurotoxicity studies). Dividing the POD_{HED} by the composite UF of 300 yielded **a chronic RfD of 2×10^{-2} mg/kg-day for 1,2,4-TMB.**

10. Confidence in the Chronic Oral 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 low to medium (see above). The database for 1,2,4-TMB includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. However, confidence in the database for 1,2,4-TMB is low to medium because it lacks chronic, multi-generation reproductive/developmental and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute.

Reflecting the confidence in the study and the database and the uncertainty surrounding the application of the available PBPK model for the purposes of a route-to-route extrapolation, the overall confidence in the RfD for 1,2,4-TMB is low.

11. Oral Reference Dose (RfD) for 1,2,3-TMB for Effects Other Than Cancer

The oral database is inadequate to derive an RfD for 1,2,3-TMB. No chronic, subchronic, or short-term oral exposure studies were found in the literature. However, as discussed in Sections 1.1.6 and B.2, the toxicokinetic and toxicity similarities between 1,2,3-TMB and 1,2,4-TMB support adopting the RfD for 1,2,4-TMB as the RfD for 1,2,3-TMB. 1,2,3-TMB is observed to elicit the same neurotoxic effects in rats (decreased pain sensitivity) as 1,2,4-TMB following subchronic inhalation exposures, and the calculated RfCs for these two isomers are equal: 5×10^{-2} mg/m^3. In addition to the outlined similarities in toxicokinetics, the qualitative metabolic profiles for the two isomers are similar such that first-pass metabolism through the liver is not expected to differ greatly between 1,2,4-TMB and 1,2,3-TMB. Therefore, **the chronic RfC of 2×10^{-2} mg/kg-day derived for 1,2,4-TMB was adopted as the RfD for 1,2,3-TMB** based on the determination of sufficient similarity between the two isomers with regard to chemical properties, toxicokinetics, and toxicity.

12. Confidence in the Chronic Oral RfD for 1,2,3-TMB

The chronic oral RfD for 1,2,4-TMB was adopted as the chronic oral RfD for 1,2,3-TMB; thus, confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium (see above). The database for 1,2,3-TMB includes acute, short-term, and subchronic studies in rats and mice. However, confidence in the database is low to medium because

it lacks chronic, multi-generation reproductive/developmental, developmental toxicity, or developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,2,3-TMB, the overall confidence in the RfD for 1,2,3-TMB is low.

13. Oral Reference Dose (RfD) for 1,3,5-TMB for Effects Other Than Cancer

The oral database is inadequate to derive an RfD for 1,3,5-TMB. No chronic, oral exposure study was found in the literature. However, one subchronic oral gavage study was identified that observed effects on multiple clinical chemistry parameters and monocyte levels (Koch Industries, 1995b). However, following an external peer review of this study (see Appendix F), it was concluded that the Koch Industries (1995b) study was not suitable as the basis for quantifying the noncancer human health risk following oral exposure. The most critical shortcoming of this study was its failure to investigate the neurotoxicity of 1,3,5-TMB.

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 2×10^{-2} 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 between the two isomers with regard to chemical properties, toxicokinetics, and toxicity.

Confidence in the Chronic Oral RfD for 1,3,5-TMB

The chronic oral RfD for 1,2,4-TMB was adopted as the chronic oral RfD for 1,3,5-TMB; thus confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium (see above). The database for 1,3,5-TMB includes acute, short-term, and developmental toxicity studies in rats and mice. However, confidence in the database is low to medium because it lacks chronic, multi-generation reproductive/developmental and developmental neurotoxicity studies, and the studies supporting the critical effect predominately come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,3,5-TMB, the overall confidence in the RfD for 1,3,5-TMB is low.

14. Evidence of Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess carcinogenic potential" of TMBs. No chronic inhalation studies

that investigated cancer outcomes were identified in the literature for 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. One cancer study in which rats were exposed to 1,2,4-TMB via oral gavage at one experimental dose of 800 mg/kg-day observed marginal increases in total malignant tumors and head tumors (e.g., neuroesthesioepitheliomas), but provided no statistical analyses of the results (Maltoni et al., 1997). A number of methodological issues limit the utility of this study (e.g., only one dose group and 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 elevations were observed. Therefore, **a quantitative cancer assessment for TMBs was not conducted.**

15. Susceptible Populations and Lifestages

No chemical-specific data that would allow for the identification of populations or lifestages with increased susceptibility to TMB exposure exist. However, some inferences can be made based on the toxicokinetics of TMB isomers. 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 and rates of glucuronidation and sulfation conjugation are reduced in children up to 1 year in age, and renal clearance is reduced in infants up to 2 months of age (Ginsberg et al., 2004). Therefore, as CYP P450 mono-oxygenase activities, the rate of glucuronidation and sulfation, and renal clearance appear to be decreased in early life, newborns and young infants may experience higher and more persistent blood concentrations of 1,2,3-TMB, 1,2,4-TMB, 1,3,5-TMB, and/or their respective metabolites compared with adults at similar exposure levels. Additionally, those with pre-existing respiratory diseases (e.g., asthma) may be more sensitive to the respiratory irritative and inflammatory effects resulting from exposure to TMB isomers.

16. Key Issues Addressed in the Assessment: Adoption of 1,2,4-TMB Toxicity Values for the 1,3,5- and 1,2,3-TMB Isomers

The toxicity database for 1,3,5-TMB was inadequate for derivation of either a reference concentration or a reference dose. The chemical, toxicokinetic, and toxicological properties of the individual isomers are sufficiently similar to one another to support adoption of 1,2,4-TMB's reference values for 1,3,5-TMB (see Section 2.3.5). Both isomers are similar in their (1) chemical properties (e.g., blood:tissue partition coefficients), (2) toxicokinetic properties (i.e., absorption, metabolism, and excretion profiles), and (3) toxicity profiles across studies utilizing multiple durations of exposure and multiple endpoints (i.e., neurological, respiratory, maternal, and fetal effects). Therefore, given these similarities, the RfC and RfD derived for 1,2,4-TMB were adopted as the RfC and RfD for 1,3,5-TMB.

The toxicity database for 1,2,3-TMB was inadequate for derivation of a reference dose. No chemical-specific PBPK model is available for 1,2,3-TMB, and therefore, no route-to-route extrapolation can be performed on which to derive an RfD from the RfC for 1,2,3-TMB. The chemical, toxicokinetic, and toxicological properties of the individual isomers are sufficiently similar to one another to support adoption of 1,2,4-TMB's reference dose for 1,2,3-TMB (see Section 2.5.2). Both isomers are similar in their (1) chemical properties (e.g., blood:air and tissue:air partition coefficients), (2) toxicokinetic properties (i.e., the degree of absorption into the bloodstream between the two isomers indicates the internal blood dose metrics for 1,2,3-TMB would be similar to those calculated for 1,2,4-TMB by that isomer's available PBPK model), and (3) toxicity profiles (i.e., the observation that both isomers affected pain sensitivity to an equal degree and that the two isomer's RfCs for this effect were equal). Therefore, given these similarities, the deficiencies in the 1,2,3-TMB oral database, and the lack of a 1,2,3-TMB PBPK model with which to perform a route-to-route extrapolation, the RfD derived for 1,2,4-TMB was adopted as the RfD for 1,2,3-TMB.

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

The literature search strategy used to identify primary, peer-reviewed literature pertaining to TMBs was conducted using the databases and keywords listed in Table LS-1. References from health assessments developed by other national and international health agencies were also examined. Other peer-reviewed information, including review articles, literature necessary for the interpretation of TMB-induced health effects, and independent analyses of the health effects data were retrieved and included in the assessment where appropriate. EPA requested public submissions of additional information in April 2008; no submissions in response to the data call-in were received. A comprehensive literature search was last conducted in December 2011.

Figure LS-1 depicts the literature search and study selection strategy and the number of references obtained at each stage of the literature screening. Approximately 4,300 references were obtained from the chemical name, keyword, and metabolite searches for 1,2,4-TMB, 1,2,3-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). From this full list of references, there were 218 references that were considered for inclusion in the Toxicological Review.

Selection of studies for inclusion in the Toxicological Review was based on consideration of the extent to which the study was informative and relevant to the assessment and general study quality considerations. In general, the relevance of health effect studies was evaluated as outlined in the Preamble and EPA Guidance (*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, 1994b)). From the list of "considered" references, 161 full text publications were identified as providing relevant information for use in the development of this document, and included 30 studies in humans (e.g., occupational epidemiologic studies, workplace exposure studies, and controlled human exposures), 63 inhalation or oral animal studies, and 68 other studies (e.g., studies that provided supporting information on mode of action, chemical properties, and susceptible subpopulations).

The references that are cited in the document, as well as those that were considered but not included in the Toxicological Review of TMBs, can be found within the Health and Environmental Research Online (HERO) website[3]. This site contains HERO links to lists of references, including bibliographic information and abstracts, which were considered for inclusion in the Toxicological Review of TMBs.

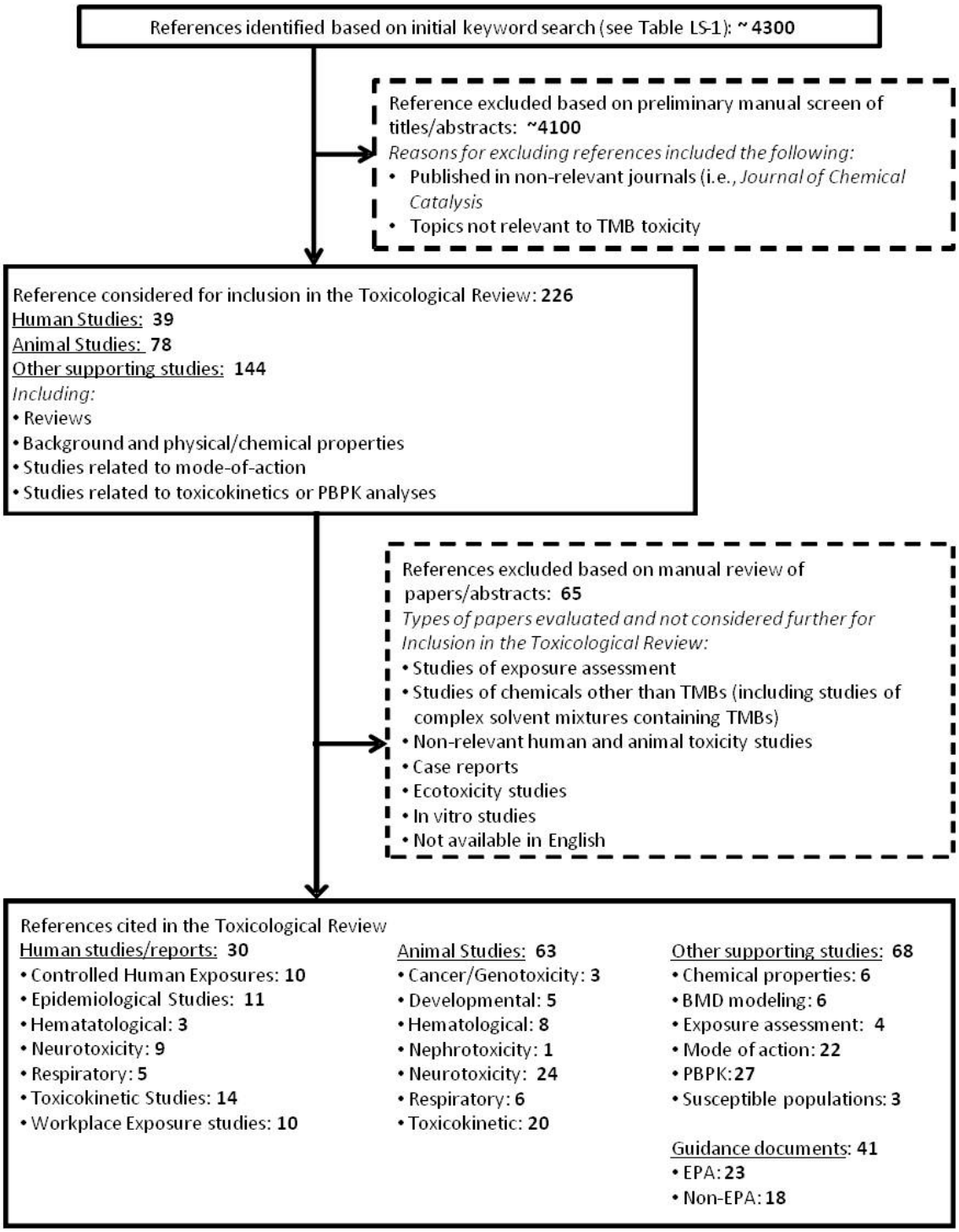
Table LS-1: Details of the search strategy employed for TMBs

Databases	Keywords[a,b]
EBSCO DISCOVERY SERVICE: HERO SCI NLM TOXLINE WOS	**Chemical name, CASRN, and synonym search:** 1,2,4-trimethylbenzene, OR pseudocumene, OR 95-63-6; 1,2,3-trimethylbenzene, OR hemimellitene, OR 526-73-8; 1,3,5-trimethylbenzene, OR mesitylene, OR 108-67-8 **Keyword search:** neurotoxicity, genotoxicity, developmental toxicity, inflammation, irritation, toxicokinetics, pbpk, mode of action, white spirit, C9, C9 fraction, JP-8 **Additional search on specific metabolites:** 2,3-dimethylbenzoic acid, OR 26998-80-1; 2,3-dimethylhippuric acid, OR 187980-99-0; 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; 2,6-dimethylbenzoic acid OR 632-46-2; 2,6-dimethylhippuric acid OR 187980-98-9; 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-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

[a]Potentially relevant publications on TMBs were identified through a literature search conducted with the EBSCO Discovery Service feature of Health and Environmental Research Online (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).

[b]Literature search was performed using related words (i.e., lemmatization) of included search terms. Search terms were entered into the EBSCO Discovery Service portal with no qualifiers and the results from individual search engines were returned and exported to HERO.

[3] HERO is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 600,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.



Note: Some references may provide information on more than one topic, and therefore, may be included in more than one study type. Accordingly, the sum of the references for subcategories of studies is not expected to equal the number of references for the larger category.

Figure LS-1. Literature search and study selection strategy for TMBs.

1. HAZARD IDENTIFICATION

1.1. Synthesis of Evidence

1.1.1. Neurological Effects

There is evidence in humans and animals that inhalation exposure to trimethylbenzenes (TMBs) induces neurotoxic effects. The human evidence comes from occupational studies involving complex volatile organic compound (VOC) mixtures that include TMBs; thus, effects cannot be attributed to any TMB isomer specifically. Prevalence rates of neuropsychological symptoms increased with exposure duration in dockyard painters, with symptoms related to motor coordination exhibiting the strongest association (Chen et al., 1999); similarly, a significant association between exposure and impaired performance in short term memory (symbol digit substitution) and motor speed/ coordination (finger tapping) tests was observed in shipyard painters exposed to TMBs (isomers were not specified) and other solvents (Lee et al., 2005). A significant, positive association between exposure symptoms (e.g., abnormal fatigue) and 1,2,4-TMB exposure, but not exposure to lower levels of 1,2,3-TMB or 1,3,5-TMB, 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, 30% 1,3,5-TMB, and unspecified amounts of 1,2,3-TMB (listed as possibly present) (Battig et al. (1956), as reviewed by MOE (2006) and Baettig et al. (1958)).

Additional evidence suggests damage or dysfunction of the inner ear and increased occurrence of vertigo following exposure to TMBs and other organic solvents in paint and varnish factories (Sulkowski et al., 2002). Increased reaction time was significantly and consistently associated with exposure 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 TMBs was reported in human volunteers exposed for 2 hours to either: 300 mg/m^3 white spirit (WS, corresponding to 11 mg/m^3 1,2,4-TMB); 11 or 123 mg/m^3 1,2,4-TMB; 123 mg/m^3 1,2,3-TMB; or 123 mg/m^3 1,3,5-TMB. However, effects on the central nervous system (CNS) were based on measures of overt CNS depression (heart rate and pulmonary ventilation) and a subjective rating of CNS symptoms (i.e., headache, fatigue, nausea, dizziness, and intoxication) (Järnberg et al., 1997a; Järnberg et al., 1996). For full details of the epidemiologic and controlled human exposures studies

(including human subjects research ethics procedures), see individual study summary tables in Appendix B.

In two studies examining the toxicokinetics of TMBs following controlled human exposures to 5–150 mg/m^3 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB, no neurological abnormalities in routine clinical examinations were reported following exposure, although results data or details regarding the specific tests performed were not provided (Kostrzewski et al., 1997; Kostrewski and Wiaderna-Brycht, 1995). Studies identifying an association between occupational exposure to TMB isomers and neurological effects are limited due to an inability to attribute effects due to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB individually versus those due to the other isomers or additional constituents within the mixture. The studies detailing controlled exposures to human volunteers are also limited for evaluating neurotoxicity to TMBs due to a lack of methods to adequately assess CNS function and a lack of no-exposure controls, short exposure duration, and exposure of individual subjects to different concentrations of TMB isomers.

In animals, there is consistent evidence of neurotoxicity following inhalation exposure, and to a lesser extent following oral exposure, to either 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB; a summary of the evidence pertaining to neurotoxic effects for TMBs is shown in Tables 1-1 and 1-2 for inhalation and oral exposures, respectively. This information is presented graphically in Figures 1-1 to 1-4.

Pain sensitivity

Decreased pain sensitivity has been observed following inhalation exposure to TMBs in multiple studies conducted in male Wistar rats (Table 1-1; Figures 1-1 – 1-3). 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. In short-term exposure studies, the animals were subjected to an additional environmental challenge, namely foot shock, which itself decreases pain sensitivity. Short-term TMBs exposure without the foot shock challenge did not result in statistically significant effects on pain sensitivity in the hot plate test several weeks after exposures had ended. Decreases in pain sensitivity have been observed at concentrations ≥ 123 mg/m^3 or ≥ 492 mg/m^3 following subchronic exposure to 1,2,4-TMB or 1,2,3-TMB, respectively (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Korsak and Rydzyński, 1996). Decreased pain sensitivity after a foot shock challenge was observed at concentrations ≥ 492 mg/m^3 following short-term exposure to 1,2,4-TMB (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b), 1,3,5-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001), or 1,2,3-TMB (Wiaderna et al., 1998; Korsak and Rydzyński, 1996), although changes were not observed at 492 mg/m^3 1,2,3-TMB (latencies 75% longer than controls were not statistically significant) in another short-term exposure study (Gralewicz and Wiaderna, 2001).

In the subchronic study (Korsak and Rydzyński, 1996), inhalation of 1,2,4-TMB or 1,2,3-TMB 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, 1998; Gralewicz et al., 1997b), decreases in pain sensitivity after foot shock challenge following exposure to TMB isomers were non-monotonic. Differences in experimental design (discussed below) may account for the lack of monotonicity in these short-term studies, in contrast to the observations in Korsak and Rydzyński (1996). Similar to the subchronic study, acute exposures to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB induced concentration-dependent decreases in pain sensitivity, with EC_{50} values of 4,172, 5,682,and 5,963 mg/m^3 for increased latency to paw-lick compared to controls, respectively (Korsak and Rydzyński, 1996; Korsak et al., 1995).

The decreases in pain sensitivity measured in the subchronic and acute studies were observed immediately after exposure (Korsak and Rydzyński, 1996; Korsak et al., 1995), with no significant effects persisting 2 weeks after subchronic exposures were terminated (i.e., increases in latency were reduced from 95 to 12% or from 78 to 13% of controls at 1,230 mg/m^3 1,2,4- or 1,2,3-TMB, respectively) (Korsak and Rydzyński, 1996; Korsak et al., 1995). In contrast, performance in the hot plate test after foot shock challenge was significantly impaired following short-term exposure to the TMB isomers when tested 51 days after exposure (Wiaderna et al., 1998) (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b), indicating a persistence of these pain sensitivity- related effects.

The addition of a foot shock challenge to the hot plate tests following short-term (i.e., 4-week), inhalation exposure to TMB isomers makes these experiments somewhat distinct from those performed following subchronic exposure, as the foot shock challenge can elicit a cognitive response from the animals in later hot plate test trials (see below)(Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b). In the short-term studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b), treatment-related, statistically significant changes at ≥ 492 mg/m^3 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB were observed 24 hours after rats were given a foot shock; no consistent, significant effects at any concentration were observed immediately following foot shock. Additionally, no statistically significant effects were observed prior to foot shock at 50 days post-exposure; studies did tend to observe increases in latency in non-shocked rats that were not statistically significant at ≥ 492 mg/m^3 1,2,4-TMB (up to 206% longer than controls), 1,3,5-TMB (up to 215% longer than controls), or 1,2,3-TMB (up to 95% longer than controls), but these responses were highly variable and not consistently observed across studies. As foot shock alone is known to cause transient reductions in pain sensitivity, these findings suggest that inhalation exposure to TMBs prolongs foot shock-induced reductions in pain sensitivity. However, although a lengthening of the foot shock-induced decrease in pain sensitivity by TMBs exposure is the most likely reason for the observed effects, and, accordingly, these responses are discussed in this context herein, this is not

the only possible explanation. It is also plausible that cognitive effects resulting from TMBs exposure could contribute to the responses observed 24 hours after foot shock. Specifically, control groups may better associate the hot plate environment with the previously-applied aversive stimulus and more quickly withdraw their paws than their TMB-exposed counterparts, who may exhibit a decreased fear response or shorter retention of that fear-associated memory. Alternatively, since this test paradigm can cause the hot plate test apparatus to become associated with the effects of foot shock, inducing stress-related responses in the shocked animal such that subsequent exposure to the hot plate test apparatus alone can reduce sensitivity to pain (possibly via the release of endogenous opioids), prior TMBs exposure could amplify this effect. From the data available, the relative contribution(s) of these behaviors to the observed effects cannot be easily distinguished. Despite the possible overlap between contributing neurological processes in this test paradigm, these observations are still regarded as significant and adverse, and clearly indicate a persistence of neurological effects long after TMBs exposures have ceased.

Substantial differences in study design between short-term and subchronic studies make it impossible to distinguish the particular aspects of the pain sensitivity phenotype that appear to be latent and only manifest with an environmental challenge from those that appear to be reversible. Regardless, 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. The overall database indicates that TMB isomers are similar in their capacity to decrease pain sensitivity following inhalation exposure (Table 1-1; Figures 1-1 – 1-3). Pain sensitivity was not examined following oral exposure.

Neuromuscular function and coordination

Human exposures to solvent mixtures containing 1,2,4-TMB (Lammers et al., 2007) or multiple TMB isomers [(Battig et al., 1956), as reviewed by MOE (2006) and (Lee et al., 2005; Sulkowski et al., 2002; Bättig et al., 1958)] result in effects that suggest alterations to neuromuscular function and balance, including increased reaction time and vertigo. Animal studies using rotarod performance, which tests motor coordination, balance, and overall neuromuscular function, indicate that inhalation of TMB isomers can affect neuromuscular system function (Table 1-1; Figures 1-1 and 1-2). Significant decreases in rotarod performance were observed at 1,230 mg/m^3 1,2,4-TMB and ≥ 493 mg/m^3 1,2,3-TMB when tested immediately after exposure for 13 weeks (Korsak and Rydzyński, 1996); significant decreases in performance were also observed at 1,230 mg/m^3 after 4 or 8 weeks of exposure to 1,2,3-TMB or 1,2,4-TMB, respectively. This impaired function was still evident at 2 weeks post-exposure and, while not statistically significant for 1,2,4-TMB, may indicate long-lasting neuromuscular effects of subchronic exposures to 1,2,4-TMB and 1,2,3-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 1,250–45,000 mg/m^3 1,2,4-TMB (MOE, 2006; Henderson, 2001). Similarly, acute exposure to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB resulted in decreased performance in rotarod tests immediately following

exposure, with EC_{50} values of 3,779 mg/m^3, 4,693 mg/m^3, and 4,738 mg/m^3, respectively (Korsak and Rydzyński, 1996; Korsak et al., 1995). These results indicate that 1,2,4-TMB and 1,3,5-TMB are similar in their ability to impair neuromuscular function, balance, and coordination while 1,2,3-TMB exposure may elicit effects at lower concentrations compared to the other two isomers. 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 Rydzyński, (1996) and Korsak et al. (1995) appear to have been conducted 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, see Table B-30, Appendix B) 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 Rydzyński, (1996) and Korsak et al. (1995) are supported by 2- to 3-fold increases in latency to paw-lick that, although not statistically significant, were observed 50 days after termination of short-term exposures to 492 mg/m^3 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB (Gralewicz and Wiaderna, 2001); increases of this magnitude were not present in the studies evaluating multiple concentrations of the isomers (Wiaderna et al., 2002, 1998; Gralewicz et al., 1997b).

Motor function and/or anxiety

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, but not 1,2,3-TMB, in male rats (Table 1-1; Figures 1-1–1-3); however, open field locomotion following injections with the stimulant, amphetamine, were amplified by prior 1,2,3-TMB exposure, but not by prior 1,2,4-TMB exposure (Lutz et al., 2010) Altered behaviors and locomotion in open field tests can be attributed to anxiety responses due to open spaces and bright light, as well as changes to motor system function. Factors other than anxiety and motor function (e.g., interpretation of olfactory or visual cues) may also contribute to alterations in open field behavior, but these are unlikely to be drivers of the responses. As all but one of the studies (Lutz et al., 2010) observed animals for only 5 or 10 minutes, these experimental tests are insufficient to identify the relative contribution(s) of the various neurological responses to the observed increases in open field behaviors. Thus, EPA has concluded that decreased anxiety and/or increased motor function are the most likely explanations for the TMB-induced effects.

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 (Lutz et al., 2010; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b). Statistically significant increases in horizontal locomotion were observed in short-term studies assessing open field behavior following inhalation exposure to 1,2,4-TMB or 1,3,5-TMB

(Lutz et al., 2010; Gralewicz and Wiaderna, 2001). Non-monotonic increases in grooming were reported following short-term exposure to 1,2,4-TMB, although changes in horizontal locomotion were not statistically significant (increases of 3–35% were also non-monotonic) (Gralewicz et al., 1997b). No statistically significant effects on open field activity have been observed following short-term exposure of male rats to 1,2,3-TMB (Lutz et al., 2010; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998). Open field locomotion following injections with the stimulant amphetamine was amplified by previous short-term exposure to 1,2,3-TMB, but not 1,2,4-TMB (which actually tended to inhibit amphetamine-induced increases in activity at 492 mg/m^3), suggesting possible effects of 1,2,3-TMB on sensitization-type responses. As open field testing was conducted 14 or 25 days after termination of exposure in these studies and TMB isomers are cleared rapidly from the body following the end of inhalation exposures (Section B.2, Appendix B), the results suggest persistence of the effects of 1,2,4-TMB and 1,3,5-TMB on anxiety and/or motor function following clearance of the toxic moiety from the nervous system.

Slight, transient increases in locomotor activity were also observed in open field tests immediately following acute, oral exposure to the TMB isomers (Table 1-2; Figure 1-4). Significant increases in locomotor activity—measured as number of squares crossed after exposure compared with prior to exposure—were observed at 3,850 mg/kg for 1,2,4-TMB and 1,2,3-TMB, and at ≥ 1,920 mg/kg for 1,3,5-TMB, with minimal concentration-effect or time-effect relationships and negligible differences in the magnitude of the change in activity between isomers (Tomas et al., 1999b). Increases in locomotor activity were biphasic in nature. At early timepoints following exposure, increased locomotor activity was associated with perturbed motor coordination and tremor, whereas after 90 minutes, this apparent motor ataxia progressed to hind limb paralysis, full immobility, and respiratory distress (e.g., tachypnea), leading to several deaths by 24 hours (Tomas et al., 1999b).

As mentioned previously, 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 inconsistent with observations in open field tests. Decreased motor activity was observed in male rats immediately after exposure to 5,000 mg/m^3 1,2,4-TMB (McKee et al., 2010). Decreased motor activity was also reported in rats acutely exposed via inhalation 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 individual isomers. 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, as well as the differing isomer concentrations, 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. The results for 1,2,3-TMB are difficult to interpret, as no effects were observed following short term inhalation exposure while acute oral exposure elicited responses consistent with 1,2,4-TMB and 1,3,5-TMB. Although an explanation for this disparity is lacking, these data highlight a potential difference between 1,2,3-TMB and the other isomers, regarding altered motor function and/or anxiety.

Cognitive function

Cognitive function following exposure to TMB isomers alone has not been evaluated in humans or following oral exposure in animals; controlled 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 1,2,4-TMB or 1,3,5-TMB via inhalation in animal studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b). Similarly, although one study indicates a significant decrement in radial maze performance following exposure to 123 mg/m^3 1,2,3-TMB t(Wiaderna et al., 1998), higher concentrations had no effect(Wiaderna et al., 1998), preventing interpretations regarding the significance of this finding. In contrast, effects on cognitive function in passive and active avoidance tests of conditioning behaviors were consistently observed across multiple studies in male rats 6-8 weeks following short-term inhalation exposure to the TMB isomers, although clear concentration-effect relationships were not observed (Table 1-1; Figures 1-1–1-3). Comparing the results of the behavioral tests reveals that there are differences in cognitive effects reported for each TMB isomer, as well as differences in the concentrations at which the cognitive effects were observed.

In the passive avoidance tests, rats were conditioned to avoid stepping down from a small, elevated platform (the impulse of rats is to step down in order to escape the bright light and constrained, elevated space of the platform) through the use of a brief series of foot shocks applied on the lower level. It is important to clarify that these tests are distinct from tests of pain sensitivity and that observations of decreased step down latency in these tests do not contrast with the increases in paw lick latency observed in hot plate tests; in fact, they may be complementary (see below; note: the foot shocks used are of a much shorter duration than those used to induce decreased pain sensitivity in the hot plate tests). Decreases in step-down latency in passive avoidance tests, particularly at 7 days following foot shock conditioning, were observed 6-7 weeks after short-term inhalation exposure to ≥ 123 mg/m^3 1,2,3-TMB and 1,3,5-TMB or ≥ 492 mg/m^3 1,2,4-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b). Differences in latency prior to foot shock were not observed. Decreases in latency were consistently observed and similar in magnitude across all studies at 7 days post foot shock, although the decreases were not statistically significant for 1,2,4-TMB or 1,2,3-TMB in the study by Gralewicz and Wiaderna (2001). At 3 days post-foot shock, decreases in latency were less consistent (i.e., statistically significant decreases were observed at 123 mg/m^3 1,2,3-TMB and at

492 mg/m^3 1,2,4-TMB, but not at other concentrations, and were not observed following exposure to 1,3,5-TMB), and only 123 mg/m^3 1,2,3-TMB was shown to have an effect at 1 day post-foot shock. In these tests, the effects occurring several days following conditioning with foot shock are most likely attributable to a reduced ability to inhibit motor reactions (or a lowered motor threshold) in response to the fear-inducing environment. Alternative explanations involve possible contributions of the following in the TMBs exposed rats: a diminished fear response to the foot shock; decreased pain sensitivity leading to a less effective negative reinforcement by the (less painful) foot shock; or diminished retention of the fear-associated memory (i.e., from the foot shock). However, as statistically significant changes were observed ≤ 24 hours following foot shock only after exposure to 123 mg/m^3 1,2,3-TMB, neither diminished fear responses to the foot shock nor decreases in pain sensitivity are likely to be the sole driver(s) of these effects. This suggests that, in this particular test paradigm, TMBs exposure causes latent effects on neurological functions associated with the persistence of adaptive behaviors to a fear-inducing stimulus. Despite the consistency of the results at 7 days post-foot shock, these tests are insufficient to pinpoint whether the effects of TMBs exposure are specific to diminished memory retention, increased impulsivity, and/ or decreased motor control.

Reduced performance in two-way active avoidance tests was observed in male rats following short-term inhalation exposure to ≥ 492 mg/m^3 1,2,4-TMB (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b), ≥ 123 mg/m^3 1,3,5-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001), and at 492 mg/m^3 1,2,3-TMB (Wiaderna et al., 1998);. The effects of TMBs were particular to the learning component of the test (acquisition/ training session), rather than the memory component (retention session 7 days later) (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998). The conditioning or training of active avoidance behaviors was based on avoiding a painful foot shock (the unconditioned stimulus) upon presentation of a tone (conditioned stimulus). Similar to the interpretation of results from passive avoidance tests, it is unclear whether and to what extent potential alterations in locomotor activity (rats had to shuttle between compartments) and/ or pain sensitivity following exposure to TMB isomers could contribute to learning deficits in these tests.

Acute inhalation exposure studies provide some support for the observed effects of TMB isomers 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 5,000 mg/m^3 1,2,4-TMB (McKee et al., 2010) or to 4,800 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 the TMB isomers. 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 and 1,2,3-TMB, as the effects following exposure to 1,3,5-TMB were more consistent and sometimes occurred at lower concentrations than those

reported following exposure to the other two isomers. Overall, however, these differences were slight.

Controlled human exposure studies suggest that exposures of ≤ 123 mg/m^3 of the TMB isomers do not cause overt CNS depression (measured as heart rate and respiration) (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 mice, CNS depression has been observed following acute inhalation exposure to > 25,000 mg/m^3 1,3,5-TMB, with similar effect levels for 1,2,4-TMB (ACGIH, 2002).

Electrocortical activity

Neurophysiological evidence from short-term inhalation studies in animals, as well as supportive evidence from acute oral and injection studies, suggests that exposures to TMB isomers at lower concentrations (at least for 1,2,4-TMB) may affect parameters associated with brain excitability. Decreases in a particular component of electrocortical arousal (i.e., spike-wave discharge, SWD, bursts 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 (statistically significant at 1,230 mg/m^3), suggesting persistent functional changes in the rat CNS (Gralewicz et al., 1997a). Altered EEG patterns can be induced by anesthetics as well as stimuli that produce arousal, and may precede other measures of neurotoxicity (U.S. EPA, 1998). In recordings from rats that were awake, but immobile (not exhibiting pronounced exploratory activity, as determined by EEG morphology), statistically significant decreases in the frequency of SWD episodes were observed at 24 hours following short-term exposure to 492 mg/m^3 1,2,4-TMB (decreases that were not statistically significant were also observed at ≥492 mg/m^3 1,2,4-TMB at 30 and 120 days after exposure) (Gralewicz et al., 1997a).

Complementing these findings, dose-related decreases in the duration and number of SWD bursts (termed high-voltage spindles) were observed at ≥ 240 mg/kg of the TMB isomers subsequent to acute oral exposure (Tomas et al., 1999a) (Table 1-2; Figure 1-4). The stronger and more persistent effects on electrocortical activity followed a pattern of 1,2,3-TMB > 1,3,5-TMB > 1,2,4-TMB (Tomas et al., 1999a). Similarly, electrophysiological alterations in cortical and hippocampal EEGs were more pronounced following i.p. injection of 1,2,3-TMB, with 1,2,4-TMB and 1,3,5-TMB exerting lesser effects (Tomas et al., 1999c). Although it is unclear whether these changes affect related processes such as memory and seizure initiation/propagation, the observed EEG abnormalities following inhalation (Gralewicz et al., 1997a), oral (Tomas et al., 1999a), and i.p. (Tomas et al., 1999c) exposure to TMB isomers provide supportive evidence of possible acute CNS depression by TMB isomers (Tomas et al., 1999a; Tomas et al., 1999c) and indicate persistent (up to 120 days post-exposure) (Gralewicz et al., 1997a) alterations in CNS activity that may reflect an adaptive response to TMB exposure.

Neurological effects: Inhalation

Table 1-1. Evidence pertaining to neurological effects of TMBs in animals — inhalation exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)
1,2,4-TMB	
Pain sensitivity	
0, 123, 492, 1,230 mg/m^3, (recovery: 1,230 mg/m^3 at 2 wks post-exposure) 90 day; Rat, Wistar, male, N = 10 Korsak and Rydzyński (1996) Table B-30[c]	Hot plate- exposure-dependent increase in paw-lick latency which recovers by 2 weeks post-exposure: *Response after exposure:* 0, 18, 79*, 95*% *Response at 2 weeks post-exposure:* 0, ND, ND, 12%
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Hot plate- increased paw-lick latency 24 hr after foot shock: *Response at 50 days post-exposure:* 0, 206% *Response at 50 days post-exposure seconds after foot shock:* 0, 25% *Response at 51 days post-exposure 24hr after foot shock:* 0, 191*%
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 15 Gralewicz et al. (1997b), Table B-24	Hot plate- increased paw-lick latency 24 hr after foot shock[d]: *Response at 50 days post-exposure:* 0, -6, 7, -9% *Response at 50 days post-exposure seconds after foot shock:* 0,-8, 17, -11% *Response at 51 days post-exposure 24 hr after foot shock:* 0, 2, 74*, 33*%
Neuromuscular function and coordination	
0, 123, 492, 1,230 mg/m^3, (recovery: 1,230 mg/m^3 at 2 wks post-exposure) 90 day; Rat, Wistar, male, N = 10 Korsak and Rydzyński (1996), Table B-30	Rotarod- exposure-dependent increase in failures at 13 weeks which does not recover by 2 weeks post-exposure: *Response after 13 weeks of exposure:* 0, 10, 20, 40*% *Response at 2 weeks post-exposure:* 0, ND, ND, 30%
Motor function and/or anxiety	
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 15 Lutz et al. (2010), Table B-35	Open field- increased horizontal locomotion (distance traveled); no overall effects with amphetamine challenge[e]: *Response at 2 weeks post-exposure with no challenge:* 0, 100, 84, 154*% *Response to single amphetamine injection challenge:* 0, 90, -25, 69% *Response to challenge after conditioning:* 0, 43, -50, 31%
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Open field- increased horizontal locomotion (number of crossings): *Response at 25 days post-exposure:* 0, 61*% *No change in exploration (rearings) or grooming episodes*

Table 1-1. (Continued): Evidence pertaining to neurological effects of TMBs in animals — inhalation exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 15 Gralewicz et al. (1997b), Table B-24	Open field- increased grooming at middle concentration: *Response at 25 days post-exposure:* 0, 82, 147*, 76% *No change in horizontal locomotion (number of crossings) or exploration*
Cognitive function	
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 1 Gralewicz and Wiaderna (2001), Table B-26	Passive avoidance- decreased step-down latency 7 days post-foot shock[f]: *Response at 39 days post-exposure prior to foot shock:* 0, 34% *Response at 42 days post-exposure 1 day after foot shock:* 0, -23% *Response at 44 days post-exposure 3 days after foot shock:* 0,-51 % *Response at 48 days post-exposure 7 days after foot shock:* 0, -43% [Note: statistical significance 7 days after foot shock was noted after the highest and lowest responder from each group was excluded] Active avoidance- decreased performance during training (learning): *Trials to reach avoidance criteria at 54-60 days post-exposure:* 0, 58*% *No differences were noted during retraining (retention)* Radial maze- no notable change in performance 14-18 days post-exposure
0, 123, 492, or 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 15 Gralewicz et al. (1997b), Table B-24	Passive avoidance- decreased step-down latency 3-7 days post-foot shock: *Response at 39 days post-exposure prior to foot shock:* 0, 26, 41, -31% *Response at 42 days post-exposure 1 day after foot shock:* 0, 95, -28, -87% *Response at 44 days post-exposure 3 days after foot shock:* 0, 7, -67*, -36% *Response at 48 days post-exposure 7 days after foot shock:* 0, -20, -79*, -47*% Active avoidance- decreased performance during acquisition (learning)[g]: *Slower increases in avoidance performance across trials*: $p < 0.003$ *Non-significant decrease in total avoidance responses*: $p = 0.08$ Radial maze- no notable change in performance 14-18 days post-exposure
Electrocortical activity	
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 9 Gralewicz et al. (1997a), Table B-25	EEG recordings[h]- decreased spike wave discharge (SWD) bursts/ hr: *Response at 120 days post-exposure:* 0, 13, -35, -55*% *No change in global arousal level or in SWD/hr at 1 or 30 days post-exposure*

Table 1-1. (Continued): Evidence pertaining to neurological effects of TMBs in animals — inhalation exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)
1,2,3-TMB	
Pain sensitivity	
0, 123, 492, 1,230 mg/m^3, (recovery: 1,230 mg/m^3 at 2 wks post-exposure) 90 days; Rat, Wistar, male, N = 10 Korsak & Rydzyński (1996), Table B-30	Hot plate- exposure-dependent increase in paw-lick latency which recovers by 2 weeks post-exposure: *Response after exposure:* 0, 22*, 68, 78*% *Response at 2 weeks post-exposure:* 0, ND, ND, 13%
0, 492 mg/m^3 4 weeks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Hot plate- no statistically significant change in paw-lick latency: *Response at 50 days post-exposure:* 0, 95% *Response at 50 days post-exposure seconds after foot shock:* 0, -1% *Response at 51 days post-exposure 24 hr after foot shock:* 0, 75%
0, 123, 492, 1,230 mg/m^3 4 weeks; Rat, Wistar, male, N = 15 Wiaderna et al. (1998), Table B-42	Hot plate- increased paw-lick latency 24 hr after foot shock at middle concentration: *Response at 50 days post-exposure:* 0, -28, -13, -12% *Response at 50 days post-exposure seconds after foot shock:* 0, -9, -16, -15% *Response at 51 days post-exposure 24 hr after foot shock:* 0, -19, 45*, 8%
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; Rat, Wistar, male, N = 10 Korsak and Rydzyński (1996), Table B-30	Rotarod- exposure-dependent increase in failures at 13 weeks which does not recover by 2 weeks post-exposure: *Response after 13 weeks of exposure:* 0, 20, 40*, 70*% *Response at 2 weeks post-exposure:* 0, ND, ND, 50*%
Motor function and/or anxiety	
0, 123, 492, 1,230 mg/m^3 4 weeks; Rat, Wistar, male, N = 15 Lutz et al. (2010), Table B-35	Open field- statistically significant increase in horizontal locomotion (distance traveled) only after amphetamine challenge[e]: *Response at 2 weeks post-exposure with no challenge:* 0, 96, 85, 115% *Response to single amphetamine injection challenge:* 0, 15, 198*, 111% *Response to challenge after conditioning:* 0, -21, 103*, 41%
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Open field- no change in horizontal locomotion (crossings): *Response at 25 days post-exposure:* 0, -9% *No change in exploration (rearings), or grooming*

Table 1-1. (Continued): Evidence pertaining to neurological effects of TMBs in animals — inhalation exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 15 Wiaderna et al. (1998), Table B-42	Open field- no significant change in horizontal locomotion (crossings): *Response at 25 days post-exposure*: 0, 19, 51, 37% *No statistically significant change[j] in exploration (rearings) or grooming*
Cognitive function	
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Active avoidance- decreased performance during training (learning): *Trials to reach avoidance criteria at 54-60 days post-exposure:* 0, 53*% *No differences were noted during retraining (retention)* Passive avoidance- no significant change in step down latency[f]: *Response at 39 days post-exposure prior to foot shock:* 0, -39% *Response at 42 days post-exposure 1 day after foot shock:* 0, -40% *Response at 44 days post-exposure 3 days after foot shock:* 0,-23 % *Response at 48 days post-exposure 7 days after foot shock:* 0, -28% Radial maze- no notable change in performance 14-18 days post-exposure
0, 123, 492, or 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 15 Wiaderna et al. (1998), Table B-42	Passive avoidance- decreased step-down latency after foot shock: *Response at 39 days post-exposure prior to foot shock:* 0, -41, -37, 19% *Response at 42 days post-exposure 1 day after foot shock:* 0, -74*, -52, -43% *Response at 44 days post-exposure 3 days after foot shock:* 0, -54*, -49, -14% *Response at 48 days post-exposure 7 days after foot shock:* 0, -50*, -62*, -37% Active avoidance- decreased performance during training (learning): *Trials to reach avoidance criteria at 54-60 days post-exposure:* 0, 3, 41*, 14% *No statistically significant differences noted during retraining (retention)* Radial maze- decreased performance at low concentration[j]: *Increased errors on trial day 3*: 0, 32*, -28, -4% *& day 5:* 0, 30*, -16, 1% *No notable change in trial duration at any day (14-18 days post-exposure)*

Table 1-1. (Continued): Evidence pertaining to neurological effects of TMBs in animals — inhalation exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)
1,3,5-TMB	
Pain sensitivity	
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Hot plate- increased paw-lick latency 24 hr after foot shock: *Response at 50 days post-exposure:* 0, 215% *Response at 50 days post-exposure seconds after foot shock:* 0, 26% *Response at 51 days post-exposure 24 hr after foot shock:* 0, 246*%
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 12 Wiaderna et al. (2002), Table B-43	Hot plate- increased paw-lick latency 24 hr after foot shock at middle concentration: *Response at 50 days post-exposure:* 0, -6, 36, 24% *Response at 50 days post-exposure seconds after foot shock:* 0, -14, 8, -4% *Response at 51 days post-exposure 24 hr after foot shock:* 0, -4, 68*, 18%
Motor function and/or anxiety	
0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz and Wiaderna (2001), Table B-26	Open field- increased horizontal locomotion (number of crossings): *Response at 25 days post-exposure:* 0, 65*% *No change in exploration (rearings) or grooming*
Cognitive function	
0, 123, 492, 1,230 mg/m^3 4 wks; Rat, Wistar, male, N = 12 Wiaderna et al. (2002), Table B-43	Passive avoidance- decreased step-down latency 7 days post-foot shock: *Response at 39 days post-exposure prior to foot shock:* 0, -5, 146, 40% *Response at 42 days post-exposure 1 day after foot shock:* 0, 99, 108, 113% *Response at 44 days post-exposure 3 days after foot shock:* 0, -32, -41, -40% *Response at 48 days post-exposure 7 days after foot shock:* 0, -47*, -53*, -43*% Active avoidance- decreased performance during training (learning): *Trials to reach avoidance criteria at 54-60 days post-exposure:* 0, 40*, 35*, 50*% Radial maze- no notable change in performance 14-18 days post-exposure

Table 1-1. (Continued): Evidence pertaining to neurological effects of TMBs in animals — inhalation exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)
Cognitive function (continued) 0, 492 mg/m^3 4 wks; Rat, Wistar, male, N = 11 Gralewicz & Wiaderna (2001), Table B-26	Passive avoidance- decreased step-down latency 7 days post-foot shock[g]: *Response at 39 days post-exposure prior to foot shock:* 0, -3% *Response at 42 days post-exposure 1 day after foot shock:* 0, -61% *Response at 44 days post-exposure 3 days after foot shock:* 0,-65% *Response at 48 days post-exposure 7 days after foot shock:* 0, -57*% [Note: statistical significance 3 days after foot shock was noted after the highest and lowest responder from each group was excluded] Active avoidance- decreased performance during training (learning): *Trials to reach avoidance criteria at 54-60 days post-exposure:* 0, 65*% Radial maze- no notable change in performance 14-18 days post-exposure

*Significantly different from controls (p< 0.05).

Notes: For studies other than Korsak and Rydzyński (1996), % change from control calculated from digitized data using Grab It! XP software; ND= Not determined

[a]Rotarod and hot plate tests were administered immediately after termination of exposure or following a 2 week recovery period by Korsak and Rydzyński (1996). EEG recordings were acquired prior to exposure and one, 30, or 120 days after exposure by Gralewicz et al. (1997a). Motor behavior in an open field (tested for 30 min) was assessed 14 days after exposure and re-tested following single and multiple (to induce sensitization) injections with amphetamine for 120 min by Lutz et al. (2010). For the remaining studies (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b): radial maze tests were administered prior to exposure and on days 14–18 after exposure; open field activity (tested for 5–10 minutes) was assessed prior to exposure and on day 25 after exposure; passive avoidance was tested on days 35–48 after exposure; hot plate sensitivity was assessed on days 50 and 51 after exposure; and active avoidance tests were administered on or after day 54 post-exposure.

[b]In instances where authors reported exposures in ppm, EPA converted these values to mg/m^3. See Appendix B (Table B-1) for conversion factor, and individual study summary tables for ppm values.

[c]Tables referenced in Study Design and Reference column correspond to study summary tables in Appendix B

[d]Observations of hot plate latency were made prior to (L1); immediately following (L2); or 24hr after foot shock (L3). Values for L3 in Graleiwicz et al. (1997b) were determined from reported values for L1 and the ratio of L3/L1 x 100.

[e]No challenge= prior to amphetamine challenge, evaluated for 30 min, and reported as Block 1: statistical significance indicated in study text only; amphetamine challenge-induced activity was measured following a single injection or following a single injection challenge after conditioning with 5 daily injections and evaluated for 120 min

[f]Results of passive avoidance tests in Gralewicz and Wiaderna (2001) may reflect adjusted data where, due to large individual differences, 2 rats (the highest and lowest responders to foot shock) in each group were excluded. As a result, the exact magnitude of change is assumed to be somewhat inaccurate and statistical comparisons of the modified groups are provided in the above evidence table only as notes.

[g]At 54 days post-exposure, TMB-exposed rats were slower to increase the percentage of avoidance responses across blocks (1 block = 5 trials). This reduction in avoidance responses across blocks appeared to be lowest (although not statistically significant) at 1,230 mg/m^3. Rats were also observed to have a lower (p = 0.08) number of avoidance responses in the whole 30-trial session.

[h]Electroencephalograms (EEGs) were recorded at electrodes implanted in the fronto-parietal cortex and the dorsal hippocampus (one recording from each region was analyzed for each rat).

[i]Dose-dependent increases in exploration and nonlinear increases in grooming were not statistically significant

[j]Data represents % change relative to control in same trial day, but statistical significance determined by the authors based on comparison to trial day 1 responses within the same group.

Neurological effects: Oral

Table 1-2. Evidence pertaining to neurological effects of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB in animals — oral exposures

Study Design[a,b] and Reference	Assay and Results (as response relative to control)			
1,2,4-TMB				
Motor function and/or anxiety				
0, 960, 1,920, 3,850 mg/kg single oral gavage Rat, Wag/Rij, male, N = 10 Tomas et al. (1999b), Table B-40	Open field- transient increases in locomotor activity: *Response at 20 min after exposure relative to pre-injection controls:* 0, 34.1, 57.8, 60.6*% *No significant changes were reported at 10, 30, 40, 50, 60, or 70 min*			
Electrocortical activity				
0, 240, 960, 3,850 mg/kg, single oral gavage Rat, Wag/Rij, male, N = 6 Tomas et al. (1999a), Table B-39	EEG recordings[d]- inhibition of the duration and number of high voltage spindle episodes (response relative to vehicle control):			
		20 min	**40 min**	**60 min**
	Duration	0, -72, -58, -83%	0, -80*, -97*, -45%	0, 11, -67, -45%
	Number	0, -26, -44,-62*%	0, -53*,-88*,-73*%	0, 7, -53*, -22%
1,2,3-TMB				
Motor function and/or anxiety				
0, 960, 1,920, 3,850 mg/kg single oral gavage Rat, Wag/Rij, male, N = 10 Tomas et al. (1999b), Table B-40	Open field- transient increases in locomotor activity: *Response at 20 or 30 min after exposure relative to pre-injection controls:* 0, 30.9, 26.5, 56.1*% (increased 65.6*% at 30 min in at the highest concentration *No significant changes were noted at 10, 40, 50, 60, or 70 min*			
Electrocortical activity				
0, 960, 3,850 mg/kg, single oral gavage Rat, Wag/Rij, male, N = 6 Tomas et al. (1999a), Table B-39	EEG recordings[d]– inhibition of the duration and number of high voltage spindle episodes (response relative to vehicle control):			
		20 min	**40 min**	**60 min**
	Duration	0, -86, -97*, -76*%	0, -95, -98*, -97*%	0, -81, -94*, -99*%
	Number	0, -71*, -86*, -48%	0, -84*,-93*,-86*%	0, -70*,-99*,-96*%

Table 1-2 (Continued): Evidence pertaining to neurological effects of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB in animals — oral exposures

<table>
<tr><th>Study Design[a,b] and Reference</th><th colspan="4">Assay and Results (as response relative to control)</th></tr>
<tr><td colspan="5">1,3,5-TMB</td></tr>
<tr><td colspan="5">Motor function and/or anxiety</td></tr>
<tr><td>0, 960, 1,920, 3,850 mg/kg single oral gavage
Rat, Wag/Rij, male, N = 10
Tomas et al. (1999b), Table B-40</td><td colspan="4">Open field- transient increases in locomotor activity:
Response at 20 min after exposure relative to pre-injection controls: 0, 0, 46.7*, 42.4*% (increased 65–70% at 40–60 min at the highest concentration
No significant changes were noted at 10, 30, or 70 min</td></tr>
<tr><td colspan="5">Electrocortical activity</td></tr>
<tr><td rowspan="4">0, 240, 960, 3,850 mg/kg, single oral gavage
Rat, Wag/Rij, male, N = 6
Tomas et al. (1999a), Table B-39</td><td colspan="4">EEG recordings[d]- inhibition of the duration and number of high voltage spindle episodes (response relative to vehicle control):</td></tr>
<tr><td></td><td>20 min</td><td>40 min</td><td>60 min</td></tr>
<tr><td>Duration</td><td>0, -76*, -79,-86%</td><td>0, -85*,-97*,-95*%</td><td>0, -66*,-94*,-88*%</td></tr>
<tr><td>Number</td><td>0, -57,- 67, -77%</td><td>0,-52*,-93*,-91*%</td><td>0,-49*,-91*, -89*%</td></tr>
</table>

*Significantly different from controls ($p < 0.05$).

Note: % change from control calculated from digitized data using Grab It! XP software.

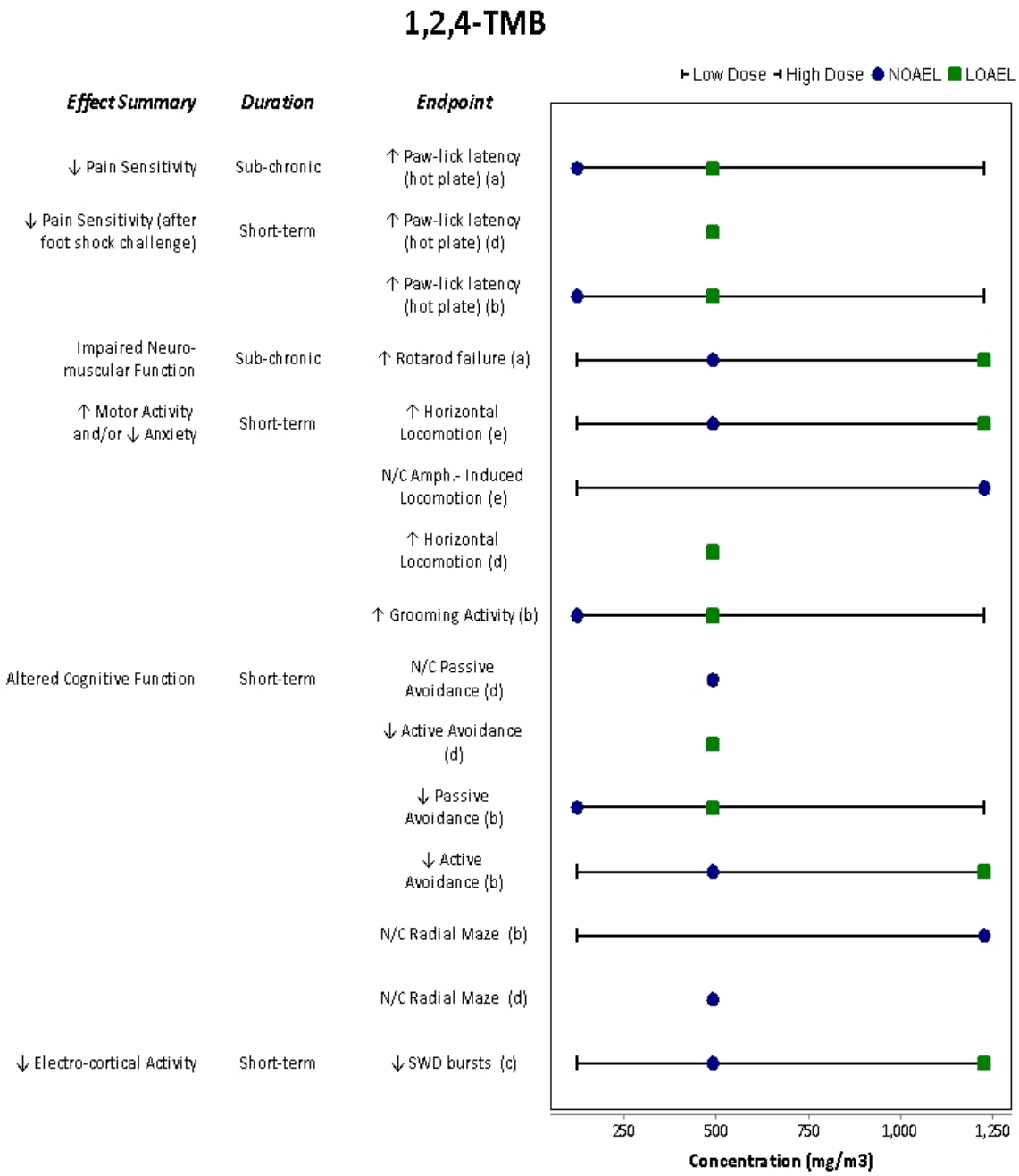
[a]Locomotor activity in open field tests and electrocortical arousal were assessed prior to exposure and immediately after exposure every 10 minutes for up to 70 minutes.

[b]In instances where authors reported exposures in ppm, EPA converted these values to mg/m^3. See Appendix B (Table B-1) for conversion factor, and individual study summary tables for ppm values.

[c]Tables referenced in Study Design and Reference column correspond to study summary tables in Appendix B.

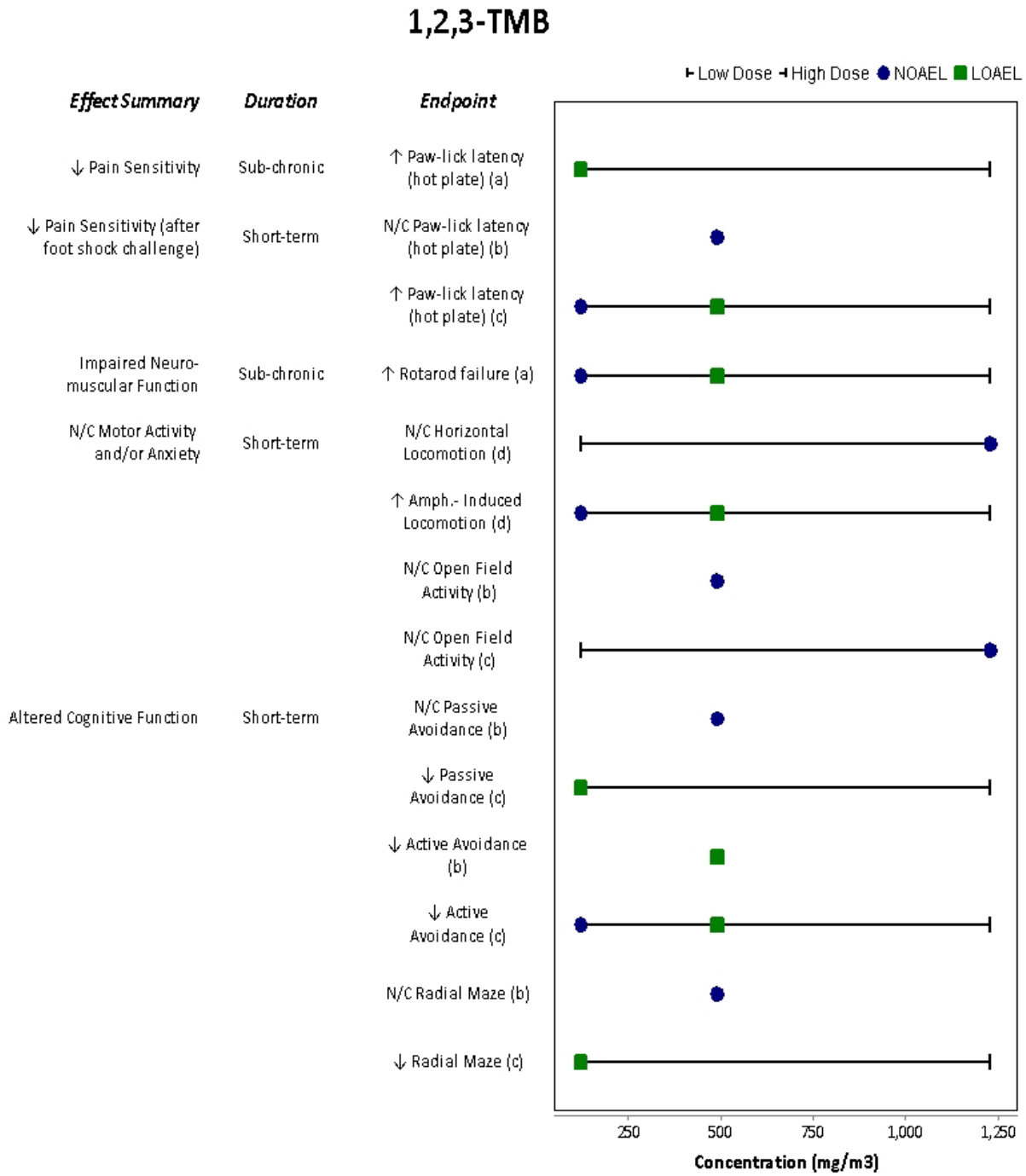
[d]Electroencephalograms (EEGs) were recorded prior to exposure and at 20, 40, and 60 minutes after exposure via electrodes implanted in the fronto-parietal cortex.

Exposure Response Arrays



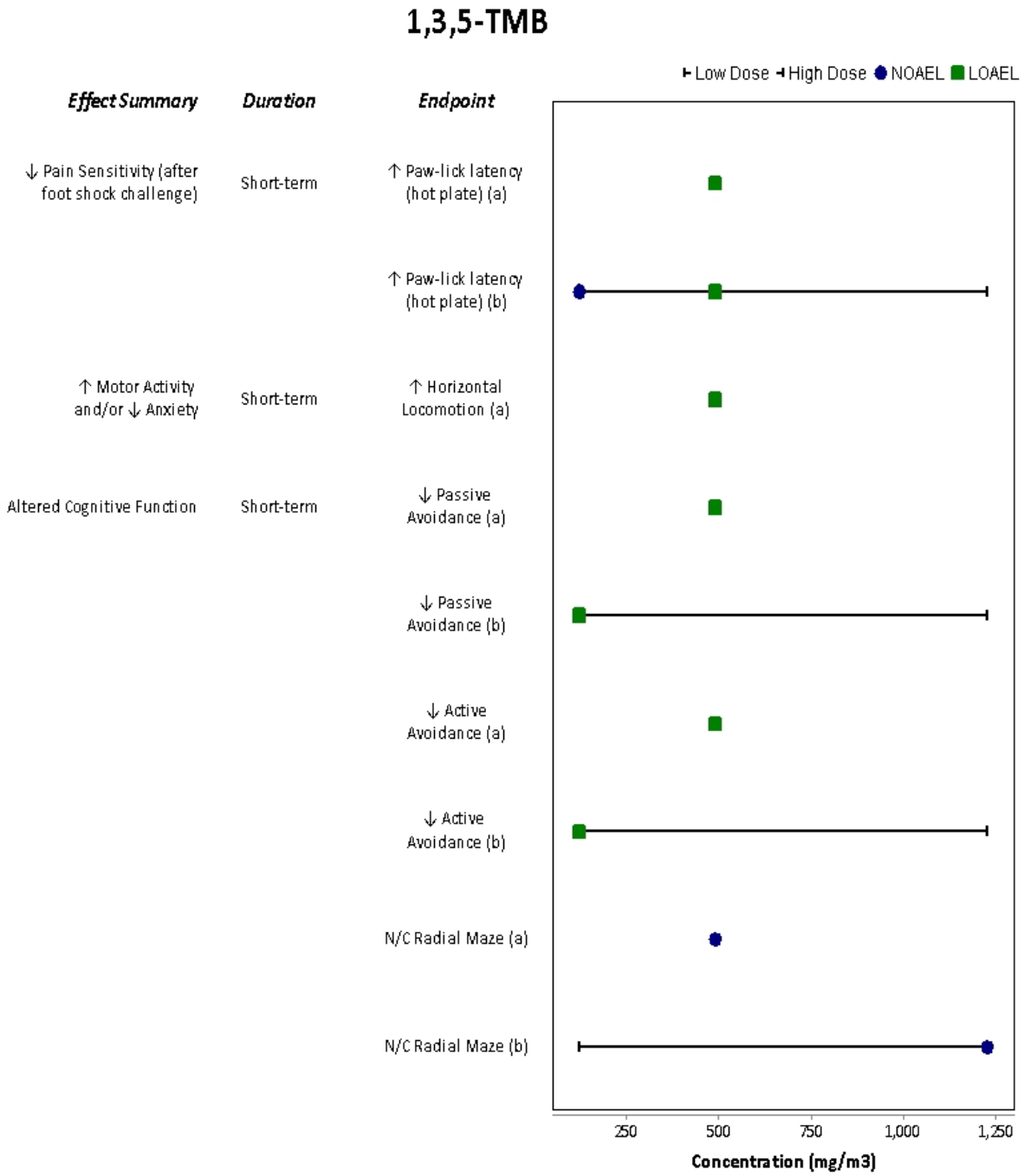
Note: Solid lines represent range of exposure concentrations. (a) Korsak and Rydzyński (1996); (b) Gralewicz et al. (1997b); (c) Gralewicz et al. (1997a); (d) Gralewicz and Wiaderna (2001); (e) Lutz et al. (2010). All effects are in male Wistar rats.

Figure 1-1. Exposure response array of neurological effects following inhalation exposure to 1,2,4-TMB.



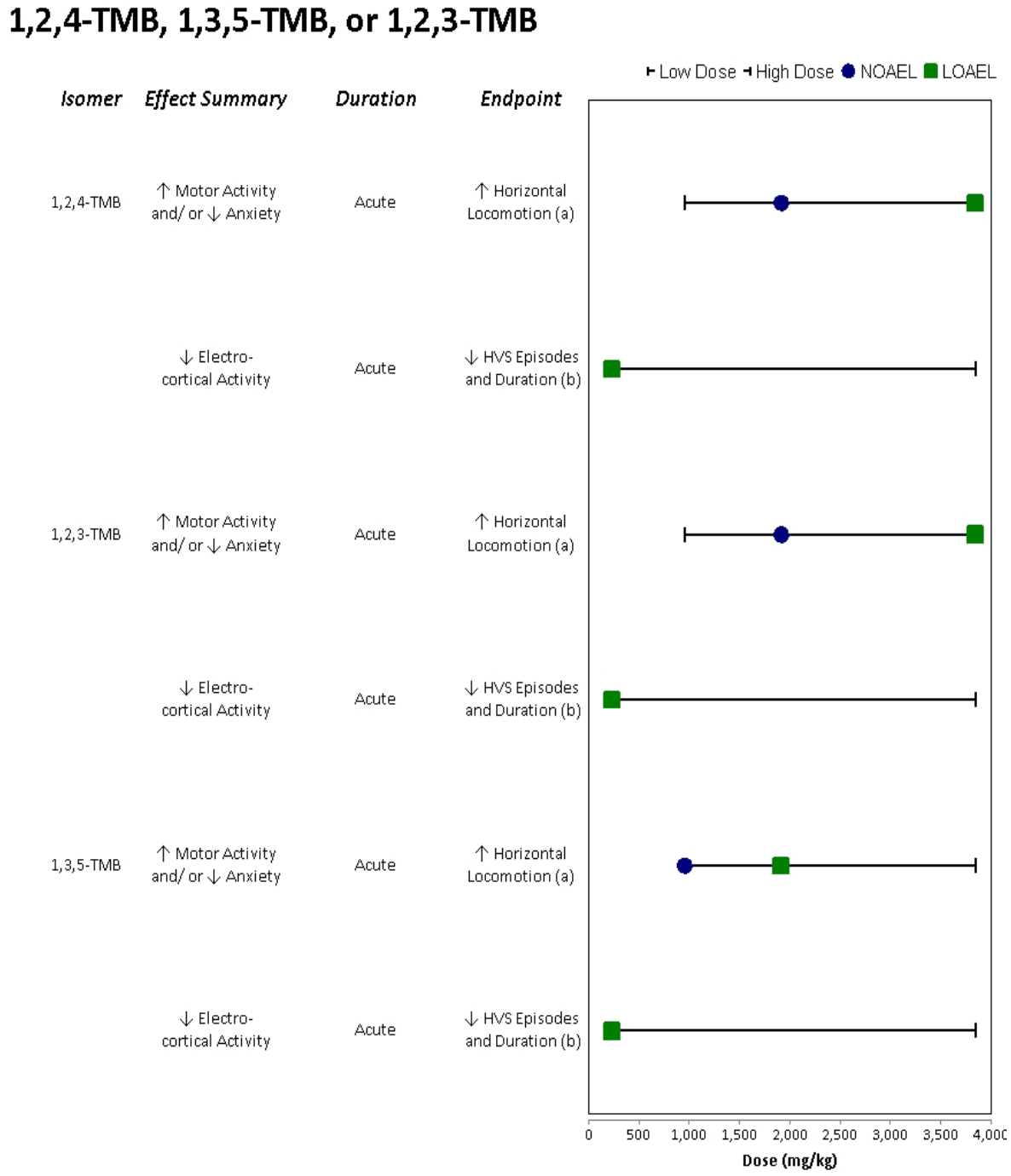
Note: Solid lines represent range of exposure concentrations. (a) Korsak and Rydzyński (1996) ; (b) Gralewicz and Wiaderna (2001); (c) Wiaderna et al. (1998); (d) Lutz et al. (2010). All effects are in male Wistar rats.

Figure 1-2. Exposure response array of neurological effects following inhalation exposure to 1,2,3-TMB.



Note: Solid lines represent range of exposure concentrations. (a) Gralewicz and Wiaderna (2001); (b) Wiaderna et al. (2002). All effects are in male Wistar rats.

Figure 1-3. Exposure response array of neurological effects following inhalation exposure to 1,3,5-TMB.



Note: Solid lines represent range of exposure concentrations. (a) Tomas et al. (1999a); (b) Tomas et al. (1999b). All effects are in male WAG/Rij (Tomas et al. (1999a)) or Wistar (Tomas et al. (1999b)) rats.

Figure 1-4. Exposure response array of neurological effects following oral exposure to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB.

1.1.1.1. *Mode of Action Analysis – Neurological Effects*

The observation of neurotoxicity following acute-, short-term-, and subchronic-duration exposure to TMB (Lutz et al., 2010; Lammers et al., 2007; Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996; Korsak et al., 1995) may indicate that TMB perturbs normal neurotransmission in exposed animals, although the specific key events necessary for TMB-induced neurotoxicity are not established. Although mechanistic and mode-of-action data is lacking for TMBs, structurally similar compounds like toluene and xylene have been more thoroughly characterized and it is reasonably assumed that TMBs would operate through a similar mechanism in producing the resultant neurotoxicological effects. 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/m^3]) have been shown to decrease spatial learning and memory, increase dopamine-mediated locomotor activity, increase 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 TMBs (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Tomas et al., 1999a; Tomas et al., 1999c; Gralewicz et al., 1997b; Gralewicz et al., 1997a). 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). Perturbation of the phospholipids on the cell membrane could indirectly affect the binding of neurotransmitters to the catecholamine or other 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 (Andersson et al., 1981). Based on effect levels with other related solvents (e.g., toluene – see Balster (1998)), it is 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) observed 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 that the observation of solvent-induced ROS production may be 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. This observation of ROS production in rat synaptosomes may potentially explain the observed TMB-induced neurotoxicity in acute, short-term, and subchronic inhalation studies.

1.1.1.2. *Summary of Neurological Effects*

Neurotoxicity is associated with exposure to TMBs based on evidence in humans exposed to mixtures containing TMBs and in animals exposed to individual TMB isomers. All three TMB isomers are taken up in humans (Järnberg et al., 1998, 1997a; Järnberg et al., 1996), and occupational studies involving exposure to TMBs and other VOCs show neuropsychological effects (Chen et al., 1999), deficits in short term memory and reduced motor speed/coordination (Lee et al., 2005), abnormal fatigue (Norseth et al., 1991), and nervousness, anxiety, and/or vertigo [(Battig et al., 1956), as reviewed by MOE (2006) and (Bättig et al., 1958)]. These effects, however, cannot be attributed to any specific compound. None of the available human studies have addressed the

potential for latent neurological effects and no studies examined the potential for neurological effects in sensitive populations.

There is strong, consistent evidence of neurotoxicity in male Wistar rats exposed to any TMB isomer via inhalation across multiple concentrations and multiple durations; however, the studies were all conducted at the same institute (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996; Korsak et al., 1995). By gavage, similar effects were observed (e.g., altered EEG recordings; increased locomotor activity in open field tests) (Tomas et al., 1999a; Tomas et al., 1999b), although testing by this route was not as extensive as by inhalation.

The spectrum of observed 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 (including pain sensitivity) involve a contributing component of motor system function. It is notable that none of the identified studies on individual TMB isomers employed protocols capable of distinguishing effects on motor activity alone (e.g., the majority of studies used open field tests 5-10 minutes in duration); thus, it remains to be determined whether TMBs exposure specifically affects motor system function. Some endpoints exhibited clear exposure-response relationships, including measures of pain sensitivity and neuromuscular function, when tested immediately after exposure. Most other endpoints did not show a clear concentration-effect relationship, although the direction and magnitude of responses was relatively consistent across studies. In most cases, effects at 1,230 mg/m^3 were less robust than those observed at lower TMB concentrations (i.e., responses were nonlinear). However, nonlinear relationships are not uncommon for solvents and, as they were observed across multiple studies using each of the three isomers, they are considered to be biologically-relevant observations rather than experimental artifacts. Latent neurological effects following TMBs exposure were consistently observed, but were difficult to characterize as deficits in a single neurological function. For example, latent measures of pain sensitivity following TMBs exposure, although consistent, were only statistically significant when the rats were challenged with a foot shock on the prior day. The most likely explanation for this observation is that TMBs exposure extends the duration of foot shock-induced decreases in pain sensitivity, since the immediate response to foot shock was similar across groups; yet, it cannot be ruled out that TMBs exposure could alter cognitive function, resulting in the observed responses. In summary, the evidence supports a determination that TMBs are neurotoxic following inhalation or oral exposure, based on consistency and coherency of effects in animals and humans, biological plausibility, evidence of delayed-onset and/ or latent neurological effects in animals several weeks following exposure, and observed exposure-response relationships in animals tested immediately after exposure.

1.1.2. Respiratory Effects

There is evidence in humans and animals that inhalation exposure to TMBs induces respiratory toxicity. The human evidence comes from occupational and residential studies involving complex VOC mixtures that include TMBs; thus, effects cannot be attributed to any TMB isomer specifically. TMB isomers are associated with increased measures of respiratory irritation, such as laryngeal and/or pharyngeal irritation (Norseth et al., 1991) and asthmatic bronchitis [(Battig et al., 1956), as reviewed in MOE (2006) and Baettig et al. (1958)] following occupational exposures. Residential exposures have demonstrated significant associations between 1,2,4-TMB and asthma (Billionnet et al., 2011). 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 to TMB isomers of up to 25 ppm (123 mg/m^3). For full details of the epidemiologic and controlled human exposures studies (including human subjects research ethics procedures), see individual study summary tables in Appendix B.

In animals, there is consistent evidence of respiratory toxicity following inhalation exposure of rodents to the TMB isomers (Table 1-3; Figure 1-5). Markers of inflammation and irritation in the lungs of rats have been observed following subchronic inhalation exposures of Wistar rats to 1,2,4-TMB or 1,2,3-TMB. Increases in immune and inflammatory cells in bronchoalveolar lavage (BAL) fluid have been observed following subchronic exposures of male Wistar rats to 1,2,4-TMB at concentrations ≥ 123 mg/m^3 (Korsak et al., 1997). Specifically, the number of cells in the BAL fluid of exposed rats was increased for both total cells (≥ 123 mg/m^3) and macrophages (≥ 492 mg/m^3). However, some attenuation of these effects was observed at high concentrations (i.e., at 1,230 mg/m^3) compared to lower concentrations. For example, the number of macrophages was increased 2.7-fold relative to control at 492 mg/m^3, but only 2.2-fold at 1,230 mg/m^3. This may indicate either adaptation to the respiratory irritation effects of 1,2,4-TMB, saturation of metabolic pathways, or immune suppression at higher doses. Subchronic exposure of male Wistar rats also significantly increased the BAL fluid content of polymorphonuclear leukocytes and lymphocytes; however the specific concentrations eliciting these significant increases were not reported by study authors. A small, but not significant, decrease in cell viability (all cells) was observed following subchronic exposure to 1,2,4-TMB at ≥ 123 mg/m^3 (Korsak et al., 1997).

In addition to increases in immune and inflammatory cells in BAL fluid following exposure to 1,2,4-TMB, histopathological alterations characterized by increases in lymphatic tissue in the lower respiratory tract have also been observed following subchronic exposures of male and female Wistar rats to 1,2,4-TMB or 1,2,3-TMB (Korsak et al., 2000a, b). Significant proliferation of peribronchial lymphatic tissue was observed in male rats exposed to 123 mg/m^3 1,2,3-TMB or 492 mg/m^3 1,2,4-TMB and female rats exposed to 123 and 492 mg/m^3 1,2,3-TMB, although trend analysis demonstrated that these increases were not concentration-dependent. Non-concentration dependent increases in interstitial lymphocytic infiltrations were also observed in male rats

exposed to 492 mg/m^3 1,2,4-TMB. However, statistically significant increases in interstitial lymphocytic infiltrations observed in male and female rats exposed to 1,230 mg/m^3 1,2,3-TMB or 1,2,4-TMB, respectively, were concentration-dependent based on trend analysis.

In some 1,2,4-TMB or 1,2,3-TMB-exposed rats exhibiting peribronchial lymphatic proliferation, the bronchial epithelium lost its cuboidal shape and formed lymphoepithelium. However, this formation of lymphoepithelium was apparently non-monotonic and not dependent on concentration. Alveolar macrophages were increased in both sexes exposed to 1,230 mg/m^3 1,2,4-TMB (significant only for males), with trend analysis demonstrating concentration-dependence across the entire concentration range. Goblet cells were statistically significantly increased in a concentration-dependent manner in female rats exposed to ≥ 492 mg/m^3 1,2,3-TMB. When the incidences of all pulmonary lesions were analyzed in aggregate, lesions were significantly increased in males at 492 mg/m^3 1,2,4-TMB, but not at any concentration in females. However, trend-analysis demonstrated significant increases in aggregate pulmonary lesions in both sexes across the entire concentration range. In rats exposed to 1,2,3-TMB, the aggregate incidences of pulmonary lesions were not statistically significantly increased at any single concentration in males or females. Male rats, however, did exhibit a concentration-dependent increase in aggregate lesions according to trend analysis. 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 exposures of both 123 and 492 mg/m^3), decreased mucoprotein (13% decrease at 123 mg/m^3 exposure), increased lactate dehydrogenase (170% and 79% increase at 123 and 492 mg/m^3, respectively) and increased acid phosphatase activity (47–75% increase at ≥ 123 mg/m^3) were observed in animals exposed to 1,2,4-TMB, suggesting pulmonary irritation or inflammation. All of these effects also exhibited either some attenuation of effect at high concentrations compared to lower concentrations. Therefore, some adaptation to the respiratory irritation effects of 1,2,4-TMB may be occurring.

Decreased respiration, a symptom of sensory irritation, has been observed in male BALB/C mice during acute inhalation exposures to the TMB isomers for 6 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 1 or 2 minutes of exposure (Korsak et al., 1997; Korsak et al., 1995). The concentration of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB that was observed to result in a 50% depression in the respiratory rate (RD_{50}) was similar between the three isomers: 578, 541, or 519 ppm (2,844, 2,662, or 2,553 mg/m^3), respectively.

Table 1-3. Evidence pertaining to respiratory effects of TMBs in animals — inhalation exposures

Study design[a] and reference	Results
1,2,4-TMB	
Pulmonary inflammation/irritation	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, male, N = 6-7 Korsak et al. (1997), Table B-31	Increased total bronchoalveolar cell count with evidence of attenuation at high exposure. *Response relative to control:* 0, 202***, 208**, 131*%
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, male, N = 6-7 Korsak et al. (1997), Table B-31	Increased macrophage count with evidence of attenuation at high exposure. *Response relative to control:* 0, 107, 170**, 116**%
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, male and female, N = 10 Korsak et al. (2000a), Table B-32	Increase in number of pulmonary lesions. *Response relative to control:* Incidences not reported, thus calculation of response relative to control not possible; authors report statistically significant increases at 492 and 1,230 mg/m^3.
Clinical chemistry effect	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, male, N = 10 Korsak et al. (1997), Table B-31	Increased acid phosphatase activity with evidence of attenuation at high exposure. *Response relative to control:* 0, 47*, 74*, 45*%
Sensory irritation (decreased respiration)	
1,245, 3,178, 5,186, 6,391, 9,486 mg/m^3, 6 min Mouse, BALB/C, male, N = 8–10 Korsak et al. (1997); Korsak et al. (1995), Tables B-31 and B-29	Decreased respiratory rate as measured during first minute of exposure. *Response relative to control:* RD_{50} = 2,844

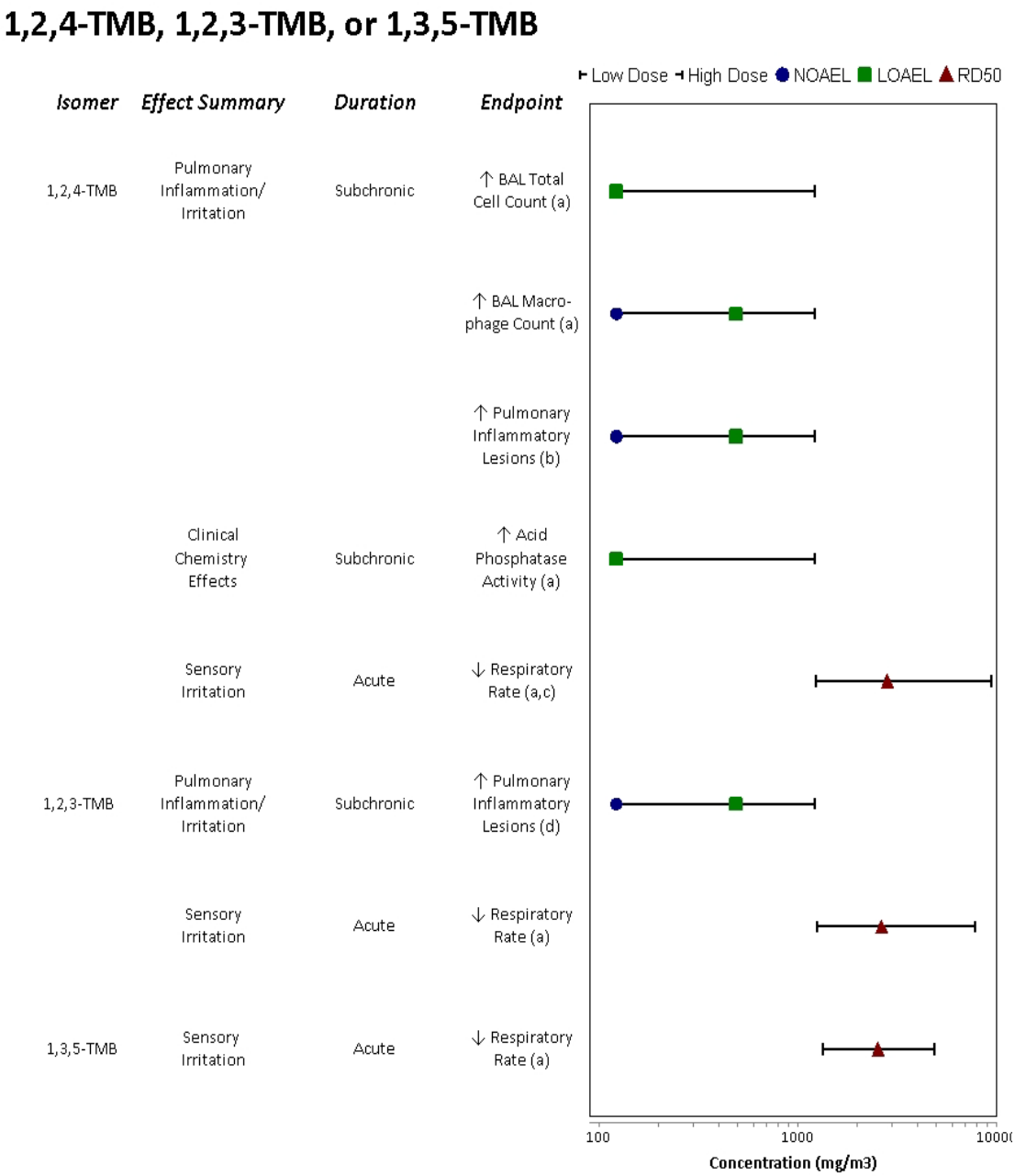
Table 1-3 (Continued): Evidence pertaining to respiratory effects of TMBs in animals — inhalation exposures

Study design[a] and reference	Results
1,2,3-TMB	
Pulmonary inflammation/irritation	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, male and female, N = 10 Korsak et al. (2000b), Table B-33	Increase in number of pulmonary lesions. *Response relative to control:* Incidences not reported, thus calculation of response relative to control not possible; authors report statistically significant increases at 492 and 1,230 mg/m^3.
Sensory irritation (decreased respiration)	
1,255, 2,514, 4,143, 7,828 mg/m^3, 6 min Mouse, BALB/C, male, N = 8–10 Korsak et al. (1997); Tables B-31	Decreased respiratory rate as measured during first minute of exposure. *Response relative to control:* RD_{50} = 2,662
1,3,5-TMB	
Sensory irritation (decreased respiration)	
1,348, 2,160, 2,716, 3,597, 4,900 mg/m^3, 6 min Mouse, BALB/C, male, N = 8–10 Korsak et al. (1997), Table B-31	Decreased respiratory rate as measured during first minute of exposure. *Response relative to control:* RD_{50} = 2,553

*, **, *** Statistically different from controls at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

[a]In instances where authors reported exposures in ppm, EPA converted these values to mg/m^3. See Appendix B (Table B-1) for conversion factor, and individual study summary tables for ppm values.

[b]Tables referenced in Study Design and Reference column correspond to study summary tables in Appendix B



Note: Solid lines represent range of exposure concentrations. (a) Korsak et al. (1997); (b) Korsak et al. (2000a); (c) Korsak et al. (1995); (d) Korsak (2000b). Y-axis is displayed on a logarithmic scale. All subchronic effects are in male Wistar rats, except for increased pulmonary lesions, which occur in both male and female Wistar rats; acute effects are in BALB/c mice.

Figure 1-5. Exposure response array of respiratory effects following inhalation exposure to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB.

1.1.2.1. *Mode of Action Analysis – Respiratory Effects*

Data regarding the potential mode of action for the respiratory effects resulting from TMB inhalation exposures are limited and the key events for TMB-induced respiratory toxicity are not established. However, the available toxicity data suggest that TMB isomers act as potent acute respiratory irritants and induce inflammatory responses following longer exposures (i.e., subchronic) in animals. Korsak et al. (1995) and Korsak et al. (1997) have suggested that decreased respiratory rate following TMB inhalation exposure is indicative of irritation, and proposed that respiratory irritants such as TMB may activate a "sensory irritant receptor" on the trigeminal nerve ending in the nasal mucosa leading to an inflammatory response. Korsak et al. (1997; 1995) further suggested that activation of this irritant receptor follows either adsorption of the agonist, or adsorption and chemical reaction with the receptor. The authors referenced a proposed model for the receptor protein that includes two main binding sites for benzene moieties and a thiol group. Further, they suggested that in the case of organic solvents (i.e., toluene, xylene, and 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 inhalation exposure of rats to 1,2,4-TMB, inflammatory cell (i.e., macrophages, polymorphonuclear leukocytes, and lymphocytes) numbers were increased along with markers of their activation (i.e., 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 inhalation exposures in rats. However, many of these effects were not observed to be concentration-dependent in repeat exposure studies (i.e., no progression of effect over an order of magnitude of concentrations), suggesting that there may be adaptation to respiratory irritation that occurs following extended inhalation exposure to TMB. The processes responsible for the respiratory inflammatory responses observed in subchronically exposed animals are unknown. However, a major inflammatory mediator, interleukin 8 (IL-8), was increased 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 and 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).

1.1.2.2. *Summary of Respiratory Effects*

Respiratory toxicity is associated with inhalation exposure to TMBs based on evidence in humans and animals. All three TMB isomers are taken up by humans (Järnberg et al., 1998, 1997a; Järnberg et al., 1996), and occupational and residential studies involving exposure to TMBs and other VOCs suggest an association between TMB exposure and asthmatic symptoms (Billionnet et al., 2011; Battig et al., 1956) and sensory irritation (Norseth et al., 1991). These effects, however, cannot be attributed to any specific compound.

There is strong, consistent evidence of respiratory toxicity in male and female Wistar rats exposed to any TMB isomer via inhalation across multiple concentrations and multiple durations, although the studies were conducted at the same institute (Korsak et al., 2000a, b; Korsak et al., 1997; Korsak et al., 1995). Some endpoints (i.e., BAL macrophages and alkaline phosphatase) showed concentration-dependence at low- and mid-exposures, 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. In summary, the evidence supports a determination that TMBs are respiratory toxicants following inhalation exposure, based on consistency and coherency of effects observed in humans and animals, biological plausibility, and observed exposure-response relationships.

1.1.3. Reproductive and Developmental Effects

There are no studies in humans that investigated the reproductive or maternal toxicity of the TMB isomers by any route of exposure. 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 Sprague-Dawley rat dams following inhalation exposure during gestation to 1,2,4-TMB or 1,3,5-TMB (Saillenfait et al., 2005) (Table 1-4; Figure 1-6). Dams exposed to 2,952 mg/m^3 1,2,4-TMB gained only 50% of the weight gained by control animals, whereas dams exposed to 2,952 mg/m^3 1,3,5-TMB gained only 25% of the weight gained by controls. Decreased maternal

food consumption (across GD6–GD21) was also observed at ≥ 2,952 mg/m^3 1,2,4-TMB and ≥ 1,476 mg/m^3 1,3,5-TMB, although the magnitude of the difference compared to controls (88-83% and 92-75% of controls, respectively) was modest relative to the observed decreases in maternal weight gain. The decrease in food consumption at 1,476 mg/m^3 1,3,5-TMB (92% relative to controls) was not considered to be a marker of adversity given no accompanying decrease in maternal weight gain was observed 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 by any route of exposure. Developmental toxicity (reported as decreased fetal body weight) has been observed in male and female rats 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 significantly) by 5–13% at concentrations of > 2,952 mg/m^3 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. Studies on the developmental or reproductive effects of 1,2,3-TMB by any route of exposure were not available.

Table 1-4. Evidence pertaining to reproductive and developmental effects of 1,2,4-TMB and 1,3,5-TMB in animals — inhalation exposures

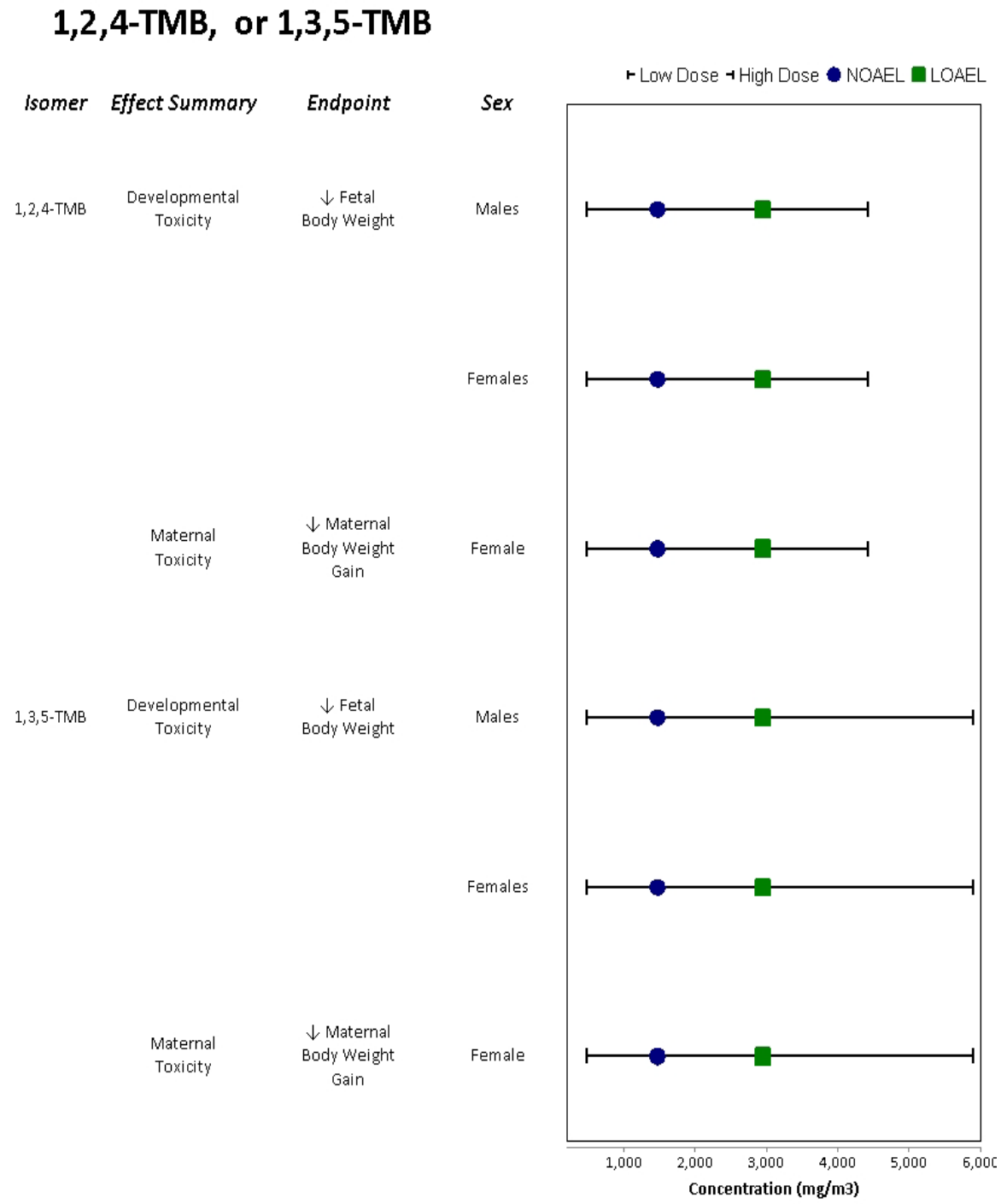
Study Design[a] and Reference	Results
1,2,4-TMB	
Developmental toxicity	
0, 492, 1,476, 2,952, 4,428 mg/m^3, GD6-GD20 (6 hr/day) Rat, Sprague-Dawley, female and male[c] Saillenfait et al. (2005), Table B-38	Decreased fetal body weight of male and female fetuses. *Response relative to control:* Male: 0, -1, -2, -5*, -11**% Female: 0, -1, -3, -5*, -12**%
Maternal toxicity	
0, 492, 1,476, 2,952, 4,428 mg/m^3, GD6-GD20 (6 hr/day) Rat, Sprague-Dawley, female, N = 24–25 dams Saillenfait et al. (2005), Table B-38	Decreased corrected maternal weight gain. *Response relative to control:* 0, +7, -7, -51**, -100**% (weight gain = 0 g)
1,3,5-TMB	
Developmental toxicity	
0, 492, 1,476, 2,952, 5,904 mg/m^3, GD6-GD20 (6 hr/day) Rat, Sprague-Dawley, female and male[a, c] Saillenfait et al. (2005), Table B-38	Decreased fetal body weight of male and female. *Response relative to control:* Male: 0, -1, -5, -7*, -12**% Female: 0, -1, -4, -6, -13**%
Maternal Toxicity	
0, 492, 1,476, 2,952, 5,904 mg/m^3, GD6-GD20 (6 hr/day) Rat, Sprague-Dawley, female, N = 24-25 dams Saillenfait et al. (2005), Table B-38	Decreased corrected maternal weight gain. *Response relative to control*: 0, +3, -31,- 76**, -159**% (weight gain = -12 g)

*, ** Statistically significantly different from controls at $p < 0.05$ and $p < 0.01$, respectively.

[a]In instances where authors reported exposures in ppm, EPA converted these values to mg/m^3. See Appendix B (Table B-1) for conversion factor, and individual study summary tables for ppm values.

[b]Tables referenced in Study Design and Reference column correspond to study summary tables in Appendix B.

[c]Number of fetuses analyzed not reported.



Note: Solid lines represent range of exposure concentrations. All effects from Saillenfait et al. (2005).

Figure 1-6. Exposure response array of reproductive and developmental effects following inhalation exposure to 1,2,4-TMB or 1,3,5-TMB.

1.1.3.1. *Summary of Reproductive and Developmental Effects*

The database for reproductive and developmental toxicity following inhalation exposure to 1,2,4-TMB and 1,3,5-TMB is limited to one animal developmental study; no studies in humans are available. Thus, these isomers may cause developmental toxicity, although this is based on only one study that demonstrated clear, exposure-related effects on fetal and maternal body weights.

1.1.4. Hematological and Clinical Chemistry Effects

There is limited evidence in humans, and stronger evidence in animals, that exposure to TMB isomers via inhalation induces hematological toxicity and alterations in clinical chemistry parameters. Alterations in blood clotting and anemia in workers exposed to a paint solvent containing 50% 1,2,4-TMB, 30% 1,3,5-TMB, and unspecified amounts of 1,2,3-TMB (listed as possibly present) was reported by Battig et al. (1956), as reviewed by MOE (2006); effects observed at 295 mg/m^3. However, as workers were exposed to a solvent mixture containing multiple TMB isomers and other VOCs, effects cannot be attributed to any TMB isomer specifically.

In animals, there is evidence of hematological toxicity following subchronic inhalation exposure to 1,2,4-TMB or 1,2,3-TMB and short-term inhalation exposure to 1,3,5-TMB (Table 1-5; Figures 1-7 and 1-8). Subchronic exposures to 1,2,4-TMB or 1,2,3-TMB have been shown to result in hematological effects and changes in serum chemistry in rats (Korsak et al., 2000a, b). In male rats exposed to 1,230 mg/m^3 1,2,4-TMB or 1,2,3-TMB, red blood cells (RBC) counts were significantly decreased 23 and 15%, respectively. The observed alterations in RBCs were concentration-dependent as determined by trend analysis. Exposure to 1,2,4-TMB or 1,2,3-TMB did not significantly decrease RBCs in female rats, but trend analysis demonstrated that decreases in RBC counts in female rats exposed to 1,2,3-TMB were concentration dependent, with a maximum decrease of 9% at 1,230 mg/m^3. RBCs in both sexes were observed to still be depressed relative to controls 2 weeks following termination of exposure to both isomers, but these decreases were not statistically significant.

White blood cell (WBC) counts were significantly increased 80% in male rats and increased 30% (not statistically significant) in female rats exposed to 1,230 mg/m^3 1,2,4-TMB. After a two-week follow-up after termination of exposure, WBC counts had returned to normal in female rats and were slightly depressed (18%) in male rats. WBC numbers were unchanged in male rats exposed to 1,2,3-TMB, but were increased (not statistically significant) 22% in female rats exposed to 1,230 mg/m^3. After two weeks following termination of exposure, WBC counts in male and female rats had fallen to roughly 60% of controls.

Significant decreases in reticulocytes (71% decrease relative to controls) and clotting time (37% decrease relative to controls) were observed in female rats exposed to 1,230 mg/m^3 and 492 mg/m^3 1,2,4-TMB, respectively. Both of these effects were concentration-dependent across the entire-range of concentrations as determined by trend-analysis; animals fully recovered within 2

weeks after termination of exposure. Reticulocyte numbers were statistically significantly increased 60% in male rats exposed to 1,230 mg/m^3 1,2,3-TMB, with reticulocyte numbers even further increased (150%) two weeks following the termination of exposure. Reticulocyte numbers in females exposed to 1,2,3-TMB were significantly increased 77% and 100% at 123 and 492 mg/m^3, and increased 69% (not statistically significant) at 1,230 mg/m^3. Reticulocyte numbers were still increased in males and females 2 weeks after the termination of exposure to 1,2,3-TMB. Segmented neutrophils were statistically significantly decreased 29% in male rats exposed to 1,230 mg/m^3 1,2,3-TMB; statistically significant decreases of 29% and 48% were observed in female rats exposed to 492 and 1,230 mg/m^3 1,2,3-TMB. Lymphocytes were statistically increased 11% and 15% in male and female rats exposed to 1,230 mg/m^3, respectively. Numbers of segmented neutrophils and lymphocytes returned to control values 2 weeks after termination of exposure.

Exposure to TMB isomers was also observed to have an effect on clinical chemistry markers that possibly indicate hepatic injury. Sorbitol dehydrogenase was increased at ≥ 123 mg/m^3 in male rats exposed to 1,2,4-TMB (18-23% relative to controls) and at 1,230 mg/m^3 in male rats exposed to 1,2,3-TMB (69% relative to controls)(Korsak et al., 2000a, b). However, the increases following exposure to 1,2,4-TMB were not concentration-dependent. Sorbitol dehydrogenase activity was also higher in female rats exposed to 1,2,4-TMB (19-23% relative to controls) but the increases in activity were not significantly higher when compared to controls. Sorbitol dehydrogenase activity was not affected in female rats exposed to 1,2,3-TMB. Alanine aminotransferase was decreased (23% relative to controls) and alkaline phosphatase was increased (42-45% relative to controls) at 1,230 mg/m^3 and ≥ 492 mg/m^3 (respectively) in female rats exposed to 1,2,3-TMB. Absolute iver weights were only observed to increase (9%) in male rats exposed to 1,230 mg/m^3 1,2,3-TMB, and no histopathological changes were observed in either sex exposed to 1,2,3-TMB or 1,2,4-TMB. Therefore, the adversity of the observed changes in clinical chemistry parameters is unclear.

An increase (30% relative to controls) 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 5 weeks) (Wiglusz et al., 1975b; Wiglusz et al., 1975a). The adversity of aspartate aminotransferase is uncertain 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.

Acute inhalation exposures of male Wistar rats to 1,500–6,000 mg/m^3 1,3,5-TMB for 6 hours did not result in substantial effects on hemoglobin or RBC or WBC count (Wiglusz et al., 1975b). 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 1 day following in rats exposed to 6,000 mg/m^3, 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/m^3 for 6 hours did not

reveal any consistent pattern in the levels of aspartate or alanine aminotransferases, although alkaline phosphatase was statistically increased 84% in rats 7 days following exposure to 3,000 mg/m^3 (Wiglusz et al., 1975a).

Slight alterations in clinical chemistry parameters and differential white blood cell counts were also observed in rats following subchronic, oral exposure to 1,3,5-TMB (Table 1-6; Figure 1-9) (Koch Industries, 1995b). While no hematological parameters (i.e., RBC counts, hematocrit) were observed to differ between exposed rats and controls, the number of monocytes were observed to increase (100-200% increase) in male rats exposed to ≥ 200 mg/kg-day 1,3,5-TMB. Additionally, a number of clinical chemistry parameters were altered in exposed rats. In female rats exposed to 600 mg/kg-day, sodium and chloride levels were statistically significantly decreased (2.3 and 2.7%, respectively) relative to controls, and cholesterol and phosphorus were statistically significantly increased (41% and 23%, respectively). In male rats, exposure to 600 mg/kg-day resulted in a significant decrease (19%) in glucose levels, and significant increases in phosphorus levels and alkaline phosphatase activity (17% and 46%, respectively). In a related, preliminary study (Koch Industries, 1995a), hematological and clinical chemistry effects were also observed following 14 days of oral exposure. Female Sprague Dawley rats exposed to either 150 or 600 mg/kg-day 1,3,5-TMB had increased cholesterol levels, and high-dose males exhibited increased white blood cell counts with corresponding increased neutrophil and lymphocyte numbers.

Table 1-5. Evidence pertaining to hematological and clinical chemistry effects of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB in animals — inhalation exposures

Study Design[a] and Reference	Results
1,2,4-TMB	
Hematological toxicity	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000a), Table B-32	Decreased red blood cells in males only. *Response relative to control:* 0, -1, -15, -23**% (recovery = 24% decrease)
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000a), Table B-32	Increased white blood cells in males only. *Response relative to control:* 0, 2, 4, 80**% (recovery = 18% decrease)
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000a), Table B-32	Decreased reticulocytes in females only. *Response relative to control:* 0, -51, -49, -71*% (recovery = 65% increase)
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000a), Table B-32	Decreases in clotting time in females only. *Response relative to control:* 0, -23, -37**, -27*% (recovery = 60% increase)
Clinical chemistry effects	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000a), Table B-32	Non-monotonic increases in sorbitol dehydrogenase in males only. *Response relative to control:* 0, 73**, 74*,73**%

Table 1-5 (Continued): Evidence pertaining to hematological and clinical chemistry effects of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB in animals — inhalation exposures

Study Design[a] and Reference	Results
1,2,3-TMB	
Hematological toxicity	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Decreased red blood cells in males only. *Response relative to control:* 0, 8, 6, -15*% (recovery = 9% decrease)
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Decreased segmented neutrophils in males and females. *Response relative to control:* *Males:* 0, 2, -17, -29*% (recovery = 11% increase) *Females:* 0, -15, -29*, -48*% (recovery = 15% decrease)
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Increased lymphocytes in males and females. R*esponse relative to control:* *Males:* 0, 1, 6, 11**% (recovery = 11% decrease) *Females:* 0, 6, 10, 15**% (recovery = 3% increase)
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Increased reticulocytes in males and females (non-monotonic). *Response relative to control:* *Males:* 0, -25, 36, 61*% (recovery = 146**% increase) *Females:* 0, 77*, 100**, 69% (recovery = 162**% increase)
Clinical chemistry effects	
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Decreased alanine aminotransferase in females only. *Response relative to control:* 0, -1, -6, -23*%
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Increased alkaline phosphatase in females only. *Response relative to control:* 0, 20, 45*, 42*%
0, 123, 492, 1,230 mg/m^3, 90 days (6 hr/day, 5 days/week) Rat, Wistar, female and male, N = 10 Korsak et al. (2000b), Table B-33	Increased sorbitol dehydrogenase in males only. *Response relative to control:* 0, 44, 56, 69*%

Table 1-5 (Continued): Evidence pertaining to hematological and clinical chemistry effects of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB in animals — inhalation exposures

Study Design[a] and Reference	Results
1,3,5-TMB	
Hematological toxicity	
1,500, 3,000, 6,000 mg/m^3, 6 hr Samples collected 0, 1, 7, 14, and 28 days post exposure Rat, Wistar, male, N = 5.8 Wiglusz et al. (1975b), Table B-44	Increased segmented neutrophilic granulocytes (1–28 days post-exposure). *Response relative to control:* *Day 0:* 0, 59, 118, 95% *Day 1:* control response not reported *Day 7:* control response not reported *Day 14:* 0, 15, 184, 94% *Day 28:* 0, -20, 124, 1%
Clinical chemistry effects	
3,000 mg/m^3, 5 weeks (6 hr/day, 6 days/week) Samples collected 1, 3, 7, 14, and 28 days during exposure Rat, Wistar, male, N = 6 Wiglusz et al. (1975a), Table B-45	Increased aspartate aminotransferase on day 14. *Response relative to control (day 14):* 12*%
300–3,000 mg/m^3, 6 hr, Samples collected 0, 2, 7, 14 and 28 days post exposure Rat, Wistar, male, N = 6 Wiglusz et al. (1975a), Table B-45	Increased alkaline phosphatase on day 7 post-exposure. *Response relative to control (on day 7 :*0, -0.1, 0.03, 84*%

*, ** Statistically different from controls at $p < 0.05$ and $p < 0.01$, respectively.

[a]In instances where authors reported exposures in ppm, EPA converted these values to mg/m^3. See Appendix B (Table B-1) for conversion factor, and individual study summary tables for ppm values.

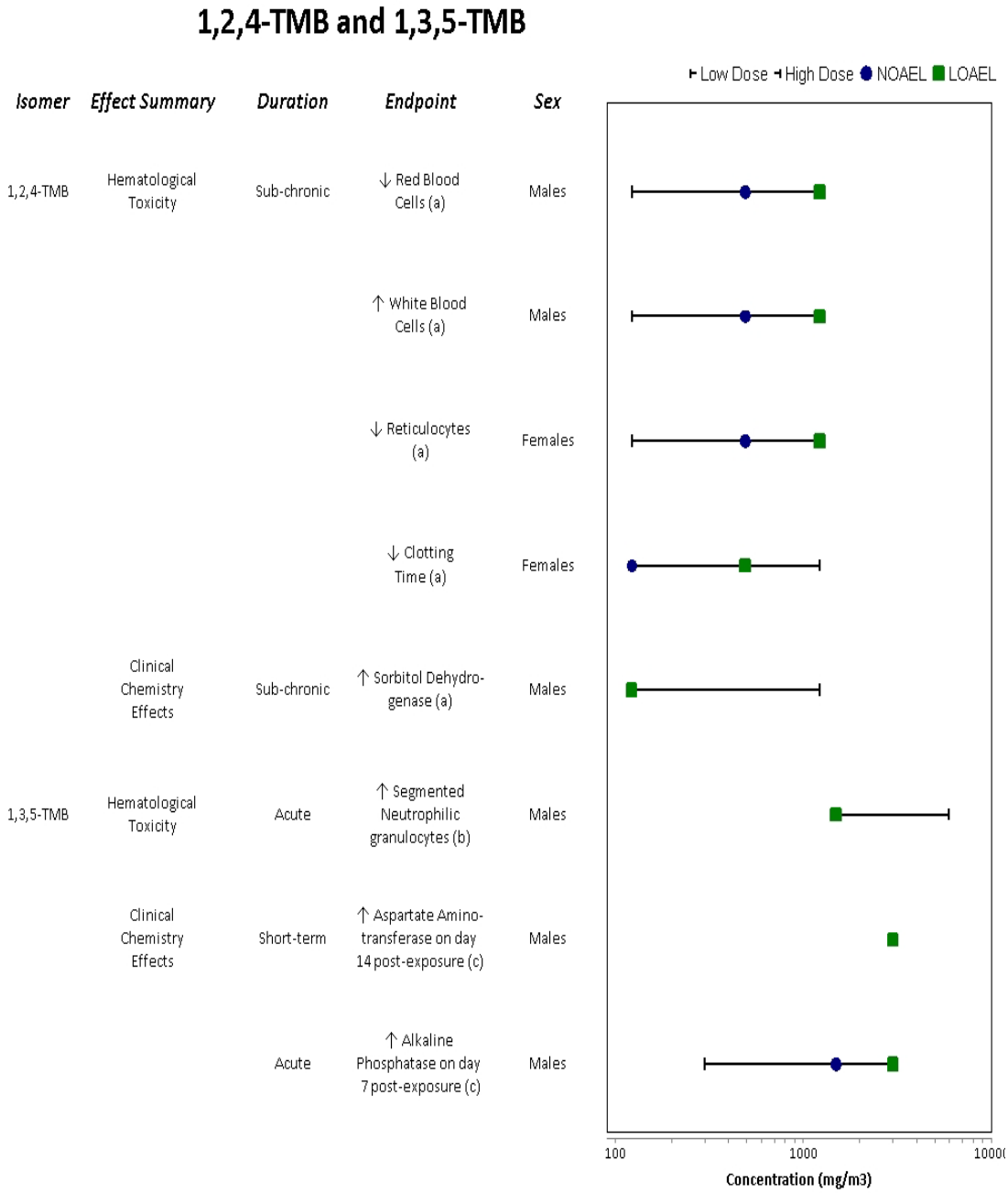
[b]Tables referenced in Study Design and Reference column correspond to study summary tables in Appendix B.

Table 1-6. Evidence pertaining to hematological and clinical chemistry effects of 1,3,5-TMB in animals — oral exposures

Study Design and Reference	Results
1,3,5-TMB	
Hematological toxicity	
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b), Table B-28[a]	Increased monocyte levels in males only *Response relative to control:* Male: 0, 100, 200*, 100*% (recovery = 100% increase)
Clinical chemistry effects	
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b), Table B-28	Increased phosphorus levels in males and females *Response relative to control:* Male: 0, 3, 8, 17*% (recovery = 11% decrease) Female: 0, 0, 5, 23*% (recovery = 13% decrease)
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b), Table B-28	Decreased sodium levels in females only *Response relative to control:* 0, 0, 0, -2*% (recover = 1% decrease)
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b), Table B-28	Decreased chloride levels in females only *Response relative to control:* 0, 0, 0, -3*% (recovery = 1% increase)
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b), Table B-28	Increased cholesterol levels in females only *Response relative to control:* 0, -3, 7, 41*% (recovery = 21% decrease)
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b) Table B-28	Decreased glucose levels in males only *Response relative to control:* 0, -10, -9, -19*% (recovery = 12% increase)
0, 50, 200, 600 mg/kg-day, 90 days (once daily, 5 days/week) Rat, Sprague-Dawley, female and male, N = 10 Koch Industries (1995b), Table B-28	Increased alkaline phosphatase activity in males only *Response relative to control:* 0, 5, 13, 46*% (recovery = 28% decrease)

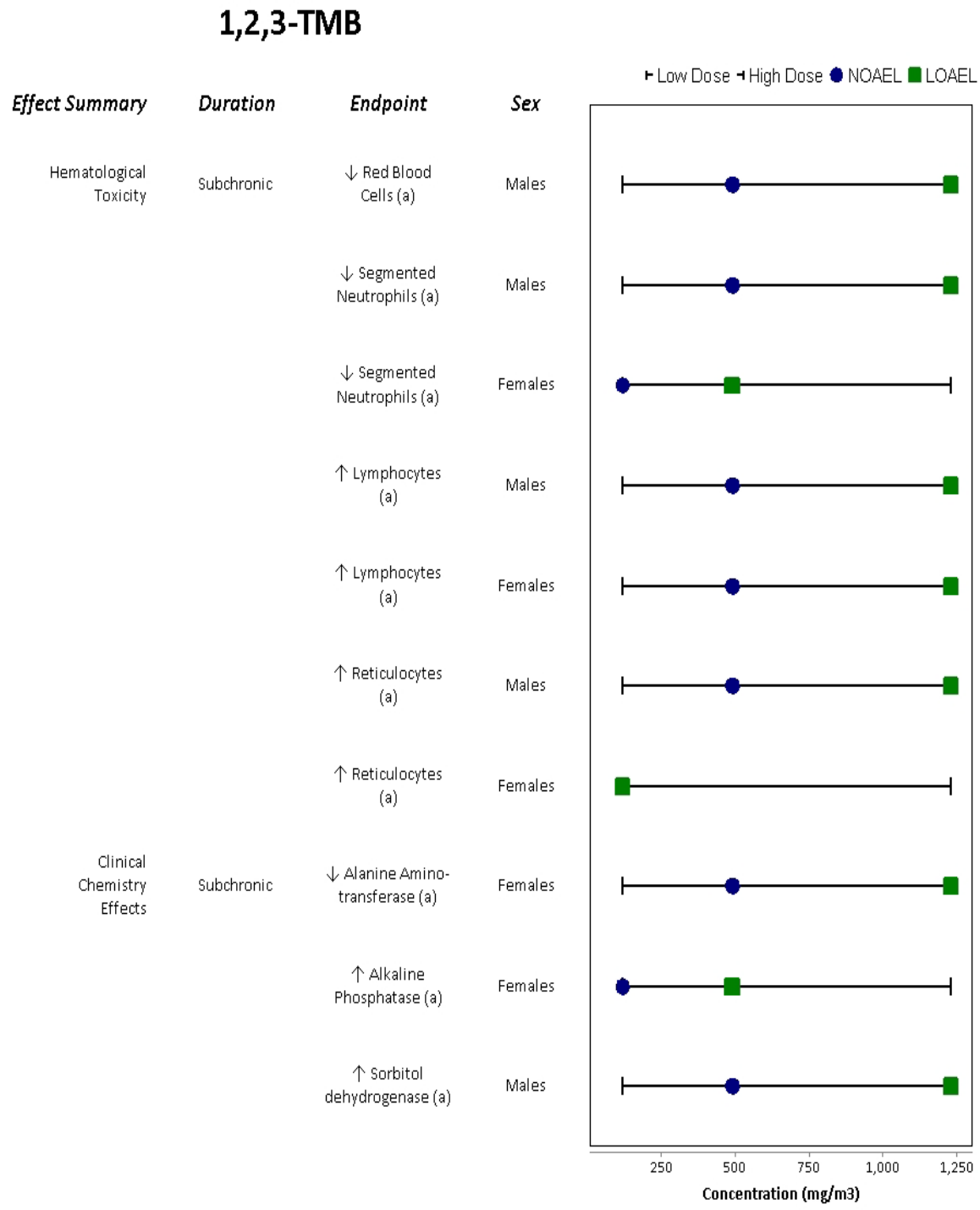
*, ** Statistically different from controls at $p < 0.05$ and $p < 0.01$, respectively.

[a] Tables referenced in Study Design and Reference column correspond to study summary tables in Appendix B.



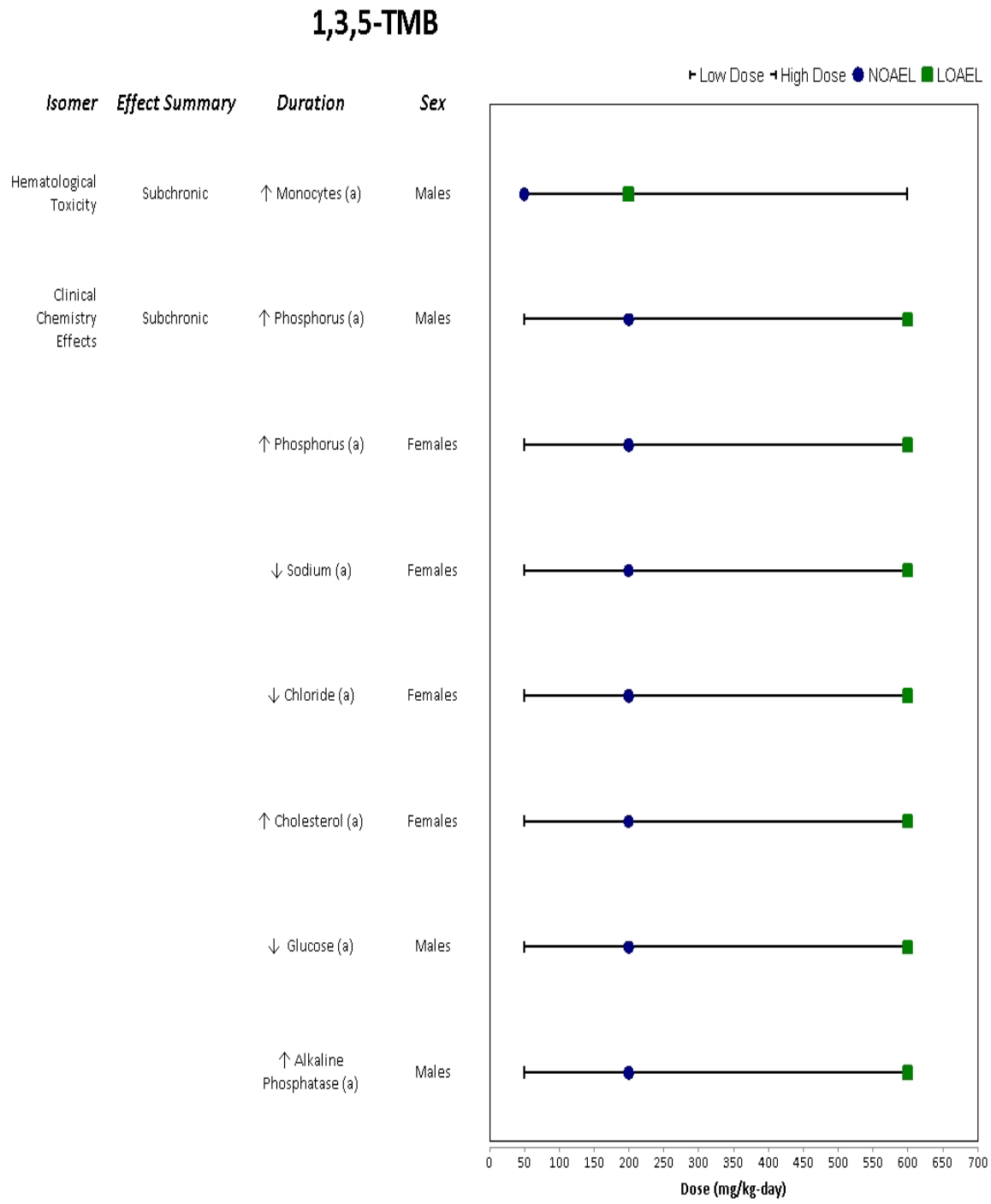
Note: Solid lines represent range of exposure concentrations. (a) Korsak et al. (2000a); (b) Wiglusz et al. (1975b); (c) Wiglusz et al. (1975a). Y-axis is displayed on a logarithmic scale.

Figure 1-7. Exposure response array of hematological and clinical chemistry effects following inhalation exposure to 1,2,4-TMB or 1,3,5-TMB.



Note: Solid lines represent range of exposure concentrations. (a) Korsak et al. (2000b).

Figure 1-8. Exposure response array of hematological and clinical chemistry effects following inhalation exposure to 1,2,3-TMB.



Note: Solid lines represent range of exposure concentrations. (a) Koch Industries (1995b).

Figure 1-9. Exposure response array of hematological and clinical chemistry effects following oral exposure to 1,3,5-TMB.

1.1.4.1. *Mode of Action Analysis – Hematological and Clinical Chemistry Effects.*

The mode of action for TMB-induced hematological and clinical chemistry effects has not been established. Increased sorbitol dehydrogenase activity is a marker for hepatic injury (Ramaiah, 2007) and therefore, underlying hepatotoxicity could explain its increase in rats exposed to 1,2,4-TMB or 1,2,3-TMB. However, absolute and relative liver weights were not observed to increase with inhalation exposure to 1,2,4-TMB, and microscopic histopathological analysis of the liver did not demonstrate any observable changes following exposure to either isomer. Similarly, although increased cholesterol levels and alkaline phosphatase levels could indicate hepatic dysfunction, no gross or histopathological lesions were observed in animals orally exposed to 1,3,5-TMB. 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 in Korsak et al. (2000a; 1997).

1.1.4.2. *Summary of Hematological and Clinical Chemistry Effects*

Hematological and clinical chemistry toxicity was observed following inhalation and oral exposure to TMBs based on evidence 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 (Battig et al., 1956), as reviewed in MOE (2006) and Baettig et al. (1958). Although this study reported hematological effects (alterations in clotting and anemia), exposure was to a mixture of TMB isomers and other VOCs. Therefore, it is impossible to attribute the effects to any TMB isomer. There is evidence of hematological effects in male and female Wistar rats following inhalation exposure (Korsak et al., 2000a, b), that are roughly analogous to those observed in humans. Additionally, there is some evidence of hematological and clinical chemistry effects in male and female Sprague-Dawley rats following oral exposure (Koch Industries, 1995b).

In summary, the evidence supports a determination that 1,2,4-TMB and 1,2,3-TMB result in hematological toxicity following inhalation exposure, based on consistency and coherency of effects across species (human and rats). The general lack of data on hematological effects following exposure to 1,3,5-TMB precludes a determination of hazard to humans for this isomer, although it is reasonably anticipated given the observed effects following 1,2,4-TMB or 1,2,3-TMB exposure.

1.1.5. Carcinogenicity

There are no studies in humans that investigated the carcinogenic potential of the TMB isomers by any route of exposure. 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 4 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 EPA performed the Fisher's exact test on the incidences calculated from the reported percentages of animals bearing tumors in the control and exposed animals, no statistically significant elevations in tumor incidence relative to controls were observed.

Janik-Spiechowicz et al. (1998) investigated the genotoxicity of TMB isomers by measuring three genotoxic endpoints: mutation frequency in bacteria, micronucleus formation in mice, and sister chromatid exchanges in mice. Neither 1,2,4-TMB or 1,3,5-TMB induced gene mutations in any *Salmonella typhimurium* strain tested (TA102, TA100, TA98, and TA97a). However, 1,2,3-TMB induced gene mutations in all four strains in absence of rat S9 fraction. When cells were incubated in the presence of S9, 1,2,3-TMB did not induce gene mutation, indicating possibly that 1,2,3-TMB itself is the primary mutagen. No isomer induced 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, but not 1,2,3-TMB, exhibited a statistically significant reduction in the ratio of polychromatic erythrocytes to normochromatic erythrocytes, indicating bone marrow cytotoxicity. All three isomers significantly increased the frequency of sister chromatid exchanges (SCEs) in Imp:BALB/c mice following i.p. injection, with 1,2,4-TMB eliciting the more significant response. These results appear to have occurred at doses that did not induce significant bone marrow cytotoxicity.

In summary, very little genotoxicity data are available on TMBs. Janik-Spiechowicz et al. (1998) observed varying results in the Ames mutation assay in Salmonella, with 1,2,3-TMB, but not 1,2,4-TMB or 1,3,5-TMB, inducing gene mutations. Results for the in vivo assays for micronucleus and SCE formation were consistent across isomers: TMB isomers were observed to induce SCEs, but not micronuclei in mouse bone marrow cells. 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 isomer (1,2,3-TMB) demonstrating a positive result for gene mutation and positive SCE results for all three isomers, there is inadequate evidence to conclude that any isomer is directly genotoxic.

1.1.6. Similarities Among TMB Isomers Regarding Observed Inhalation and Oral Toxicity

In the existing toxicological database for 1,2,3-TMB, 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 these three isomers in male and female Wistar rats, although some important differences also exist (Table 1-7).

In acute studies investigating respiratory irritative effects (i.e., decreased respiratory rate), the RD_{50} for the three isomers were very similar, ranging from 2,553 to 2,844 mg/m^3 (Korsak et al., 1997). Measures of acute inhalation neurotoxicity, namely EC_{50} values for decreases in rotarod performance (4,694 and 4,738 mg/m^3) and pain sensitivity (5,683 5,963 mg/m^3), were also similar for 1,2,4-TMB and 1,3,5-TMB, respectively (Korsak and Rydzyński, 1996). However, the EC_{50} values for both measures were lower following exposure to 1,2,3-TMB (3.779 and 4,172 mg/m^3, respectively). The observation that 1,2,3-TMB may be slightly more neurotoxic than 1,2,4-TMB or 1,3,5-TMB was also observed following acute oral and injection exposures. Although all three isomers were observed to result in altered EEG readings, stronger and more persistent effects followed a pattern of 1,2,3-TMB > 1,3,5-TMB > 1,2,4-TMB following oral exposures (Tomas et al., 1999a) and 1,2,3-TMB > 1,2,4-TMB > 1,3,5-TMB following i.p. injections (Tomas et al., 1999c). Acute exposure to both 1,2,4-TMB and 1,2,3-TMB affected motor function and/or anxiety at similar exposure levels, whereas 1,3,5-TMB appeared to be slightly more potent, although the magnitude of the response across isomers suggests that this difference is negligible (Tomas et al., 1999b).

In short-term neurotoxicity studies, a qualitatively similar pattern of effects (inability to learn passive and/or active avoidance and decreased pain sensitivity following foot shock challenge) indicating altered neurobehavioral function was observed for TMBs, although some quantitative differences were noted (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b). Exposure to any isomer resulted in statistically significant decreases in pain sensitivity following foot shock challenge at the same concentration, although the magnitude of effect and consistency across studies was greater for 1,3,5-TMB and 1,2,4-TMB compared to 1,2,3-TMB (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b). 1,2,4-TMB and 1,3,5-TMB were also observed to increase motor function and/or decrease anxiety in open field tests, whereas 1,2,3-TMB was observed to have no statistically significant effects (Lutz et al., 2010; Wiaderna et al., 2002, 1998; Gralewicz et al., 1997b). In contrast, increased locomotor activity elicited by amphetamine was amplified following exposure to 1,2,3-TMB, but not 1,2,4-TMB (Lutz et al., 2010). All three isomers elicited effects on cognitive function, as measured by learning decrements in two-way active avoidance or by decreased fear responses in a passive avoidance test paradigm(Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b). 1,3,5-TMB was observed to be the most potent isomer in this regard, eliciting effects on both passive and

active avoidance at ≥ 123 mg/m^3. 1,2,3-TMB and 1,2,4-TMB affected passive avoidance performance at ≥ 123 and ≥ 492 mg/m^3, respectively, and both 1,2,3-TMB and 1,2,4-TMB affected the ability to learn active avoidance at 492 mg/m^3. For all isomers, short-term exposure to 1,230 mg/m^3 TMB was nearly always less effective (or ineffective), as compared to lower TMB concentrations, at eliciting responses (i.e., responses were nonlinear).

Following subchronic exposure to either 1,2,4-TMB or 1,2,3-TMB, both decreased pain sensitivity and decreased rotarod performance were observed. With regard to decreased pain sensitivity, although 1,2,3-TMB was observed to decrease pain sensitivity at a lower concentration than 1,2,4-TMB, the magnitude of effect was similar between isomers at every concentration (Korsak and Rydzyński, 1996). For either isomer, effects on pain sensitivity appeared to be reversible at 1,230 mg/m^3 TMB; lower concentrations were not tested. 1,2,3-TMB was more potent than 1,2,4-TMB in reducing rotarod performance. Specifically, 1,2,3-TMB elicited effects at a lower concentration and caused a greater magnitude of effect at each concentration, as well as following a period of recovery (Korsak and Rydzyński, 1996).

Similarities were also observed in 1,2,4-TMB- and 1,3,5-TMB-induced developmental and maternal effects (Saillenfait et al., 2005). Male fetal weights were significantly reduced in animals exposed gestationally to 2,952 mg/m^3 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/m^3, female fetal weights were decreased at 2,952 mg/m^3 to a similar degree (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/m^3 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%.

Lastly, 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB were observed to elicit hematological toxicity in exposed animals. Although all three isomers were observed to qualitatively affect similar hematological parameters, the direction and magnitude of effect often differed between isomers. Red blood cells were significantly decreased in male rats exposed to 1,230 mg/m^3 1,2,3-TMB (23% decrease) or 1,2,4-TMB (15% decrease) (Korsak et al., 2000a, b). Reticulocyte numbers were also altered in rats following exposure to these isomers, although 1,2,4-TMB was observed to significantly decrease reticulocytes in male rats at 1,230 mg/m^3 (71% decrease), while exposure to 1,2,3-TMB increased reticulocytes in male rats at 1,230 mg/m^3 (61% increase) and female rats at 123 and 492 mg/m^3 (77% and 100% increases, respectively). 1,2,3-TMB and 1,2,4-TMB were also altered the numbers of white blood cells in exposed animals following subchronic exposures. In male rats exposed to 1,230 mg/m^3 1,2,4-TMB, white blood cell numbers were significantly increased by 80%. Exposure to 1,230 mg/m^3 1,2,3-TMB also increased lymphocyte numbers by

11% and 15% in male and female rats, respectively. Exposure to 1,230 mg/m^3 1,2,3-TMB decreased segmented neutrophils by 29% in male rats, whereas exposure to 492 mg/m^3 and 1,230 mg/m^3 decreased neutrophil numbers in female rats by 29% and 48%, respectively. Acute exposure (six hours) to 1,500 – 6,000 mg/m^3 1,3,5-TMB was also reported to result in increased numbers of segmented neutrophils that persisted for up to 28 days post exposure (Wiglusz et al., 1975b). A summary of these comparisons across isomers is presented below in Table 1-7.

Table 1-7. Similarities between 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB regarding observed inhalation and oral toxicity

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

1.1.7. Similarities Among TMB Isomers Regarding Toxicokinetics

In the existing toxicokinetic database for 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB, important similarities have been observed in the chemical properties and absorption, distribution, metabolism, and excretion profiles for these isomers in animals and humans, although some important differences also exist.

All three isomers have very similar Log K_{ow} values (3.42–3.78), and blood:air partition coefficients reported for humans and rats in the literature are similar: 43.0 and 55.7 for 1,2,4-TMB, 66.5 and 62.6 for 1,2,3-TMB, and 59.1 and 57.7 for 1,3,5-TMB (Meulenberg and Vijverberg, 2000). This gives an indication that the three 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), although the absorption for 1,2,3-TMB was observed to be higher (7.3 μM) (Järnberg et al., 1998, 1997a; Järnberg et al., 1996). Also, the net respiratory uptake of 1,2,3-TMB, 1,2,4-TMB and 1,3,5-TMB was similar among humans (48-60%), and the respiratory uptake for 1,2,4-TMB was similar across humans and rats (50-60%)(Järnberg et al., 1996; Dahl et al., 1988). Although no data exist regarding the distribution of TMB isomers in humans, experimentally-derived tissue-specific partition coefficients were similar for all three isomers across a number of organ systems (Meulenberg and Vijverberg, 2000), strongly suggesting that the individual isomers can be expected to distribute similarly to these various organ systems. Distribution of the 1,2,4-TMB and 1,3,5-TMB throughout the body is qualitatively similar in animals, although it appears that liver and kidney concentrations for 1,2,4-TMB are greater than those for 1,3,5-TMB after both acute and short-term inhalation 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. Detailed information regarding the distribution of 1,2,3-TMB following inhalation exposure is lacking. However, similar tissue-specific partition coefficients for 1,2,3-TMB compared to 1,2,4-TMB and 1,3,5-TMB indicate a similar pattern of distribution can be reasonably anticipated (Meulenberg and Vijverberg, 2000).

All three TMB 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 amounts of inhaled TMB recovered as hippuric acid metabolites following exposure to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB was dissimilar in humans (11%, 22%, and 3%, respectively) and rats (10%, 24–38%, and 59%, respectively) (Järnberg et al., 1996; Mikulski and Wiglusz, 1975). Greater amounts of urinary benzoic acid and hippuric acid metabolites (73%) were observed after exposure to higher amounts of 1,3,5-TMB (up to 30.5 ppm) for 8 hours (Kostrzewski et al., 1997; Kostrewski and Wiaderna-Brycht, 1995). 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; mercapturic acids (~5% total dose), phenols (<1–8% total dose), and glucuronides and sulphuric acid conjugates (8–15% total dose) for 1,2,3-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, 1999; Huo et al., 1989; Wiglusz, 1979; Mikulski and Wiglusz, 1975).

In humans, the half-lives of elimination from blood were observed to be similar for all isomers in the first three phases of elimination: 1,2,4-TMB (1.3 ± 0.8 min, 21 ± 5 min, 3.6 ± 1.1 hr), 1,2,3-TMB (1.5 ± 0.9 min, 24 ± 9 min, 4.7 ± 1.6 hr), and 1,3,5-TMB (1.7 ± 0.8 min, 27 ± 5 min, 4.9 ± 1.4 hr) (Järnberg et al., 1996). The half-life of elimination for 1,3,5-TMB in the last and longest phase is much greater than those for 1,2,4-TMB or 1,2,3-TMB (120 ± 41 hr vs. 87 ± 27 and 78 ± 22 hr, respectively). Urinary excretion of unchanged parent compound was extremely low (<0.002%) for all three isomers (Janasik et al., 2008; Järnberg et al., 1997b). The difference observed in half-lives between the three isomers in the last elimination phase may be due to small sample sizes and difficulties in measuring slow elimination phases rather than a true difference in half-lives. All three isomers were eliminated via exhalation: 20–37% of the absorbed dose of 1,2,4-TMB, 1,2,3-TMB, or 1,3,5-TMB was eliminated via exhalation during exposure to 123 mg/m^3 (25 ppm) for 2 hours (Järnberg et al., 1996). At low concentrations in rats, half-life of elimination from the blood was greater for 1,2,4-TMB compared to 1,3,5-TMB (3.6 vs. 2.7 hours). 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 1,230 mg/m^3 for 6 hours) (Swiercz et al., 2003; Swiercz et al., 2002). For a full discussion of the chemical properties and toxicokinetics 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB see Appendices B.1 and B.2.

1.2. Summary and Evaluation

1.2.1. Weight of Evidence for Effects Other Than Cancer

In both humans and animals, inhalation exposure to TMBs has been shown to result in toxicity in multiple organ systems, including the nervous, respiratory, and hematological systems. In addition, developmental toxicity has been observed in animals exposed to either 1,2,4-TMB or 1,3,5-TMB. Generally, the information regarding inhalation toxicity in humans is limited for a number of reasons, including that the majority of human studies involved exposure to complex VOC mixtures containing several TMB isomers and other VOCs, and not the individual isomers themselves. Therefore, the observed health effects cannot be attributed to specific TMB isomers. However, these studies observe effects in exposed human populations that are generally analogous to effects observed in animal toxicity studies, and provide qualitative, supportive evidence for hazard identification. Currently, no human studies exist that investigate the oral toxicity of any TMB isomer. Potential limitations in the animal inhalation and oral toxicity database for TMBs include the lack of a chronic study and the fact that all of the available inhalation animal studies were conducted by the same research group: The Nofer Institute of Occupational Medicine, Lodz Poland.

The most strongly and widely supported manifestation of toxicity in humans and animals following inhalation exposure to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB is neurotoxicity. In humans exposed to TMB-containing VOC mixtures, a multitude of effects, including neuropsychological

effects (Chen et al., 1999), deficits in short-term memory and reduced motor speed/coordination (Lee et al., 2005), abnormal fatigue (Norseth et al., 1991), dysfunction of the inner ear/vertigo (Sulkowski et al., 2002), and nervousness, anxiety, and/or vertigo [Battig et al. (1956), as reviewed by MOE (2006) and Baettig et al. (1958)], have been observed. None of the available human studies have addressed the potential for latent neurological effects and no studies examined the potential for neurological effects in sensitive populations. Although the reported human symptoms do not directly parallel the animal data, exposure of male Wistar rats to the TMB isomers has been shown to consistently result in a multitude of neurotoxic effects, including decreased pain sensitivity, impaired neuromuscular function and coordination, altered cognitive function, decreased anxiety and/or increased motor function, and neurophysiological effects (e.g., decreased electrocortical activity) across multiple concentrations and durations (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996; Korsak et al., 1995).

The effects observed in the animal neurotoxicity studies are recognized in the U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) as possible indicators of neurotoxicity. The effects observed include concentration-dependent decrements in pain sensitivity in hot plate tests and neuromuscular function in rotarod tests following subchronic exposure. Although effects on pain sensitivity appeared to be reversible at the highest concentration (i.e., 1,230 mg/m^3), reversible effects occurring in occupational settings may be of high concern, particularly if they diminish a person's ability to survive or adapt to the environment [(U.S. EPA, 1998), pg.8]; such is the case for exposure to TMBs in occupations with dangerous surroundings and/ or heavy equipment, such as dockyard painters or asphalt workers. These effects are supported by additional data from short-term exposure studies that consistently identified latent effects of TMBs exposure on pain sensitivity in hot plate tests following an environmental challenge (i.e., foot shock), alongside reproducible learning decrements in passive and active avoidance experiments, altered EEG patterns, and increased locomotor activity in open field tests. Further, the data from these short-term studies clearly indicated a persistence of neurological effects several weeks after TMB exposures had ended and identified a consistent nonlinearity in many of the TMB-elicited responses (e.g., 1,230 mg/m^3 was nearly always substantially less effective than 123 or 492 mg/m^3). The neurotoxic effects are biologically plausible and analogous to effects that could occur in humans. Thus, the evidence for TMBs identifies neurotoxicity as a toxicity hazard based on consistency and coherency of effect across multiple studies and durations of exposure.

Three acute oral studies (Tomas et al., 1999a; Tomas et al., 1999b; Tomas et al., 1999c) observe similar effects as observed in the available inhalation neurotoxicity studies (i.e., increased locomotor activity and altered brain wave activity). However, these studies are also limited with regard to the range of endpoints investigated, and as such, no weight of evidence determination can be made regarding the chronic oral toxicity of the TMB isomers.

In addition to neurotoxicity, both respiratory and hematological toxicity have been observed in human populations and animals exposed to TMBs, or to mixtures containing the three isomers. In humans, occupational and residential exposure to VOC mixtures containing TMB isomers have resulted in number of effects characterized as respiratory toxicity, including asthmatic bronchitis ((Battig et al., 1956), as reviewed in MOE (2006) and Baettig et al. (1958)), asthma (Billionnet et al., 2011), or laryngeal/pharyngeal irritation (Norseth et al., 1991). Additionally, workers exposed to a VOC mixture containing 1,2,4-TMB and 1,3,5-TMB, and possibly 1,2,3-TMB, were reported to exhibit hematological effects including alterations in clotting time and anemia ((Battig et al., 1956), as reviewed in MOE (2006) and Baettig et al. (1958)). Again, as workers were exposed to complex VOC mixtures containing TMB isomers, the observed health effects cannot be attributed to any single TMB isomer.

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 concentrations, and subchronic and acute exposure durations (Korsak et al., 2000a; Korsak et al., 1997; Korsak et al., 1995). Respiratory toxicity was also observed in multiple studies involving exposure to 1,2,3-TMB (Korsak et al., 2000b; Korsak et al., 1995). Although the reported symptoms in humans (laryngeal and/or pharyngeal irritation, asthmatic bronchitis, and 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. Additionally, multiple measures of hematological toxicity have been observed in rats subchronically exposed to 1,2,4-TMB or 1,2,3-TMB, including decreased RBCs, increased WBCs, decreased clotting time, and decreased reticulocytes (1,2,4-TMB) and decreased RBCs, decreased segmented neutrophils, increased lymphocytes and increased reticulocytes (1,2,3-TMB) (Korsak et al., 2000a, b). At least two of these effects, decreased RBCs and decreased clotting time, are roughly analogous to the hematological effects (alterations in clotting and anemia) observed in occupationally exposed humans, thereby demonstrating a consistency and coherency of effect across species. Therefore, the respiratory and hematological effects observed in animals are biologically plausible and analogous to effects that could occur in exposed human populations. The available evidence for 1,2,4-TMB and 1,2,3-TMB identified respiratory and hematological toxicity as a hazard.

Currently, no human studies exist that investigate the reproductive or developmental toxicity of 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB. However, one animal study (Saillenfait et al., 2005) observed effects on fetal body weights and maternal body weight gains due to gestational exposure to 1,2,4-TMB or 1,3,5-TMB. Although the weight of evidence regarding developmental toxicity is not as strong compared to other measures of toxicity in the TMB database, these effects observed in animals are considered biologically plausible and potentially analogous to effects that could occur in humans. The available evidence for 1,2,4-TMB and 1,3,5-TMB identifies maternal and developmental toxicity as a hazard.

1.2.2. Weight of Evidence for Carcinogenicity

Under the *Guidelines for Carcinogen Risk Assessment* (2005a), the database for the TMBs provides "inadequate information to assess 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 available on the carcinogenicity of 1,2,4-TMB observed no statistically significant carcinogenic effects. No studies regarding the carcinogenicity of 1,2,3-TMB or 1,3,5-TMB were identified in the available scientific literature.

In the animal carcinogenicity study identified (Maltoni et al., 1997), involving exposure to 1,2,4-TMB by oral gavage, an increased incidence of total malignant tumors in both sexes and head cancers (predominantly 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.

Additionally, in the only study investigating the genotoxicity of TMB isomers, Janik-Spiechowicz et al. (1998) observed negative results in in vitro genotoxicity assays (i.e., Ames mutation assay in Salmonella) involving 1,2,4-TMB and 1,3,5-TMB. However, 1,2,3-TMB was observed to induce gene mutations in all *Salmonella typhimurium* strains tested. All three isomers failed to induce micronuclei in mouse bone marrow cells. Janik-Spiechowicz et al. (1998) observed an increased incidence of SCE in mice exposed to all three TMB isomers (individually); however, this observation does not provide a specific indication of mutagenic potential. Given the findings regarding the in vitro genotoxicity of the TMB isomers, and increased frequency SCEs does not provide specific indication of mutagenic potential, the evidence is inadequate to conclude that any TMB isomer is genotoxic.

1.2.3. Susceptible Populations and Lifestages

Although there are no chemical-specific data that would allow for the identification of susceptible populations and lifestages, 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 following inhalation and oral exposure 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 the TMB isomers, 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.

2. DOSE-RESPONSE ANALYSIS

2.1. Inhalation Reference Concentration for Effects Other Than Cancer for 1,2,4-TMB

The RfC (expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

2.1.1. Identification of Studies and Effects for Dose-Response Analysis for 1,2,4-TMB

The nervous, respiratory, hematological systems, as well as pregnant animals and the developing fetus, are the primary targets of inhaled 1,2,4-TMB in humans and experimental animals, and effects in these systems have been identified as hazards following inhalation exposure to 1,2,4-TMB.

The selection of studies and general procedures for dose-response analysis are outlined in Sections 6 and 7 of the Preamble. Human data are preferred over animal data for deriving reference values when possible because the use of human data is more relevant in the assessment of human health and avoids the uncertainty associated with interspecies extrapolation introduced when animal data serve as the basis for the reference value. In this case, while literature exists on the effects of 1,2,4-TMB exposure in humans, including neurological, respiratory, and hematological toxicities, no human studies are available that would allow for dose-response analysis. The human studies evaluated TMB exposures occurring as complex solvents or VOC mixtures, and this confounding 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 indicate a coherency of effects in both humans and laboratory animals.

Several studies investigating 1,2,4-TMB effects in experimental animal models were identified in the literature. No chronic studies were available, although acute, short-term,

subchronic, and developmental toxicity studies were identified. 1,2,4-TMB-induced toxicity was observed across several organ systems in three subchronic studies by Korsak et al., (2000a; 1997) and Korsak and Rydzyński (1996), and in pregnant animals and developing fetuses in a developmental toxicity study by Saillenfait et al. (2005). These four studies were the only subchronic or developmental studies identified in the peer-reviewed literature. Data from these studies pertaining to the primary hazards observed in humans and animals identified in Chapter 1 (neurological, respiratory, and hematological toxicity) or in animals only (maternal and developmental toxicity) were considered as candidate critical effects for the purpose of determining the point of departure (POD) for derivation of the inhalation RfC for 1,2,4-TMB. Neurotoxicity was also observed in both acute and short-term inhalation studies and respiratory toxicity was also observed in acute studies. However, the high concentrations used in acute studies and the short exposure durations employed in both acute and short-term studies limit their utility for the quantitation of chronic human health effects. Nevertheless, as with the human mixture studies, these studies provide qualitative information regarding hazard identification, especially the observation of the consistency and coherency of these effects across the 1,2,4-TMB database.

The three subchronic studies by Korsak et al., (2000a; 1997) and Korsak and Rydzyński (1996), and the developmental toxicity study by Saillenfait et al. (2005), adequately supported dose response analysis. All four studies exposed rats, a common model for human response, by inhalation, to 1,2,4-TMB (reported as ≥ 97-99% pure [impurities not reported]). All studies used at least three exposure levels, spaced approximately threefold apart. All controls were exposed under similar conditions to untreated air. The durations of exposure, subchronic or gestational, were suitable for the effects under evaluation: neurological, developmental, and short-term general toxicity. In addition, the persistence of some outcomes after termination of exposure was investigated. Typical numbers of animals per exposure group for these study designs were used: at least 10/group for the subchronic studies [Korsak et al., (2000a; 1997), Korsak and Rydzyński (1996)]; and 25/group for the developmental study (Saillenfait et al. (2005). Regarding exposure characterization, Korsak et al. (2000a) and Saillenfait et al. (2005) reported actual concentrations, as measured by gas chromatography, to be within 10% of target concentrations. This increases the confidence in the overall adequacy of these studies. Although Korsak and Rydzyński (1996) and Korsak et al. (1997) did not report actual, measured concentrations, these studies used the same exposure methodology as Korsak et al. (2000a); suggesting that it is likely that the actual concentrations in these studies were also within 10% of target concentrations. Target and actual concentrations are presented in Table 2-1.

Table 2-1. Target and actual inhalation concentrations, and internal blood dose metrics of 1,2,4-TMB calculated using the available rat PBPK model (Hissink et al., 2007)

Reference	Species/ sex	Body weight (kg)[a]	Exposure concentration (mg/m^3)[b]	Internal dose – average weekly venous blood concentration (mg/L)
Korsak and Rydzyński (1996)	Rat, male	0.387	123	0.1272
		0.404	492	0.8666
		0.403	1,230	5.4424
Korsak et al. (1997)	Rat, male	0.383	123	0.1272
		0.409	492	0.8661
		0.416	1,230	5.4274
Korsak et al. (2000a)	Rat, male	0.390	123 (129)	0.1339
		0.399	492 (492)	0.8671
		0.389	1,230 (1,207)	5.2481
	Rat, female	0.243	123 (129)	0.1335
		0.230	492 (492)	0.8899
		0.229	1,230 (1,207)	5.5189
Saillenfait et al. (2005)	Rat: Female (pregnant dam); Male and female (fetuses)	--	492 (492)	n/a
		--	1,476 (1,471)	n/a
		--	2,952 (2,913)	n/a
		--	4,428 (4,408)	n/a

[a]For Korsak et al. (2000a; 1997), exposure group-specific terminal body weights from those studies were used to calculate internal dose metrics; for Korsak and Rydzyński (1996) the average of the exposure group-specific body weights reported in Korsak et al. (2000a; 1997) were used in internal dose metric calculations. For Saillenfait et al. (2005), body weights were not provided so the PBPK model was not used to derive internal dose metrics for this study

[b] For Korsak et al. (2000a) and Saillenfait et al. (2005), values in parentheses are actual concentrations, as measured by gas chromatography

Rat PBPK model (Hissink et al., 2007)

These subchronic and developmental toxicity studies examined 1,2,4-TMB-induced toxicity in multiple organ systems and neurological, respiratory, hematological, maternal, or developmental toxicity endpoints that demonstrated statistically significant increases or decreases relative to control were considered for the derivation of the RfC for 1,2,4-TMB (Table 2-2). The endpoints included decreased pain sensitivity in male rats (Korsak and Rydzyński, 1996), increased BAL total cells in male rats (Korsak et al., 1997), increased inflammatory lung lesions, decreased RBCs, and increased WBCs in male rats and decreased reticulocytes and clotting time in female rats (Korsak et

al., 2000a), and decreased fetal weight (males and females) and decreased maternal weight gain (Saillenfait et al., 2005). Increases in BAL polymorphonuclear leukocytes and lymphocytes observed in the Korsak et al. (1997) study were not considered for RfC derivation due to a lack of reporting of exposures at which statistically significant increases occurred. Additionally, Korsak et al. (1997) reported that 123 mg/m^3 was the LOAEL for increased BAL total cells, but the NOAEL for increased BAL macrophages. Therefore, increased BAL macrophages were not considered for RfC derivation as these effects were not observed at concentrations that elicited an increase in total BAL cells. 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.

Impaired neuromuscular function and coordination, measured as performance deficits on the rotarod apparatus, was also observed in rats exposed to 1,2,4-TMB. The use of rotarod data from Korsak and Rydzyński (1996) was initially considered as a candidate critical effect for 1,2,4-TMB. However, upon critical evaluation of the exposure-response information in the study, it was determined that rotarod performance was reported in a manner that reduced the confidence in the observed effect levels. The most widely used and accepted measure of rotarod performance in rodents is latency to fall from the rotating rod (Brooks and Dunnett, 2009; Kaspar et al., 2003; Bogo et al., 1981), typically with an arbitrary upper limit on the maximum latency allowed to prevent confounding by fatigue. The primary limitation for these data was that rotarod performance was presented as percent of failures to last 2 minutes on the apparatus. Although the quantal percent failures data can provide useful information, these measures require an arbitrary selection of the length of time required for successful performance; there is no scientific consensus on an optimal time for this parameter. In addition, when identifying effect levels based on the data presented by Korsak and Rydzyński (1996), latencies on the rod of 1 second versus 119 seconds would be treated identically as failures when, in fact, they indicate very different levels of neurological dysfunction (Bogo et al., 1981). This adds uncertainty when trying to extrapolate to a concentration associated with a minimally adverse effect. Finally, this quantal presentation of data does not allow for interpretations related to intra-rat and intra-group variability in performance. Due to these reporting limitations, impaired neuromuscular function and coordination, measured as performance deficits on the rotarod apparatus, was considered to be less informative than the data supporting decreases in pain sensitivity, and thus, was excluded from consideration for derivation of the RfC for 1,2,4-TMB.

Table 2-2. Endpoints considered for the derivation of the RfC for 1,2,4-TMB

Endpoint	Species/ sex	Exposure concentration (mg/m^3)				
Neurological endpoints		**0**	**123**	**492**	**1,230**	
Decreased pain sensitivity (measured as latency to paw-lick, in seconds)[b]	Rat, male	15.4 ± 5.8[a] (n = 9)	18.2 ± 5.7 (n = 10)	27.6 ± 3.2** (n = 9)	30.1 ± 7.9** (n = 10)	
Hematological endpoints		**0**	**123**	**492**	**1,230**	
Decreased RBCs ($10^6/mm^3$)[c] (10^6 cells per 100 µL)	Rat, male	9.98 ± 1.68 (n = 10)	9.84 ± 1.82 (n = 10)	8.50 ± 1.11 (n = 10)	7.70 + 1.38** (n = 10)	
Increased WBCs ($10^3/mm^3$)[c] (10^3 cells per 100 µL)		8.68 ± 2.89 (n = 10)	8.92 ± 3.44 (n = 10)	8.30 ± 1.84 (n = 10)	15.89 ± 5.74** (n = 10)	
Decreased reticulocytes (%)[c]	Rat, female	3.5 ± 2.6 (n = 10)	1.7 ± 2.0 (n = 10)	1.8 ± 0.9 (n = 10)	1.0 ± 0.6* (n = 10)	
Decreased clotting time (s)[c]		30 ± 10 (n = 10)	23 ± 4 (n = 10)	19 ± 5** (n = 10)	22 ± 7* (n =10)	
Respiratory endpoints		**0**	**123**	**492**	**1,230**	
Increased BAL total cells ($10^6/cm^3$)[d]	Rat, male	1.93 ± 0.79 (n = 6)	5.82 ± 1.32*** (n = 6)	5.96 ± 2.80** (n = 7)	4.45 ± 1.58* (n = 7)	
Increased inflammatory lung lesions[c]		[e] (n = 10)	[e] (n =10)	[e] (n = 10)	[e] (n = 10)	
Developmental endpoints		**0**	**492**	**1,476**	**2,952**	**4,428**
Decreased fetal weight (g)[f,g]	Rat, male	5.86 ± 0.34	5.79 ± 0.30	5.72 ± 0.49	5.55 ± 0.48*	5.20 ± 0.42**
	Rat, female	5.57 ± 0.33	5.51 ± 0.31	5.40 ± 0.45	5.28 ± 0.40*	4.92 ± 0.40**

Table 2-2 (Continued): Endpoints considered for the derivation of the RfC for 1,2,4-TMB

Endpoint	Species/ sex	Exposure concentration (mg/m^3)				
Maternal endpoints		**0**	**492**	**1,476**	**2,952**	**4,428**
Decreased maternal weight gain (g)[f]	Rat, female	29 ± 12 (n = 24)	31 ± 14 (n = 22)	27 ± 12 (n = 22)	15 ± 17** (n= 22)	0 ± 14** (n = 24)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[a]Values are expressed as mean ± 1 SD. Korsak and Rydzyński (1996) does not explicitly state that the reported measures of variance in Table 1 of that reference are standard deviations. However, independent analysis conducted by EPA confirms that the reported measures of variance are standard deviations.

[b] Adapted from Korsak and Rydzyński (1996)

[c] Adapted from Korsak et al. (2000a)

[d] Adapted from Korsak et al. (1997)

[e]Incidences for individual exposure groups not reported; however, based on qualitative information reported in the study (i.e., that female rats exhibited a statistically significant increase in inflammatory lung lesions at 492 mg/m^3), a NOAEL of 123 mg/m^3 was identified.

[f] Adapted from Saillenfait et al. (2005)

[g] Numbers of fetuses not explicitly reported. See maternal weight gain for number of litters.

2.1.2. Methods of Analysis for 1,2,4-TMB

This assessment uses PBPK model estimates of internal blood dose metrics coupled with the benchmark dose (BMD) approach, when possible, to estimate a POD for the derivation of an RfC for 1,2,4-TMB (see Section B.3 of Appendix B and Section C.1 of Appendix C for details regarding PBPK model estimates and BMD modeling, respectively). As dosimetry can often be non-linear due to metabolic saturation, and internal dose metrics are expected to correlate more closely to toxic response than external concentrations (Mclanahan et al., 2012), this assessment used the PBPK model-estimated internal dose metrics for dose-response modeling.

A deterministic rat PBPK model (Hissink et al., 2007) was used to convert non-continuous external inhalation concentrations (in mg/m^3) of 1,2,4-TMB to the internal blood dose metric of average weekly venous blood concentration (in mg/L) of 1,2,4-TMB for Korsak et al., (2000a; 1997) and Korsak and Rydzyński (1996) only (see Table 2-1). Weekly average venous blood 1,2,4-TMB concentration was chosen as the internal dose metric on which to base the POD as it is assumed that the parent compound is the toxic moiety of interest and that average venous blood concentration of 1,2,4-TMB is assumed to adequately represent the target tissue dose across the multiple tissues of interest. The use of concentration of parent compound in venous blood as the relevant dose metric in non-metabolizing, non-first pass organs is recommended by Aylward et al. (2011). Furthermore, toluene-induced neurological effects in the brain are provided by Aylward et al. (2011) as an example of a chemically induced toxic endpoint for which this dose metric is relevant. As discussed in Section 1 (*Mode of Action Analysis – Neurotoxic Effects*), 1,2,4-TMB is reasonably expected to have a mode of action for neurotoxic effects similar to toluene, further supporting the selection of venous blood concentration as the relevant internal dose metric.

One consequence of using PBPK model-estimated internal dose metrics as the dose inputs for BMD modeling was the necessity of dropping the high exposure group in all datasets modeled. During the validation and optimization of the animal PBPK model (Hissink et al., 2007) against available animal toxicokinetic datasets, the model accurately reproduced venous blood concentrations of 1,2,4-TMB following repeated (6 hours/day, 5 days/week, 4 weeks) exposures to 123 or 492 mg/m^3 (see Section B.3.3.2, Appendix B). However, the PBPK model consistently overpredicted venous blood concentrations following exposure to 1,230 mg/m^3. It was concluded that the optimized animal PBPK model produces acceptable simulations of venous blood 1,2,4-TMB concentrations for chronic exposures of up to 100 ppm [492 mg/m^3] in rats following inhalation exposure to 1,2,4-TMB (Section B.3.3.2, Appendix B). Therefore, as the model-estimated internal blood dose metrics at the high concentration are not representative of empirically observed blood concentrations, using the high-dose model estimates as dose inputs for BMD modeling is not appropriate. The decision to drop the high concentration results in a loss of information regarding dose-response characteristics at high concentrations and a reduction in the number of available

dose-response models to fit to the data (due to the number of model parameters > exposure groups). However, this methodology is preferred over inclusion of demonstrably inaccurate internal blood dose metrics that result from high exposure concentrations. Additionally, this methodology still allows for BMD modeling of these endpoints, which is preferred over use of the NOAEL/LOAEL approach.

After calculation of internal blood dose metrics, those dose metrics were used as the dose inputs for BMD modeling. As the Hissink et al. (2007) PBPK model was not parameterized for pregnant animals and did not include a fetal compartment, internal dose metrics were not calculated from Saillenfait et al. (2005). Instead, actual exposure concentrations were used for these endpoints.

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 a BMD and its associated 95% lower confidence limit (BMDL) corresponding to a selected benchmark response (BMR). For continuous data (i.e., decreased pain sensitivity, increased BAL total cells, decreased RBCs, decreased reticulocytes, and decreased clotting time) from the Korsak and Rydzyński (1996) and Korsak et al. (2000a; 1997) studies, and maternal weight gain from Saillenfait et al. (2005), no information is available regarding the change in these responses that would be considered biologically significant, thus a BMR equal to a 1 standard deviation change in the control mean was used in modeling these endpoints, consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). For the decreased male and female fetal body weight endpoints identified from the Saillenfait et al. (2005) study, a BMR of 5% relative deviation from the control mean was selected. A 5% decrease in fetal body weight relative to control was determined to be a minimal, biologically significant response. This determination is based on the fact that decreased body weight gain in fetuses and/or pups is considered indicative of altered growth, which has been identified by EPA as one of the four major manifestations of developmental toxicity (U.S. EPA, 1991). In addition, a 10% decrease in adult body weight in animals is generally recognized as a biologically significant response associated with identifying a maximum tolerated dose, but since fetuses and/or pups are generally recognized as a susceptible lifestage, and thus are assumed to be more greatly affected by decreases in body weight than adult animals, a 5% decrease in fetal body weight is considered a biologically significant response. Finally, in humans, reduced birth weight is associated with a series of adverse effects including neonatal and postnatal mortality, coronary heart disease, arterial hypertension, chronic renal insufficiency, and diabetes mellitus (Barker, 2007; Reyes and Mañalich, 2005). For these reasons, the selection of a BMR of 5% for decreased fetal body weight was considered reasonable. Additionally, a BMR equal to a 1 standard deviation change in the control mean was also selected for the BMD modeling of both fetal body weight and maternal body weight gain to facilitate comparisons across assessments [see EPA's *Benchmark Dose Technical Guidance* (2012b)].

Some endpoints for 1,2,4-TMB were not modeled for a variety of reasons, including equal responses at all exposure groups (e.g., increased BAL total cells and decreased reticulocytes), responses only in the high exposure group with no changes in responses in lower exposure groups (e.g., increased WBCs), and absence of incidence data (e.g., increased inflammatory lung lesions). Additionally, some datasets were modeled, but no model provided estimated BMDLs that were considered to be biologically plausible (e.g., decreased clotting time). In cases where BMD modeling was not feasible or modeling failed to appropriately describe the dose-response characteristics, the NOAEL/LOAEL approach was used to identify a POD. Detailed modeling results are provided in Section C.1 of Appendix C.

Because an RfC is a toxicity value that assumes continuous human inhalation exposure over a lifetime, data derived from inhalation studies in animals dose metrics need to be adjusted to account for the noncontinuous exposures used in these studies. This is addressed by calculation of internal dose metrics for the Korsak et al., (2000a; 1997) and Korsak and Rydzyński (1996) studies. For the Saillenfait et al. (2005) study, rats were exposed to 1,2,4-TMB for 6 hours/day for 15 consecutive days (GD6–GD20). Therefore, the duration-adjusted PODs for developmental/maternal effects were calculated as follows:

$$\mathbf{POD_{ADJ}\ (mg/m^3) = POD\ (mg/m^3) \times hours\ exposed\ per\ day/24\ hours}$$

For example, for decreased fetal weight in males, the POD_{ADJ} would be calculated as follows:

$$\mathbf{POD_{ADJ}\ (mg/m^3) = 1{,}640.07\ mg/m^3 \times 6\ hours/24\ hours}$$

$$\mathbf{POD_{ADJ}\ (mg/m^3) = 410\ mg/m^3}$$

For the derivation of an RfC based upon animal data, the calculated POD_{ADJ} values are converted to human equivalent concentrations (HECs) using the available human PBPK model (Hissink et al., 2007) for the selected endpoints from the Korsak et al., (2000a; 1997) and Korsak and Rydzyński (1996) studies. The human PBPK model was run (as described in Appendix B), assuming a continuous (24 hours/day, 7 days/week) exposure, to estimate a human POD_{HEC} that would result from the same weekly average venous blood concentration reflected in the POD_{ADJ} in animals (Table 2-3). As the selected endpoints from Saillenfait et al. (2005) (i.e., decreased fetal body weight, and maternal body weight gain) are assumed to result primarily from systemic distribution of 1,2,4-TMB, and the Hissink et al. (2007) PBPK model is not parameterized for pregnant animals and did not include a fetal compartment, the human equivalent concentration (HEC) for 1,2,4-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 EPA's *RfC Methodology* (U.S. EPA, 1994b). DAFs are ratios of animal and human physiologic parameters, and are dependent on the nature of the contaminant (i.e., particle or gas) and the target site (i.e., respiratory tract or remote to the portal-of-entry [i.e., systemic]) (U.S. EPA, 1994b). For gases with

systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

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

where:

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

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

DAF = 57.7 (Järnberg and Johanson, 1995)**/59.1** (Meulenberg and Vijverberg, 2000)

DAF = 0.98

In cases where the animal blood:air partition coefficient is lower than the human value, resulting in a DAF < 1, the calculated value is used for dosimetric adjustments (U.S. EPA, 1994b). For example, the HEC for decreased female fetal body weight (reported in Saillenfait et al. (2005)) is calculated as follows:

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

$$\mathbf{POD_{HEC} = POD_{ADJ}\ (mg/m^3) \times 0.98}$$

$$\mathbf{POD_{HEC} = 403.2\ mg/m^3 \times 0.98}$$

$$\mathbf{POD_{HEC} = 395.1\ mg/m^3}$$

The calculated POD_{HEC} (mg/m^3) values for all endpoints considered for candidate value derivation are presented in Table 2-3.

Table 2-3. Summary of derivation of points of departure for 1,2,4-TMB

Endpoint/Reference	Species/sex	Model; BMR or NOAEL/LOAEL	POD[a]	Candidate POD_{ADJ}[a]	Candidate POD_{HEC} (mg/m^3)
Neurological endpoints					
Decreased pain sensitivity (Korsak and Rydzyński, 1996)	Rat, male	Exponential M4; 1 SD	0.086	0.086	15.8
Hematological endpoints					
Decreased RBCs (Korsak et al., 2000a)	Rat, male	Linear; 1 SD	0.499	0.499	83.9
Increased WBCs (Korsak et al., 2000a)	Rat, male	NOAEL[b]	0.867	0.867	131.5
Decreased reticulocytes (Korsak et al., 2000a)	Rat, female	NOAEL[b]	0.890	0.890	134.0
Decreased clotting time (Korsak et al., 2000a)	Rat, female	NOAEL[b]	0.134	0.134	24.4
Respiratory endpoints					
Increased BAL total cells (Korsak et al., 1997)	Rat, male	LOAEL[b]	0.127	0.127	23.2
inflammatory lung lesions (Korsak et al., 2000a)	Rat, male	NOAEL[b]	0.134	0.134	24.4
Developmental endpoints					
Decreased fetal weight Saillenfait et al. (2005)	Rat, male	Linear, 5% RD	1,640.07	410	401.8
	Rat, female	Linear, 5% RD	1,612.89	403.2	395.1
Maternal endpoints					
Decreased maternal weight gain (Saillenfait et al., 2005)	Rat, female	Exponential M3, 1SD	2,247.99	562	550.8

[a] Values are weekly average venous blood 1,2,4-TMB concentration (mg/L) for Korsak et al. (2000a; 1997) and Korsak and Rydzyński (1996). See Appendix B for details on PBPK modeling, Values are in mg/m^3 for Saillenfait et al. (2005)

[b] No model was able to fit data adequately, or data were not modeled. NOAEL/LOAEL method used to identify a POD

2.1.3. Derivation of Candidate RfC Values for 1,2,4-TMB

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002) §4.4.5], also described in the Preamble, five possible areas of uncertainty and variability were considered in deriving the candidate RfC values for 1,2,4-TMB. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated uncertainty factor (UF) to be applied to the candidate POD_{HEC} are as follows:

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 and any residual toxicokinetic uncertainty not accounted for by the PBPK model.

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.

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 1 standard deviation change in the control mean for modeled endpoints was selected under the assumption that this BMR represents a minimal, biologically significant change for these effects. For endpoints that could not be modeled, a LOAEL to NOAEL uncertainty factor of 1 was applied as a NOAEL was used, except for increased BAL cells to which a uncertainty factor of 10 was applied due to the use of a LOAEL for this endpoint.

A subchronic to chronic uncertainty factor, UF_S, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for extrapolation from a subchronic exposure duration study to derive a chronic RfC, for all endpoints except decreases in fetal weight, to which an UF_s of 1 was applied. The 3-fold uncertainty factor is applied to the POD identified from the subchronic study on the assumption that effects observed in a similar chronic study would be observed at lower concentrations for a number of possible reasons, including potential cumulative damage occurring over the duration of the chronic study or an increase in the magnitude or severity of effect with increasing duration of exposure. For example, in the case of neurotoxicity, chronic exposures may overwhelm the adaptive responses observed after termination of subchronic exposure, potentially resulting in more severe and/or irreversible changes in neurological function. A full subchronic to chronic uncertainty factor of 10 was not applied in this case as there was evidence of reversibility of not only neurotoxic effects, but also hematological effects in rats exposed to 1,2,4-TMB for subchronic durations. Also,

the respiratory effects appeared to be inflammatory in nature. Although reversibility was not investigated for these endpoints, it is possible that adaptive mechanisms may alleviate these effects following the termination of exposure.

A database uncertainty factor, UF_D, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for database deficiencies. Strengths of the database include the three well-designed subchronic studies that observe exposure-response effects in multiple organ systems (nervous, respiratory, and hematological systems), in Wistar rats exposed to 1,2,4-TMB via inhalation. An additional strength of the database is the well-designed developmental toxicity study that investigated standard measures of maternal and fetal toxicity in a different strain of rat (Sprague-Dawley). However, the lack of a multi-generation reproductive/developmental toxicity study is a weakness of the database. EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) recommends that the database uncertainty factor take into consideration whether there is concern from the available toxicology database that the developing organism may be particularly susceptible to effects in specific organ systems. TMBs (unspecified isomer) are able to cross the placenta (Cooper et al., 2001; Dowty et al., 1976); therefore, as neurotoxicity is observed in adult animals, there is the concern that exposure to 1,2,4-TMB may result in neurotoxicity in the developing organism. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) identifies specific effects observed in adult animals (e.g., cognitive and motor function) that can also affect the developing organism exposed in utero. The Neurotoxicity Guidelines (U.S. EPA, 1998) also indicate that neurotoxicants may have greater access to the nervous system in developing organisms due to an incomplete blood-brain barrier and immature metabolic detoxifying pathways. Lastly, EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) also states that effects that may be mild or reversible in adults may produce more robust or permanent effects in offspring following developmental exposures. Therefore, there is some concern that the lack of a developmental neurotoxicity study is a deficiency in the database and that inclusion of such a study would potentially result in a lower POD than the POD for neurotoxicity identified from the available 1,2,4-TMB toxicity database. In summary, a 3-fold database UF was applied to account for the lack of both a multi-generation reproductive/developmental toxicity study and a developmental neurotoxicity study in the available database for 1,2,4-TMB.

Table 2-4 is a continuation of Table 2-3, and summarizes the application of UFs s to each POD to derive a candidate RfC value for each data set. The candidate RfC values presented in Table 2-4 are preliminary to the derivation of the organ/system-specific RfC values. These candidate RfC values are considered individually in the selection of a representative inhalation reference RfC value for a specific hazard and subsequent overall RfC for 1,2,4-TMB. Figure 2-1 presents graphically these candidate RfC values, uncertainty factors, and points of departure, with each bar corresponding to one data set described in Tables 2-3 and 2-4.

Table 2-4. Effects and corresponding derivation of candidate RfC values for 1,2,4-TMB

Endpoint/Reference		HEC (mg/m^3)[a]	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate RfC value (mg/m^3)[b]
Neurological endpoints									
Decreased pain sensitivity (Korsak and Rydzyński, 1996)		15.8	3	10	1	3	3	300	5.27×10^{-2}
Hematological endpoints									
Decreased RBCs, (Korsak et al., 2000a)		83.9	3	10	1	3	3	300	2.80×10^{-1}
Increased WBCs (Korsak et al., 2000a)		131.5	3	10	1	3	3	300	4.38×10^{-1}
Decreased reticulocytes (Korsak et al., 2000a)		134.0	3	10	1	3	3	300	4.47×10^{-1}
Decreased clotting time (Korsak et al., 2000a)		24.4	3	10	1	3	3	300	8.13×10^{-2}
Respiratory endpoints									
Increased BAL total cells (Korsak et al., 1997)		23.2	3	10	10	3	3	3,000	n/a[c]
Increased inflammatory lung lesions (Korsak et al., 2000a)		24.4	3	10	1	3	3	300	8.13×10^{-2}
Developmental endpoints									
Decreased fetal weight (Saillenfait et al., 2005)	rat, male	401.8	3	10	1	1	3	100	4.02
	(rat, female)	395.1	3	10	1	1	3	100	3.95
Maternal endpoints									
Decreased maternal weight gain (Saillenfait et al., 2005)		550.8	3	10	1	3	3	300	1.84

[a]Human equivalent concentration.

[b]As calculated by application of uncertainty factors, not rounded to 1 significant digit.

[c]Endpoint excluded for further consideration due to a $UF_{COMPOSITE}$ of 3,000. 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 composite uncertainty factor is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Although, 3,000 is generally recognized as the maximum composite uncertainty factor for RfC derivation, a candidate RfC based on the data for increased BAL total cells was not derived due to the fact that the uncertainty surrounding this endpoint was much higher than for any other endpoint.

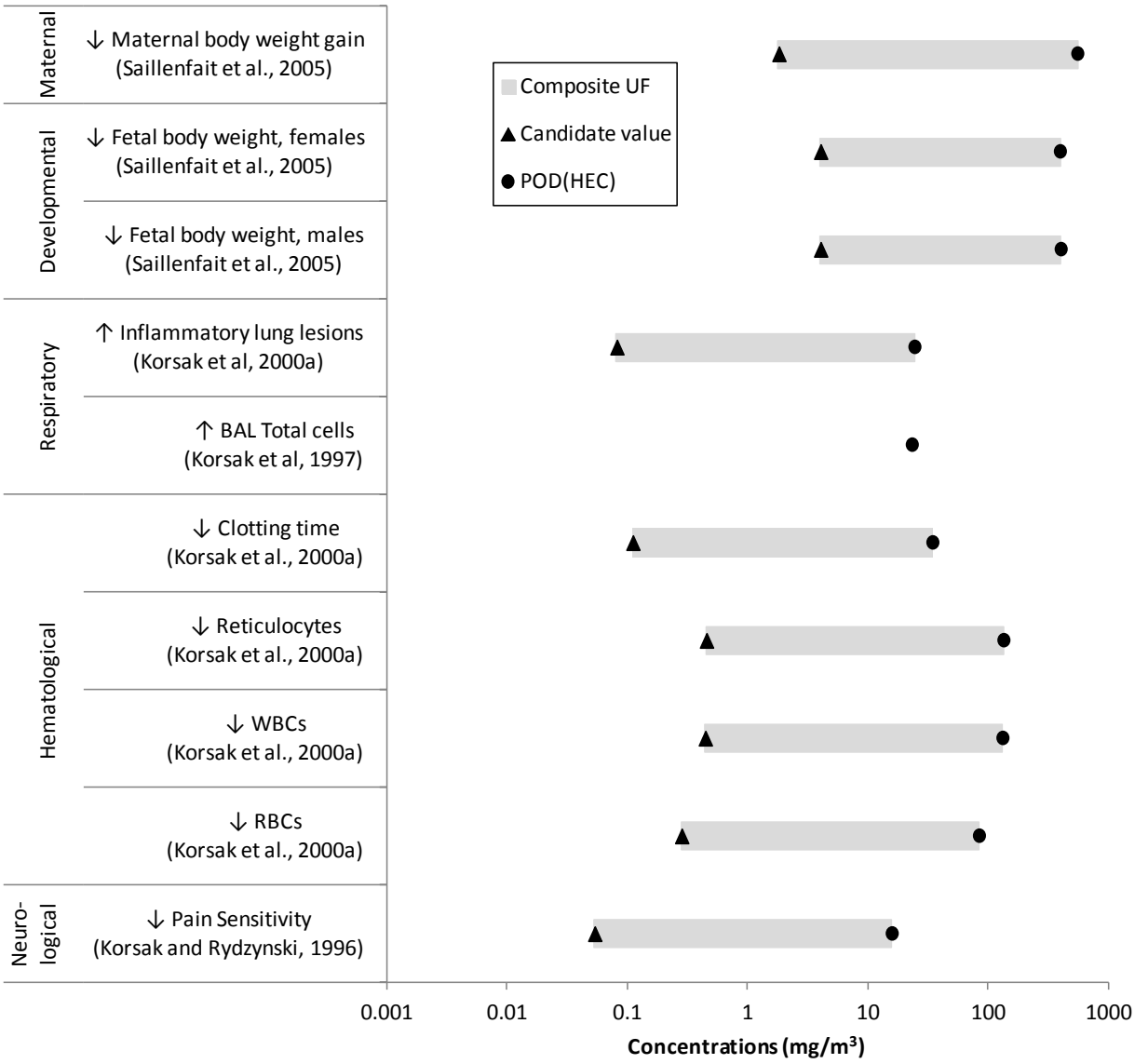


Figure 2-1. Candidate RfC values with corresponding POD and composite UF for 1,2,4-TMB.

2.1.4. Derivation of Organ/System Specific Reference Concentrations for 1,2,4-TMB

Table 2-5 distills the candidate RfC values from Table 2-4 into a single value for each organ or system. The single RfC value selected for a particular organ system was preferably chosen using biological and toxicological information regarding that endpoint. If no compelling biological information exists with which to select the primary hazard, the lowest RfC value for that organ

system was selected. These organ- or system-specific reference concentrations may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site. The individual organs and systems for which specific RfC values were derived were the neurological, hematological, and respiratory systems, along with specific RfCs derived for the pregnant animal (maternal) and developing fetus (developmental). The RfC value for the neurological system, based on decreased pain sensitivity, was selected for the proposed overall RfC for 1,2,4-TMB (see Section 2.1.5 for details). The RfC values for the hematological and respiratory systems, based on decreased clotting time and increased inflammatory lung lesions, respectively, are only slightly higher than the RfC derived for neurological effects (8×10^{-2} vs. 5×10^{-2} mg/m^3), indicating that effects in these organ systems may also be of concern. However, effects to pregnant animals and the developing fetus may be of less concern as the RfCs for these types of effects (based on decreased maternal weight gain and decreased male and female fetal weight, respectively) are much higher than those derived for other organ systems.

Table 2-5. Organ/system-specific RfCs and proposed overall RfC for 1,2,4-TMB

Effect	Basis	RfC (mg/m^3)	Exposure description	Confidence
Hematological	Decreased clotting time	8×10^{-2}	Subchronic	Low to medium
Respiratory	Increased inflammatory lung lesions	8×10^{-2}	Subchronic	Low to medium
Maternal	Decreased maternal weight gain	2	Gestational	Low to medium
Developmental	Decreased fetal weight (males and females)	4	Gestational	Low to medium
Proposed overall RfC (Neurological)	**Decreased pain sensitivity**	5×10^{-2}	**Subchronic**	**Low to medium**

2.1.5. Selection of the Proposed Overall Reference Concentration for 1,2,4-TMB

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* (U.S. EPA, 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, measured as an increased latency to paw-lick in hot plate tests, represents an alteration in

neurobehavioral function (U.S. EPA, 1998). Decreased pain sensitivity or decreased pain sensitivity following a foot shock challenge was observed in multiple studies across multiple exposure durations (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b; Korsak and Rydzyński, 1996; Korsak et al., 1995), and in the presence of other measures of altered neurobehavior, including impaired neuromuscular function and altered cognitive function. Additionally, neurological symptoms (e.g., hand tremble, weakness) were observed in worker populations exposed to complex VOC mixtures containing 1,2,4-TMB (notably, pain sensitivity has not been tested in humans), suggesting a consistency and coherency of neurotoxic effects in humans and animals following exposure to 1,2,4-TMB.

EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) note that effects that are reversible in minutes, hours, or days after the end of exposure and appear to be associated with the pharmacokinetics of the agent and its presence in the body may be of less concern than effects that persist for longer periods of time after the end of exposure. Pain sensitivity was observed to return to control levels 2 weeks after termination of subchronic 1,2,4-TMB exposure at 1,230 mg/m^3 in one study (Korsak and Rydzyński, 1996). However, the *Neurotoxicity Guidelines* also indicate that reversible effects occurring in occupational settings may be of high concern, particularly if they diminish a person's ability to survive or adapt to the environment (U.S. EPA, 1998) (pg. 8); such is the case for exposure to 1,2,4-TMB in occupations with dangerous surroundings and/ or heavy equipment, such as dockyard painters or asphalt workers.

In several short-term studies of TMBs, there is evidence indicating that decreased pain sensitivity in the presence of an additional environmental challenge (i.e., foot shock) is not rapidly reversible and is not associated with clearance of the chemical from the body. TMB isomers have been observed to clear rapidly from blood and nervous tissues (Section B.2, Appendix B), and decreased pain sensitivity following foot shock persisted 51 days after termination of short-term exposures (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b). As pointed out in *A Review of the Reference Dose and Reference Concentration Process* (U.S. EPA, 2002), "[i]t is also important to keep in mind that effects that may initially appear to be reversible may re-appear later or be predictive of later adverse outcomes." (pg. 4-16). Additionally, the *Neurotoxicity Guidelines* (U.S. EPA, 1998) state that "latent effects (those that become evident only after an environmental challenge [e.g., in this case, footshock]) have a high level of concern." The hot plate test is a relatively simple assessment that may not be sensitive enough to detect subtle changes (U.S. EPA, 1998), suggesting that the large changes observed immediately after 1,2,4-TMB exposure may represent gross effects. It is possible that, at longer durations after exposure, an environmental challenge is necessary for the more subtle perturbations that persist to become manifest at a detectable level. The latent decrements in pain sensitivity following foot shock appear to reflect a lengthening of the numbing effects of foot shock following exposure to 1,2,4-TMB weeks earlier, as the immediate increases in latency due to foot shock were unchanged by prior 1,2,4-TMB exposure.

Although these measures may be complicated by less likely, but possible, effects on cognition, the results suggest that some aspect(s) of the altered pain sensitivity phenotype fail to resolve following termination of exposure. No environmental challenge was applied in the subchronic study by Korsak and Rydzyński (1996); such an experiment may have uncovered similar latent responses. Conversely, the short-term 1,2,4-TMB exposure studies testing pain sensitivity failed to analyze hot plate latency with a foot shock challenge shortly after exposure, as these evaluations only occurred at ≥ 50 days post-exposure.

Uncertainty regarding the reversibility of pain sensitivity in non-shocked rats at all tested 1,2,4-TMB concentrations also exists. Reversibility of the pain sensitivity phenotype following subchronic exposure was only tested at the highest concentration of 1,2,4-TMB used in any study (i.e., 1,230 mg/m^3). In multiple other tests of neurological function (including pain sensitivity following a foot shock challenge), it was clearly shown that exposure to 1,2,4-TMB elicits nonlinear effects when tested some period of time after exposure, with 1,230 mg/m^3 1,2,4-TMB usually resulting in no response or a substantially reduced response as compared to lower 1,2,4-TMB concentrations (e.g., 492 mg/m^3). Thus, from the data available, a determination regarding the reversibility of 1,2,4-TMB-induced decreases in pain sensitivity at other concentrations (i.e., 492 mg/m^3) at two weeks post-exposure cannot be made with confidence.

Although it is important to consider the potential for reversibility of neurological effects, "for chronic lifetime exposures, designation of an effect as irreversible or reversible is academic, as exposure is presumed to be lifetime (i.e., there is no post-exposure period)" (U.S. EPA, 2002) (pg. 3-27). In other words, the nature of an RfC precludes the possibility of recovery of the critical effect. This supports the choice of the principal study even were all aspects of the pain sensitivity phenotype identified as transient, which, notably, does not appear to be the case. Taken as a whole, the database supports the characterization of decreased pain sensitivity associated with exposure to 1,2,4-TMB as being an effect of high concern. Given the consistency of observations from hot plate tests with or without foot shock challenge across several studies from the same research group using multiple durations of exposure in male Wistar rats, as well as the evidence and biological plausibility of similarities in neurological effects between rats and humans, there is strong evidence that neurotoxicity is the primary hazard associated with exposure to 1,2,4-TMB. Based on the above considerations, decreased pain sensitivity measured immediately after subchronic exposure is identified as an adverse neurotoxic effect and thus is an appropriate effect on which to base the RfC. Therefore, the candidate RfC for neurotoxicity based on decreased pain sensitivity was selected as the RfC for 1,2,4-TMB.

A POD_{HEC} of 15.8 mg/m^3 for decreased pain sensitivity (Korsak and Rydzyński, 1996) was used as the POD from which to derive the chronic RfC for 1,2,4-TMB (see Table 2-4). The uncertainty factors (UFs), selected and applied in accordance with the procedures described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002)

(Section 4.4.5 of the report)], were discussed previously in Section 2.1.3. Application of the **composite UF of 300** to the POD_{HEC} yields the following chronic RfC for 1,2,4-TMB:

$$\mathbf{RfC = POD_{HEC} \div UF = 15.8\ mg/m^3 \div 300 = 0.05\ mg/m^3 = 5 \times 10^{-2}\ mg/m^3}$$

(rounded to one significant digit)

2.1.6. Uncertainties in the Derivation of the Reference Concentration for 1,2,4-TMB

As presented above, the UF approach, following EPA practices and RfC guidance (U.S. EPA, 2002, 1994b), was applied to the POD_{HEC} in order to derive the chronic RfC for 1,2,4-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, duration of exposure, 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 hematological or respiratory effects would result in similar RfCs that would be equivalent when rounding to one significant digit (i.e., 2×10^{-2} mg/m^3, see Figure 2-2). Some uncertainty exists regarding the selection of the BMRs for use in BMD modeling due to the absence of information to determine the biologically significant level of response associated with the endpoints. However in cases such as this, the selection of a BMR of 1 standard deviation for continuous endpoints is supported by EPA guidance (U.S. EPA, 2012b). In addition, there is uncertainty in the estimated standard deviation for decreased pain sensitivity (Korsak and Rydzyński, 1996), which was two- to threefold higher than that estimated in the parallel evaluation of 1,2,3-TMB in the same publication. Given the lack of information concerning a biologically significant level of response for pain sensitivity, the concurrently estimated standard deviation was judged to be most relevant for characterizing this response to 1,2,4-TMB.

Uncertainty regarding the selection of particular models for individual endpoints exists as selection of alternative models could decrease or increase the estimated POD and consequently, the RfC. The selection criteria for model selection was based on a practical approach as described in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). Uncertainty may exist in the PBPK model estimates of internal blood dose metrics for the rat, and subsequent HEC calculations for humans, including parameter uncertainty, but such uncertainties would apply equally to all endpoints.

2.1.7. Confidence Statement for 1,2,4-TMB

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for*

Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b).

Confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996) is low to medium. The study is a peer-reviewed study that utilized three dose groups plus untreated controls and employed an appropriate number of animals per dose group. However, sources of uncertainty exist that reduce confidence in this study.

One area of uncertainty regarding this study is the lack of reported actual concentrations. However, as the methods by which the test atmosphere was generated and analyzed were reported in sufficient detail, and given the fact that this laboratory has used this methodology in subsequent studies (Korsak et al., 2000a, b) and achieved appropriate actual concentrations (i.e., within 10% of target concentrations), the concern regarding the lack of reported actual concentrations is minimal. The critical effect on which the RfC is based is well-supported as the weight of evidence for 1,2,4-TMB-induced neurotoxicity is coherent across species (i.e., human and rat) and consistent across multiple exposure durations (i.e., acute, short-term, and subchronic) (Gralewicz and Wiaderna, 2001; Chen et al., 1999; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996; Norseth et al., 1991).

The database for 1,2,4-TMB includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. However, confidence in the overall database is low to medium because it lacks chronic, multi-generation reproductive/developmental, and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. The overall confidence in the RfC for 1,2,4-TMB is low to medium.

2.2. Inhalation Reference Concentration for Effects Other Than Cancer for 1,2,3-TMB

2.2.1. Identification of Studies and Effects for Dose-Response Analysis for 1,2,3-TMB

The nervous, hematological, and respiratory systems are the primary targets of inhaled 1,2,3-TMB in humans and experimental animals, and effects in these systems have been identified as hazards following inhalation exposure to 1,2,3-TMB. Although literature exists on the effects of 1,2,3-TMB exposure in humans, including neurological, hematological, and respiratory toxicities, no human studies are available that would allow for dose-response analysis. The human studies evaluated TMB exposures occurring as complex solvents 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, hematological, and respiratory toxicity of TMB isomers in humans and indicate a coherency of effects in both humans and laboratory animals.

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

The two subchronic studies by Korsak et al. (2000b) and Korsak and Rydzyński (1996) are adequate for dose-response analysis. Both studies exposed rats, a common model for human response, by inhalation, to1,2,3-TMB (reported as > 97% pure [impurities not reported]). The studies used three exposure levels spaced two- to threefold apart, facilitating dose-response analysis and utilized sham-exposed controls. The subchronic durations of exposure were suitable for the effects under evaluation. In addition, the persistence of some outcomes after termination of exposure was investigated. Typical numbers of animals per exposure group for subchronic studies were used, at least 10/group. Regarding exposure characterization, Korsak et al. (2000b) reported actual concentrations, as measured by gas chromatography, to be within 10% of target concentrations. This increases the confidence in the overall evaluation and adequacy of this study. Although Korsak and Rydzyński (1996) do not report actual, measured concentrations, this study uses the same exposure methodology as Korsak et al. (2000b); suggesting that it is likely that the actual concentrations in this study were also within 10% of target concentrations. Target and actual concentrations for these studies are presented in Table 2-6.

Table 2-6. Target and actual exposure concentrations used in BMD modeling of 1,2,3-TMB endpoints considered for the derivation of the RfC

Reference	Species/ sex	Target exposure concentration (mg/m^3)	Actual exposure concentration (mg/m^3)
Korsak and Rydzyński (1996)	Rat, male	123	n/a
		492	n/a
		1,230	n/a
Korsak et al. (2000b)	Rat, male	123	128
		492	523
		1,230	1,269
	Rat, female	123	128
		492	523
		1,230	1,269

These two subchronic studies examined 1,2,3-TMB-induced toxicity in multiple organ systems and the neurological, hematological, and respiratory endpoints that demonstrated statistically significant increases or decreases relative to control were considered for the derivation of the RfC for 1,2,3-TMB (Table 2-7). These endpoints included decreased pain sensitivity in male rats (Korsak and Rydzyński, 1996), and decreased RBCs and increased reticulocytes in male rats, decreased segmented neutrophils and increased lymphocytes in male and female rats, and increased inflammatory lung lesions in female rats (Korsak et al., 2000b). Changes in liver organ weights and clinical chemistry parameters from Korsak et al. (2000b) were not further considered due to the lack of accompanying hepatocellular histopathological alterations in exposed animals. Changes in splenic organ weights were similarly not considered further due to a lack of any observed histopathological changes in that organ. Increases in reticulocytes in females were not further considered due to non-monotonicity in response (increases in high concentration animals that were not statistically significant). Increased lymphocytes were excluded from further consideration due to the unusually high standard deviations reported in the high-concentration group.

Table 2-7. Endpoints considered for the derivation of the RfC for 1,2,3-TMB

Endpoint	Species/ sex	Exposure concentration (mg/m^3) [a]			
		0	123	492	1,230
Neurological endpoints					
Decreased pain sensitivity (measured as latency to paw-lick in seconds)[b]	Rat, male	9.7 ± 2.1 (n = 30)	11.8 ± 3.8* (n = 20)	16.3 ± 6.3[c] (n = 10)	17.3 ± 3.4** (n = 10)
Hematological endpoints					
Decreased RBCs (10^6/mm^3)[d] (10^6 cells per 100 µL)	Rat, male	9.49 ± 2.03 (n = 10)	10.25 ± 1.29 (n = 10)	10.11 ± 1.27 (n = 10)	8.05 ± 1.38* (n = 10)
Decreased segmented neutrophils (%)[d]	Rat, male	24.8 ± 4.5 (n = 10)	25.4 ± 5.8 (n = 10)	20.7 ± 5.8 (n = 10)	17.7 ± 8.3* (n = 10)
	Rat, female	23.1 ± 6.1 (n = 10)	19.7 ± 3.4 (n = 10)	16.4 ± 4.2* (n = 10)	11.9 ± 7.1** (n = 10)
Increased reticulocytes (%)[d]	Rat, male	2.8 ± 1.3 (n = 10)	2.1 ± 1.7 (n = 10)	3.8 ± 2.1 (n = 10)	4.5 ± 1.8* (n = 10)
Respiratory Endpoints					
Increased inflammatory lung lesions[d]	Rat, female	[e] (n = 10)	[e] (n =10)	[e] (n = 10)	[e] (n = 10)

$^*p < 0.05$; $^{**}p < 0.01$.

[a]Values are expressed as mean ± 1 SD. Korsak and Rydzyński (1996) does not explicitly state that the reported measures of variance in Table 1 of that reference are standard deviations. However, independent analysis conducted by EPA confirms that the reported measure of variance are standard deviations.

[b] Adapted from Korsak and Rydzyński (1996)

[c] Level of significance not reported in Table 1 from Korsak and Rydzyński (1996), however the results of an ad-hoc t-test (performed by EPA) indicated significance at $p < 0.01$.

[d] Adapted from Korsak et al. (2000b)

[e] Incidences for exposure groups not reported; however, based on qualitative information reported in the study (i.e., that female rats exhibited a statistically significant increase in inflammatory lung lesions at 492 mg/m^3), a NOAEL of 123 mg/m^3 was identified.

Impaired neuromuscular function and coordination, measured as performance on the rotarod apparatus, was also observed in rats exposed to 1,2,3-TMB. See Section 2.1.1 for a detailed discussion of the uncertainties surrounding the use of this endpoint for derivation of an RfC. Due to these uncertainties, this endpoint was excluded from consideration for the derivation of the RfC for 1,2,3-TMB.

2.2.2. Methods of Analysis for 1,2,3-TMB

As discussed above in Section 2.2.1, endpoints observed in Korsak et al. (2000b) and Korsak and Rydzyński (1996) that demonstrated statistically significant ($p < 0.05$ level) increases or decreases relative to control for at least one exposure group were considered for the derivation of the RfC for 1,2,3-TMB; these effects are listed in Table 2-7. This assessment used the BMD approach, when possible, to estimate a POD for the derivation of an RfC for 1,2,3-TMB (see Section C.1 of Appendix C for detailed methodology). The BMD approach involves fitting a suite of mathematical models to the observed dose-response data using EPA's BMDS (version 2.2). Each fitted model estimates a BMD and its associated BMDL corresponding to a selected BMR. For continuous data (i.e., decreased pain sensitivity, decreased RBCs, decreased segmented neutrophils, increased reticulocytes) from the Korsak and Rydzyński (1996) and Korsak et al. (2000b) studies, no information is available regarding the change in these responses that would be considered biologically significant, and thus a BMR equal to a 1 standard deviation change in control mean was used in modeling the endpoints, consistent with the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b). The estimated BMDL is then used as the POD for deriving the RfC (Table 2-8).

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

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

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

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

$$\mathbf{POD_{ADJ}\ (mg/m^3) = 97.19\ mg/m^3 \times 6\ hours/24\ hours \times 5\ days/7\ days}$$

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

Because the majority of the selected endpoints for consideration as the critical effect (decreased pain sensitivity, decreased RBCs, decreased segmented neutrophils, increased reticulocytes) result primarily from systemic distribution of 1,2,3-TMB, and no available PBPK model exists for 1,2,3-TMB, the human equivalent concentration (HEC) for 1,2,3-TMB was calculated by the application of the dosimetric adjustment factor (DAF) for systemically acting gases (i.e., Category 3 gases), in accordance with the U.S. EPA RfC Methodology (U.S. EPA, 1994b). Additionally, although the observation of lung lesions would normally indicate portal-of-entry effects, the observation that the overwhelming majority of 1,2,3-TMB-induced effects are systemic in nature supports the determination that 1,2,3-TMB is a Category 3 gas. Other factors also support that 1,2,3-TMB is a systemically-acting toxicant, including the isomer's relatively low water-solubility and non-reactivity. Gases with these properties are expected to preferentially distribute to the lower regions of the respiratory tract where larger surface areas and thin alveolar-capillary boundaries facilitate uptake. Respiratory absorption of 1,2,3-TMB into the bloodstream has been observed to be relatively high (~60%) following inhalation exposures to humans (Järnberg et al., 1996). Therefore, increased inflammatory lung lesions are assumed to result from systemic distribution of 1,2,3-TMB in the bloodstream of exposed animals. DAFs are ratios of animal and human physiologic parameters, and are dependent on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal-of-entry [i.e., systemic]) (U.S. EPA, 1994b). For gases with systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

$DAF = (Hb/g)_A/(Hb/g)_H$

where:

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

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

DAF = 62.6 (Järnberg and Johanson, 1995)**/66.5** (Meulenberg and Vijverberg, 2000)

DAF = 0.94

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

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

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

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

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

Table 2-8 presents the calculated HECs for the candidate critical effects, selected uncertainty factors (UFs), and the resulting derivation of candidate RfCs from the two subchronic toxicity studies (Korsak et al., 2000b; Korsak and Rydzyński, 1996).

Table 2-8. Summary of derivation of points of departure for 1,2,3-TMB

Endpoint/Reference	Species/sex	Model; BMR or NOAEL/LOAEL	POD (mg/m^3)	Candidate POD_{ADJ} (mg/m^3)	Candidate POD_{HEC} (mg/m^3)
Neurological endpoints					
Decreased pain sensitivity (Korsak and Rydzyński, 1996)	Rat, male	Linear; 1 SD	97.19	17.36	16.32
Hematological endpoints					
Decreased RBCs (Korsak et al., 2000b)	Rat, male	NOAEL[a]	523	93.39	87.79
Increased segmented neutrophils (Korsak et al., 2000b)	Rat, male	Exponential M2; 1 SD	534.81	95.50	89.77
	Rat, female	Hill; 1 SD	99.21	17.72	16.66
Increased reticulocytes (Korsak et al., 2000b)	Rat, male	Linear; 1 SD	652.90	116.58	109.58
Respiratory endpoints					
inflammatory lung lesions (Korsak et al., 2000b)	Rat, male	NOAEL[a]	128	22.86	21.49

[a] No model was able to fit data adequately, or data were not modeled. NOAEL/LOAEL method used to identify a POD.

2.2.3. Derivation of Candidate RfC Values for 1,2,3-TMB

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002) §4.4.5], also described in the Preamble, five possible areas of uncertainty and variability were considered in deriving the candidate RfC values for 1,2,4-TMB. An explanation of these five

possible areas of uncertainty and variability and the values assigned to each as a designated uncertainty factor (UF) to be applied to the candidate POD_{HEC} are as follows:

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between rats and humans following inhalation exposure to 1,2,3-TMB. In this assessment, the use of a DAF to extrapolate external concentrations from rats to humans reduces toxicokinetic uncertainty in extrapolating from the rat data, but does not account for the possibility that humans may be more sensitive to 1,2,3-TMB than rats due to toxicodynamic differences. A default UF_A of 3 was thus applied to account for this remaining toxicodynamic and residual toxicokinetic uncertainty not accounted for in the DAF.

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

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR equal to a 1 standard deviation change in the control mean for modeled endpoints was selected under the assumption that this BMR represents a minimal, biologically significant change for these effects. For endpoints that could not be modeled, a LOAEL to NOAEL uncertainty factor of 1 was applied as a NOAEL was used.

A subchronic to chronic uncertainty factor, UF_S, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for extrapolation from a subchronic exposure duration study to derive a chronic RfC. The 3-fold uncertainty factor is applied to the POD identified from the subchronic study on the assumption that effects observed in a similar chronic study would be observed at lower concentrations for a number of possible reasons, including potential cumulative damage occurring over the duration of the chronic study or an increase in the magnitude or severity of effect with increasing duration of exposure. For example, in the case of neurotoxicity, chronic exposures may overwhelm the adaptive responses observed after termination of subchronic exposure, potentially resulting in more severe and/or irreversible changes in neurological function. A full subchronic to chronic uncertainty factor of 10 was not applied in this case as there was evidence of reversibility of not only neurotoxic effects, but also hematological effects in rats exposed to 1,2,4-TMB for subchronic durations. Also, the respiratory effects appeared to be inflammatory in nature. Although reversibility was not investigated for these endpoints, it is possible that adaptive mechanisms may alleviate these effects following the termination of exposure.

A database uncertainty factor, UF_D, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for database deficiencies. Strengths of the database include the two well-designed subchronic

studies that observe exposure-response effects in multiple organ systems (i.e., neurological, hematological, and respiratory effects) in Wistar rats exposed to 1,2,3-TMB via inhalation. However, the lack of a either a multi-generational reproductive/developmental toxicity study or a developmental toxicity study investigating effects due to 1,2,3-TMB exposure is a weakness of the database. Normally, the lack of both of these types of studies in a toxicity database would warrant the application of a full, 10-fold UF_D in accordance with EPA's *A Review of the Reference Dose and Reference Concentration Processes* (2002). Although there is no developmental toxicity study for 1,2,3-TMB, Saillenfait et al. (2005) investigates the developmental toxicity of the other two TMB isomers (1,2,4-TMB and 1,3,5-TMB) and observes developmental toxicity at levels much higher than those eliciting neurotoxicity, hematotoxicity, and respiratory toxicity in adult animals (Korsak studies). Given that toxic effects were observed at lower concentrations in adult animals exposed 1,2,4-TMB and 1,3,5-TMB compared with rats exposed in utero and the similarities in toxicity profiles amongst the three isomers, it is unlikely that the inclusion of a developmental toxicity study for 1,2,3-TMB would result in a POD that is lower than the POD associated with neurotoxicity for this isomer. Thus, the application of an UF to account for the lack of a developmental toxicity study is not warranted.

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

Table 2-9 is a continuation of Table 2-8, and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in Table 2-9 are

preliminary to the derivation of the organ/system-specific values. These candidate values are considered individually in the selection of a representative inhalation reference value for a specific hazard and subsequent overall RfC for 1,2,3-TMB. Figure 2-2 presents graphically these candidate values, uncertainty factors, and points of departure, with each bar corresponding to one data set described in Tables 2-8 and 2-9.

Table 2-9. Effects and corresponding derivation of candidate RfC values for 1,2,3-TMB

Endpoint/Reference		HEC (mg/m^3)[a]	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m^3)[b]
Neurological endpoints									
Decreased pain sensitivity (Korsak and Rydzyński, 1996)		16.32	3	10	1	3	3	300	5.44×10^{-2}
Hematological endpoints									
Decreased RBCs (Korsak et al., 2000b)		87.79	3	10	1	3	3	300	2.93×10^{-1}
Decreased segmented neutrophils, (Korsak et al., 2000b)	male	89.77	3	10	1	3	3	300	2.99×10^{-1}
	female	16.66	3	10	1	3	3	300	5.55×10^{-2}
Increased reticulocytes (Korsak et al., 2000b)		109.58	3	10	1	3	3	300	3.65×10^{-1}
Respiratory endpoints									
Increased inflammatory lung lesions (Korsak et al., 2000b)		21.49	3	10	1	3	3	300	7.16×10^{-2}

[a]Human equivalent concentration.

[b]As calculated by application of uncertainty factors, not rounded to 1 significant digit.

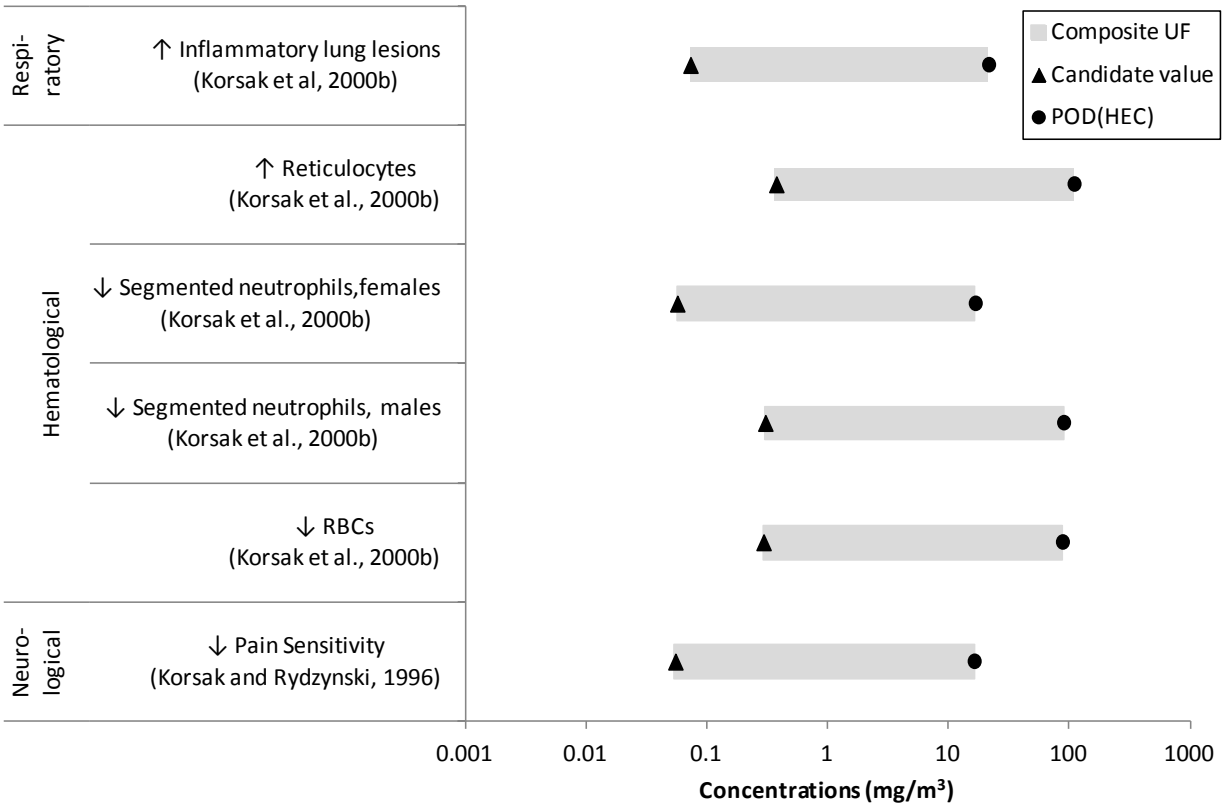


Figure 2-2. Candidate RfC values with corresponding POD and composite UF for 1,2,3-TMB.

2.2.4. Derivation of Organ/System Specific Reference Concentrations for 1,2,3-TMB

Table 2-10 distills the candidate values from Table 2-9 into a single value for each organ or system. The single RfC value selected for a particular organ system was preferably chosen using biological and toxicological information regarding that endpoint. If no compelling biological information exists with which to select the primary hazard, the lowest RfC value for that organ system was selected. These organ- or system-specific reference concentrations may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site. The individual organs and systems for which specific RfC values were derived were the neurological, hematological, and respiratory systems. The RfC value for the neurological system, based on decreased pain sensitivity, was selected for the proposed overall RfC for 1,2,3-TMB (see Section 2.2.5 for details). The RfC values for the hematological and respiratory systems, based on decreased segmented neutrophils and increased inflammatory lung lesions, were

only marginally higher than the RfC derived for neurological effects (6×10^{-2} and 7×10^{-2} mg/m^3 vs. 5×10^{-2} mg/m^3), indicating that effects in these organ systems may also be of concern.

Table 2-10. Organ/system-specific RfCs and proposed overall RfC for 1,2,3-TMB

Effect	Basis	Rfc (mg/m^3)	Exposure description	Confidence
Hematological	Decreased segmented neutrophils	6×10^{-2}	Subchronic	Low to medium
Respiratory	Increased inflammatory lung lesions	7×10^{-2}	Subchronic	Low to medium
Proposed overall RfC (Neurological)	**Decreased pain sensitivity**	5×10^{-2}	**Subchronic**	**Low to medium**

2.2.5. Selection of the Proposed Overall Reference Concentration for 1,2,3-TMB

Neurotoxicity is the most consistently observed endpoint in the toxicological database for 1,2,3-TMB. According to EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 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 an increased latency to paw-lick in hot plate tests, represents an alteration in neurobehavioral function (U.S. EPA, 1998). Decreased pain sensitivity or decreased pain sensitivity following a foot shock challenge was observed in two studies investigating short-term and subchronic exposure durations (Wiaderna et al., 1998; Korsak and Rydzyński, 1996) and in the presence of other metrics of altered neurobehavior, including impaired neuromuscular function and altered cognitive function. Additionally, neurological symptoms (e.g., hand tremble, weakness) are observed in human worker populations exposed to complex VOC mixtures containing 1,2,3-TMB (notably, pain sensitivity has not been tested in humans) indicating a consistency and coherency of neurotoxic effects in humans and animals following exposure to 1,2,3-TMB.

See Section 2.1.5 for a detailed discussion of U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) and the use of reversible and/or latent neurotoxicological endpoints in the derivation of reference values. The issues pertaining to the observed 1,2,3-TMB neurotoxicity are the same as those identified for 1,2,4-TMB. For example, although 1,2,3-TMB-induced pain sensitivity was observed to return to control levels two weeks after termination of subchronic inhalation exposure in one study (Korsak and Rydzyński, 1996), the *Neurotoxicity Guidelines* note that reversible effects occurring in occupational settings may be of high concern, particularly if they

diminish a person's ability to survive or adapt to the environment (U.S. EPA, 1998) (pg. 8). Additionally, the "designation of an effect as irreversible or reversible is academic, as exposure is presumed to be lifetime (i.e., there is no post-exposure period)" (U.S. EPA, 2002) (pg. 3-27). In other words, the nature of an RfC precludes the possibility of recovery from the critical effect. Lastly, the issues surrounding the use of an environmental challenge (i.e., foot shock) in short-term neurotoxicity studies of 1,2,3-TMB are the same as those discussed for 1,2,4-TMB in Section 2.1.5.

Taken as a whole, the database supports the characterization of decreased pain sensitivity associated with exposure to 1,2,3-TMB as being an effect of high concern. Given the consistency of observations from hot plate tests with or without foot shock challenge across several studies from the same research group using multiple durations of exposure in male Wistar rats, as well as the evidence and biological plausibility of similarities in neurological effects between rats and humans, there is strong evidence that neurotoxicity is the primary hazard associated with exposure to 1,2,3-TMB. Based on these considerations, decreased pain sensitivity observed immediately after subchronic exposure is identified as an adverse neurotoxic effect and thus is an appropriate effect on which to base the RfC. Therefore, the candidate RfC for neurotoxicity based on decreased pain sensitivity was selected as the proposed overall RfC for 1,2,3-TMB.

A POD_{HEC} of 16.3 mg/m^3 for decreased pain sensitivity (Korsak and Rydzyński, 1996) was used as the POD to derive the chronic RfC for 1,2,3-TMB. The uncertainty factors (UFs), selected and applied in accordance with the procedures described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) (Section 4.4.5 of the report), were discussed previously in Section 2.2.3. Application of this composite UF of 300 to the POD_{HEC} yields the following chronic RfC for 1,2,3-TMB:

$RfC = POD_{HEC} \div UF = 16.3\ mg/m^3 \div 300 = 0.05\ mg/m^3 = 5 \times 10^{-2}\ mg/m^3$ (rounded to one significant digit)

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

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

The critical effect selected, decreased pain sensitivity, does not introduce substantial variability into the RfC calculation as selection of alternative hematological or respiratory effects would result in similar RfCs that would be equivalent when rounding to one significant digit (i.e., 2×10^{-2} mg/m^3, see Figure 2-4). Some uncertainty exists regarding the selection of the BMRs for use in BMD modeling due to the absence of information to determine the biologically significant level of

response associated with the endpoints. However in cases such as this, the selection of a BMR of 1 standard deviation for continuous endpoints is supported by EPA guidance (U.S. EPA, 2012b). In addition, there is uncertainty in the estimated standard deviation for decreased pain sensitivity (Korsak and Rydzyński, 1996), which was two- to threefold lower than that estimated in the parallel evaluation of 1,2,4-TMB in the same publication (see Section 2.1.6.). Given the lack of information concerning a biologically significant level of response for pain sensitivity, the concurrently estimated standard deviation was judged to be most relevant for characterizing this response to 1,2,3-TMB.

Uncertainty regarding the selection of particular models for individual endpoints exists as selection of alternative models could decrease or increase the estimated POD and consequently, the RfC. The criteria for model selection was based on a practical approach as described in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b). Uncertainty may exist in the default dosimetry methods used to calculate HEC estimates, but such uncertainties would apply equally to all endpoints.

2.2.7. Confidence Statement for 1,2,3-TMB

Confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996) is low to medium. The study is a peer-reviewed study that utilized three dose groups plus untreated controls, employed an appropriate number of animals per dose group, and appropriately performed statistical analyses. However, sources of uncertainty exist that reduce confidence in this study.

One area of uncertainty regarding this study is the lack of reported actual concentrations. However, as the methods by which the test atmosphere was generated and analyzed were reported in sufficient detail, and given the fact that this laboratory has used this methodology in subsequent studies (Korsak et al., 2000a, b) and achieved appropriate actual concentrations (i.e., within 10% of target concentrations), the concern regarding the lack of reported actual concentrations is minimal. Another source of uncertainty is the fact that Korsak and Rydzyński (1996) does not explicitly state that the reported measures of variance in Table 1 of that reference are standard deviations. However, careful analysis of the reported levels of variance and magnitude of statistical significance reported indicate that the measures of variance are standard deviations. Supporting this conclusions is the observation that all other papers by Korsak et al. (2000a, b; 1997; 1995) report variance as standard deviations. The critical effect on which the RfC is based is well-supported as the weight of evidence for 1,2,3-TMB-induced neurotoxicity is coherent across multiple animals species (i.e., mouse, and rat) and consistent across multiple exposure durations (i.e., acute, short-term, and subchronic) (Lutz et al., 2010; Wiaderna et al., 1998; Korsak and Rydzyński, 1996).

The database for 1,2,3-TMB includes acute, short-term, and subchronic toxicity studies in rats and mice. However, confidence in the database is low to medium because it lacks chronic,

multi-generation reproductive/developmental, developmental toxicity, or developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. The overall confidence in the RfC for 1,2,3-TMB is low to medium.

2.3. Inhalation Reference Concentration for Effects Other Than Cancer for 1,3,5-TMB

2.3.1. Identification of Studies and Effects for Dose-Response Analysis for 1,3,5-TMB

The nervous, hematological, and respiratory systems are the primary targets of toxicity for inhaled 1,3,5-TMB in humans, whereas the nervous system in adults, pregnant females, and developing organism are the primary targets of inhaled 1,3,5-TMB in experimental animals. Effects in these systems have been identified as hazards following inhalation exposures to 1,3,5-TMB. Although literature exists on the effects of 1,3,5-TMB exposure in humans, including neurological, hematological, and respiratory toxicities, no human studies are available that would allow for dose-response analysis. The human studies evaluated TMB exposures occurring as complex solvents or VOC mixtures, and this consideration along with similar uncertainties as discussed for 1,2,4-TMB and 1,2,3-TMB limit their utility in derivation of quantitative human health toxicity values. As for the other two isomers, the human studies provide supportive evidence for the neurological toxicity of 1,3,5-TMB in humans and indicate a consistency and coherency of this effect in humans and laboratory animals.

Several studies investigating 1,3,5-TMB effects in experimental animals models were identified in the literature. No chronic or subchronic inhalation studies were identified that investigated effects in adult animals. One developmental toxicity study investigating maternal and fetal toxicity was identified in the literature (Saillenfait et al., 2005). Data from this study pertaining to the primary hazards observed animals (maternal/developmental effects) was considered as candidate critical effects for the purpose of determining the point of departure (POD) for derivation of the inhalation RfC for 1,3,5-TMB. Neurotoxicity and respiratory toxicities were also observed in acute and short-term inhalation studies investigating effects in adult animals. However, the high exposure concentrations used in acute studies and the short exposure durations employed in both acute and short-term studies limit their utility for the quantitation of chronic human health effects. Nevertheless, as with the human in which subject were exposed to mixtures containing 1,3,5-TMB, these studies provide qualitative information regarding hazard identification, especially the observation of the consistency and coherency of these effects across the 1,3,5-TMB database.

The developmental toxicity study by Saillenfait et al. (2005) is adequate for dose-response analysis. This study exposed rats, a common laboratory animal for developmental studies, by inhalation to 1,3,5-TMB (reported as 99% pure [impurities not reported]). The four exposure groups covered just over an order of magnitude, with the higher three groups spaced about twofold

apart. Typical numbers of animals per exposure group were used, 25/group. Regarding exposure characterization, Saillenfait et al. (2005) reported actual concentrations, as measured by gas chromatography, to be within 10% of target concentrations. This increases the confidence in the overall evaluation and adequacy of this study. Target and actual concentrations are provided in Table 2-11.

Table 2-11. Target and actual exposure concentrations used in BMD modeling of 1,3,5-TMB endpoints considered for the derivation of the RfC

Reference	Species/sex	Target exposure concentration (mg/m^3)	Actual exposure concentration (mg/m^3)
Saillenfait et al. (2005)	Rat, female (pregnant dam); male and female (fetuses)	492	497
		1,476	1,471
		2,952	2,974
		5,904	5,874

The Saillenfait et al. (2005) study examined 1,3,5-TMB-induced toxicity in both the pregnant animal and the developing fetus, and the observed effects that demonstrated statistically significant decreases relative to control were considered for the derivation of the RfC for 1,3,5-TMB (Table 2-12). These endpoints included decreased male and female fetal weights and decreased maternal weight gain (minus gravid uterine weight). Changes in serum chemistry parameters in rats exposed to 1,3,5-TMB in a short-term (five weeks) inhalation study (Wiglusz et al., 1975a) were not considered for derivation of the RfC due to inconsistent temporal patterns of effect and the lack of accompanying histopathology.

Table 2-12. Endpoints considered for the derivation of the RfC for 1,3,5-TMB

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

[*] $p < 0.05$; [**] $p < 0.01$

[a]Adapted from Saillenfait et al. (2005).

[b]Numbers of live fetuses not explicitly reported.

[c]Values are expressed as mean ± 1 SD.

[d]Number of dams with live litters.

2.3.2. Methods of Analysis for 1,3,5-TMB

As discussed above in Section 2.3.1, endpoints observed in Saillenfait et al. (2005) that demonstrated statistically significant ($p < 0.05$) increases or decreases relative to control for at least one exposure group were considered for the derivation of the RfC for 1,3,5-TMB; these effects are listed in Table 2-12. This assessment used the BMD approach, when possible, to estimate a POD for the derivation of an RfC for 1,3,5-TMB (see Section C.1 of Appendix C 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), and then selecting the best fitting model. Each best-fit model estimates a BMD and its associated BMDL (i.e., a 95% lower bound on the BMD) corresponding to a selected BMR.

For maternal weight gain identified from the Saillenfait et al. (2005) study, no information is available regarding the change in these responses that would be considered biologically significant, thus a BMR equal to a 1 standard deviation change in the control mean was used in modeling these endpoints, consistent with EPA's *Benchmark Dose Technical Guidanc*e (U.S. EPA, 2012b). For the decreased male and female fetal body weight endpoints identified from the Saillenfait et al. (2005) study, a BMR of 5% relative deviation from the control mean was selected (see Section 2.1.2 for a detailed discussion for the rationale for this choice). Additionally, a BMR equal to a 1 standard deviation change in the control mean was also selected for the BMD modeling

of fetal body weight to facilitate comparisons across assessments (U.S. EPA, 2012b). The estimated BMDL is then used as the candidate POD (Table 2-13).

The suitability of the above methods to determine a POD is dependent on the nature of the toxicity database for a specific chemical. In the Saillenfait et al. (2005) study, although decreased fetal body weight in males and females was considered for BMD modeling, BMDS was unable to adequately model the variance in response for this endpoint. Consequently, the NOAEL/LOAEL approach was used to identify a POD. Detailed modeling results are provided in Section C.1 of Appendix C.

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 Saillenfait et al. (2005) study, rats were exposed to 1,3,5-TMB for 6 hours/day for 15 consecutive days (GD6–GD20). Therefore, the duration-adjusted PODs for developmental/ maternal effects were calculated as follows:

$$\mathbf{POD_{ADJ}\ (mg/m^3) = POD\ (mg/m^3) \times hours\ exposed\ per\ day/24\ hours}$$

For example, for decreased fetal weight in males, the POD_{ADJ} would be calculated as follows:

$$\mathbf{POD_{ADJ}\ (mg/m^3) = 2{,}974\ mg/m^3 \times 6\ hours/24\ hours}$$

$$\mathbf{POD_{ADJ}\ (mg/m^3) = 744\ mg/m^3}$$

Because the selected endpoints for consideration as the critical effect (i.e., 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 (HEC) 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 EPA's *RfC Methodology* (U.S. EPA, 1994b). DAFs are ratios of animal and human physiologic parameters, and are dependent on the nature of the contaminant (i.e., particle or gas) and the target site (i.e., respiratory tract or remote to the portal-of-entry [i.e., systemic]) (U.S. EPA, 1994b). For gases with systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

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

where:

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

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

$$\mathbf{DAF = 55.7}\ (\text{Järnberg and Johanson, 1995})\mathbf{/43}\ (\text{Meulenberg and Vijverberg, 2000})$$

$$\mathbf{DAF = 1.3}$$

In cases where the animal blood:air partition coefficient is higher than the human value, resulting in a DAF > 1, a default value of 1 is substituted (U.S. EPA, 1994b). For example, the HEC for decreased female fetal body weight (reported in Saillenfait et al. (2005)) is calculated as follows:

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

$$\mathbf{POD_{HEC} = POD_{ADJ}\ (mg/m^3) \times 1.0}$$

$$\mathbf{POD_{HEC} = 744\ mg/m^3 \times 1.0}$$

$$\mathbf{POD_{HEC} = 744\ mg/m^3}$$

Table 2-13 presents the calculated HECs for the candidate critical effects, selected uncertainty factors (UFs), and the resulting derivation of candidate RfCs from the Saillenfait et al. (2005) developmental toxicity study.

Table 2-13. Summary of derivation of points of departure for 1,3,5-TMB

Endpoint/Reference	Species/sex	Model; BMR or NOAEL/LOAEL	POD (mg/m^3)	Candidate POD_{ADJ} (mg/m^3)	Candidate POD_{HEC} (mg/m^3)
Developmental endpoints					
Decreased fetal body weight (Saillenfait et al., 2005)	Rat, male	NOAEL[a]	2,974	744	744
	Rat, female	NOAEL[a]	2,974	744	744
Maternal endpoints					
Decreased maternal body weight gain (Saillenfait et al., 2005)	Rat, female	Power; 1 SD	1,302	326	326

[a] No model was able to fit data adequately, or data were not modeled.

2.3.3. Derivation of Candidate RfC Values for 1,3,5-TMB

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002), §4.4.5], also described in the Preamble, five possible areas of uncertainty and variability were considered in deriving the candidate RfC values for 1,2,4-TMB. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated uncertainty factor (UF) to be applied to the candidate POD_{HEC} are as follows:

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 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 and any residual toxicokinetic uncertainty.

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response in the human population following inhalation of 1,3,5-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.

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 1 standard deviation change in the control mean for modeled endpoints was selected under the assumption that this BMR represents a minimal, biologically significant change for these effects. For endpoints that could not be modeled, a LOAEL to NOAEL uncertainty factor of 1 was applied as a NOAEL was used.

A subchronic to chronic uncertainty factor, UF_S, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to decreased maternal weight to account for extrapolation from a subchronic (albeit gestational) exposure duration study to derive a chronic RfC. The 3-fold uncertainty factor is applied to the POD identified from the subchronic study on the assumption that effects observed in a similar chronic study would be observed at lower concentrations for a number of possible reasons, including potential cumulative damage occurring over the duration of the chronic study or an increase in the magnitude or severity of effect with increasing duration of exposure. A full subchronic to chronic uncertainty factor of 10 was not applied in this case as there was no observed decrease in adult body weights in rats exposed to either 1,2,4-TMB or 1,2,3-TMB for longer durations (i.e., 90 days). For decreases in fetal weight, a UF_S of 1 was applied.

A database uncertainty factor, UF_D, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for database deficiencies. Strengths of the database include the well-designed developmental toxicity study that investigated standard measures of maternal and fetal toxicity in Sprague-Dawley rats. However, the lack of a multi-generational reproductive/developmental toxicity study investigating effects due to 1,3,5-TMB exposure is a weakness of the database. EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) also recommends that the database uncertainty factor take into consideration whether there is concern from the available toxicology database that the developing organism may be particular susceptible to effects in specific organ systems. TMBs (unspecified isomer) are able to cross the placenta (Cooper et al., 2001; Dowty et al., 1976); therefore, as neurotoxicity is observed in adult animals in the available short-term 1,3,5-TMB inhalation studies, there is concern that exposure to 1,3,5-TMB may result in

neurotoxicity in the developing organism. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) identifies specific effects observed in adult animals (e.g., cognitive and motor function) that can also affect the developing organism exposed in utero. The Neurotoxicity Guidelines (U.S. EPA, 1998) also indicate that neurotoxicants may have greater access to the nervous system in developing organisms due to an incomplete blood-brain barrier and immature metabolic detoxifying pathways. Therefore, there is some concern that the lack of a developmental neurotoxicity study is a deficiency in the database and that the inclusion of such a study would potentially result in a lower POD than the POD for maternal effects identified from the available 1,3,5-TMB toxicity database. In summary, a 3-fold database UF was applied to account for the lack of both a multi-generation reproductive/developmental toxicity study and a developmental neurotoxicity study in the available database for 1,3,5-TMB.

Table 2-14 is a continuation of Table 2-13, and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in Table 2-14 are preliminary to the derivation of the organ/system-specific values. These candidate values are considered individually in the selection of a representative inhalation reference value for a specific hazard and subsequent overall RfC for 1,3,5-TMB. Figure 2-3 presents graphically these candidate values, uncertainty factors, and points of departure, with each bar corresponding to one data set described in Tables 2-13 and 2-14. Additionally, the RfC values for 1,2,4-TMB and 1,2,3-TMB are shown for comparative purposes

Table 2-14. Effects and corresponding derivation of candidate RfC values for 1,3,5-TMB

Endpoint/Reference	HEC (mg/m^3)[a]	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate RfC value (mg/m^3)[b]
Developmental endpoints								
Decreased fetal body weight, male (Saillenfait et al., 2005)	744	3	10	1	1	3	100	7.44
Decreased fetal body weight, female (Saillenfait et al., 2005)	744	3	10	1	1	3	100	7.44
Maternal endpoints								
Decreased maternal body weight gain (Saillenfait et al., 2005)	326	3	10	1	3	3	300	1.09

[a]Human equivalent concentration.

[b]As calculated by application of uncertainty factors, not rounded to 1 significant digit.

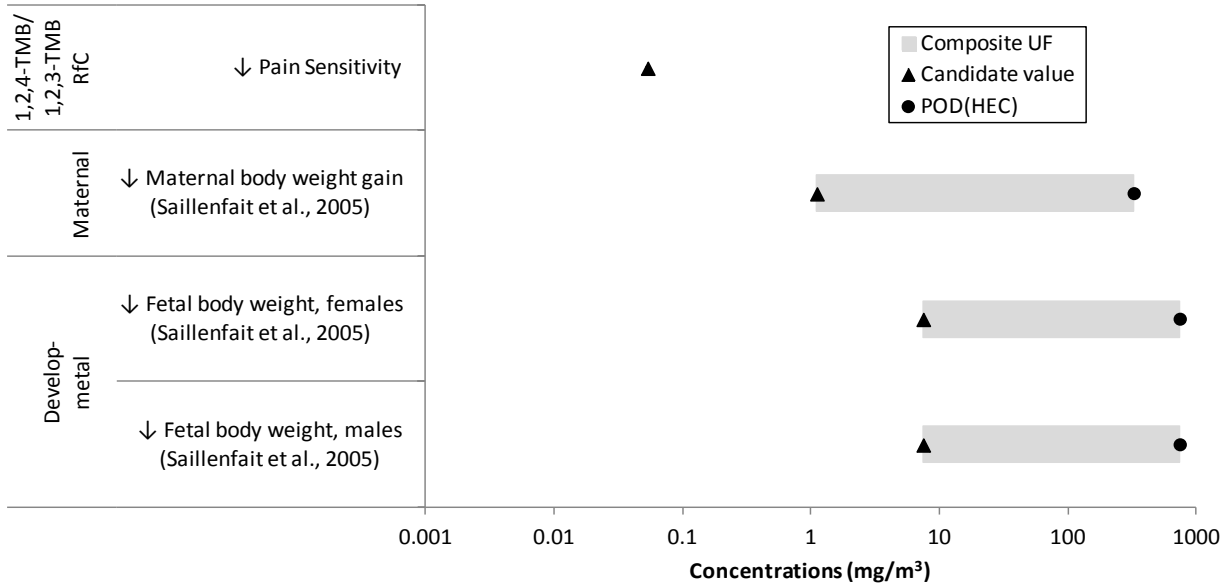


Figure 2-3. Candidate RfC values with corresponding POD and composite UF for 1,3,5-TMB.

2.3.4. Derivation of Organ/System Specific Reference Concentrations for 1,3,5-TMB

Table 2-15 distills the candidate values from Table 2-14 into a single value for each organ or system. The single RfC value selected for a particular organ system was preferably chosen using biological and toxicological information regarding that endpoint. If no compelling biological information exists with which to select the primary hazard, the lowest RfC value for that organ system was selected. These organ- or system-specific reference concentrations may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site. The individual organs and systems for which specific RfC values were derived were the pregnant animal (maternal) and developing fetus (developmental). The RfC value for maternal effects was the lowest of the derived specific RfCs using 1,3,5-TMB data. The RfC value for developmental effects was greater than that for maternal effects, indicating this effect may be of less concern. However, effects to pregnant animals and the developing fetus may be of less concern in general as the RfC values for these types of effects (based on decreased maternal weight gain and decreased male and female fetal weight, respectively) are much greater than the RfC value derived for 1,2,4-TMB based on decreased pain sensitivity (see Section 2.3.5 for details).

Table 2-15. Organ/system-specific RfCs and proposed overall RfC for 1,3,5-TMB

Effect	Basis	RfC (mg/m^3)	Exposure description	Confidence
Developmental	Decreased fetal weight (male and female	7	Gestational	Low to medium
Maternal	Decreased maternal weight gain	1	Gestational	Low to medium
Proposed overall RfC (Neurological)	**Decreased pain sensitivity (based on RfC derived for 1,2,4-TMB)**	5×10^{-2}	**Subchronic**	**Low to medium**

2.3.5. Selection of the Proposed Overall Reference Concentration for 1,3,5-TMB

Decreased maternal weight gain was identified as the most sensitive endpoint in the 1,3,5-TMB database. A POD_{HEC} of 326.0 mg/m^3 for decreased maternal weight gain from Saillenfait et al. (2005) was used to derive a candidate chronic RfC for 1,3,5-TMB as shown in Table 2-14. Uncertainty factors, selected and applied in accordance with the procedures described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), were previously discussed in Section 2.3.2. Application of this 300-fold composite UF yields the calculation of the chronic RfC for 1,3,5-TMB as follows:

$RfC = POD_{HEC} \div UF = 326\ mg/m^3 \div 300 = 1.09\ mg/m^3 = 1\ mg/m^3$ (rounded to one significant digit)

However, while Saillenfait et al. (2005) is a well-conducted developmental toxicity study that evaluates a wide range of fetal and maternal endpoints resulting from 1,3,5-TMB inhalation exposure, a number of other factors lessens its suitability for use in deriving an 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 maternal body weight gain (corrected for gravid uterine weight), is 20-fold higher than the RfC derived for 1,2,4-TMB, which is based on altered CNS function measured as decreased pain sensitivity. As discussed in Section 1.1.6, 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 isomers' neurotoxicity that are supportive of an RfC for 1,3,5-TMB that is not substantially different than the RfC derived for 1,2,4-TMB. Also supporting this conclusion is the observation that 1,2,4-TMB and 1,3,5-TMB display important similarities in regard to chemical properties and toxicokinetics, including similarities in blood:air partition coefficients, respiratory uptake, and absorption into the

bloodstream (see Section 1.1.7 and Appendices B.1 and B.2). These similarities support the conclusion that internal blood dose metrics for 1,3,5-TMB would be comparable to those calculated for 1,2,4-TMB using the available PBPK model.

Given these considerations, the use of 1,3,5-TMB-specific data for derivation of an RfC was not considered to be scientifically supported. **Thus, the chronic RfC of 5×10^{-2} mg/m^3 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 based on chemical properties, toxicokinetics, and toxicity.**

2.3.6. Uncertainties in the Derivation of the Reference Concentration 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. While the available database for 1,3,5-TMB was considered sufficient 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 20-fold higher would be derived for 1,3,5-TMB vs. that derived for 1,2,4-TMB (1 vs. 5×10^{-2} mg/m^3, respectively). Although uncertainty exists in adopting the 1,2,4-TMB RfC for 1,3,5-TMB RfC, both isomers share multiple commonalities and similarities regarding their chemical, 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 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. Therefore, while uncertainty exists in the derivation of 1,3,5-TMB's RfC, the available information regarding sufficient chemical, toxicokinetic, and toxicological similarity between the two isomers supports adopting the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB.

2.3.7. Confidence Statement for 1,3,5-TMB

The chronic RfC for 1,2,4-TMB was adopted as the RfC for 1,3,5-TMB; thus**, confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium** (see above). The database for 1,3,5-TMB includes acute, short-term, and developmental toxicity studies in rats and mice. However, confidence in the overall database is low to medium because it lacks chronic, subchronic, multi-generation reproductive/developmental toxicity, and developmental neurotoxicity studies and most of the studies supporting the critical effect come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the adoption of the RfC derived for 1,2,4-TMB as the RfC for 1,3,5-TMB, the overall confidence in the RfC for 1,3,5-TMB is low.

2.4. Oral Reference Dose for Effects Other Than Cancer for 1,2,4-TMB

The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, a LOAEL, or a 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

2.4.1. Identification of Studies and Effects for Dose-Response Analysis 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 thus this database is inadequate for the derivation of an RfD.

2.4.2. Methods of Analysis for 1,2,4-TMB

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). 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 interest. Using route-to-route extrapolation via application of PBPK models is supported by EPA guidance (U.S. EPA, 2002, 1994b) given enough data and the ability to interpret that data with regard to 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 (Section B.2, Appendix B). Further, no evidence exists that would suggest toxicity profiles would differ to a substantial degree between oral and inhalation exposures. In fact, in acute oral studies in rats (Tomas et al., 1999a; Tomas et al., 1999b), the observed neurotoxic effects of exposure to 1,2,4-TMB (i.e., alterations in motor function and electrocortical activity) are similar to effects observed following short-term exposures to 1,2,4-TMB via inhalation.

Therefore, assuming oral exposure would result in the same systemic effect as inhalation exposure (i.e., 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 (Section B.3.3.5, Appendix B), assuming continuous oral ingestion and 100% absorption of the ingested 1,2,4-TMB 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 (see Figure B-18, Appendix B). This difference became insignificant for daily doses exceeding 50 mg/kg-day.

The human PBPK model inhalation dose metric (weekly average blood concentration, mg/L) for the POD_{ADJ} (0.086 mg/L) for decreased pain sensitivity 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 then the resulting value of 6.3 mg/kg-day was used as the human equivalent dose POD (POD_{HED}) for the RfD derivation.

2.4.3. Derivation of the Reference Dose for 1,2,4-TMB

A POD_{HED} of 6.3 mg/kg-day was derived for the oral database using route-to-route extrapolation based on the neurotoxic effects (i.e., decreased pain sensitivity) observed by Korsak and Rydzyński (1996) following inhalation exposure to 1,2,4-TMB. Thus, the same uncertainty factors applied to derive the RfC (see Section 2.1.5) were also applied to derive the RfD. The uncertainty factors, selected and applied in accordance with the procedures described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) (Section 4.4.5 of the report), address five areas of uncertainty resulting in a composite UF of 300.

Application of this 300-fold composite UF yields the calculation of the chronic RfD for 1,2,4-TMB as follows:

$$\mathbf{RfD = POD_{HED} \div UF = 6.3\ mg/kg\text{-}day \div 300 = 0.02\ mg/kg\text{-}day = 2 \times 10^{-2}\ mg/kg\text{-}day}$$

(rounded to one significant digit)

2.4.4. Uncertainties in the Derivation of the Reference Dose 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 (i.e., decreased pain sensitivity) as inhalation exposure in Korsak and Rydzyński (1996), the uncertainties regarding this derivation are the same as those for the RfC for 1,2,4-TMB (see Section 2.1.6), 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 considered appropriate for the purposes of this assessment. 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.

2.4.5. Confidence Statement 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 low to medium (see Section 2.1.7). The inhalation database for 1,2,4-TMB includes acute, short-term, subchronic, and developmental toxicity studies in rats and mice. However, confidence in the database for 1,2,4-TMB is low to medium because it lacks chronic, multi-generation reproductive/developmental and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the application of the available PBPK model for the purposes of a route-to-route extrapolation, the overall confidence in the RfD for 1,2,4-TMB is low.

2.5. Oral Reference Dose for Effects Other Than Cancer for 1,2,3-TMB

2.5.1. Identification of Studies and Effects for Dose-Response Analysis for 1,2,3-TMB

No chronic or subchronic studies were identified for 1,2,3-TMB that utilized the oral route of exposure. Therefore, the available oral database for 1,2,3-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 thus this database is inadequate for the derivation of an RfD.

2.5.2. Methods of Analysis and Derivation of the Reference Dose for 1,2,3-TMB

The available oral database is inadequate to derive an RfD for 1,2,3-TMB. No chronic, subchronic, or short-term oral exposure studies were found in the literature. However, as discussed in Section 1.1.6, there are sufficient similarities between isomers regarding observed toxicological

effects that support adopting the RfD for 1,2,4-TMB as the RfD for 1,2,3-TMB. Specifically, the qualitative pattern of neurotoxic effects following short-term and subchronic inhalation exposures is similar between TMB isomers. Particularly important to this determination is that, although 1,2,3-TMB is observed to decrease pain sensitivity at lower concentrations than 1,2,4-TMB (LOAEL values of 123 vs. 492 mg/m^3, respectively), the magnitude of decreased pain sensitivity is similar for 1,2,4-TMB and 1,2,3-TMB, especially at the low- and mid-concentrations. This similarity of effect in the low-dose region of the dose-response curve is exhibited by equal RfC values derived from isomer-specific data: 5×10^{-2} mg/m^3. Additionally, given that similar patterns of neurotoxicity are observed following acute oral and subchronic inhalation exposures to 1,2,4-TMB, it is reasonable to assume that neurotoxicity profiles would not differ substantially between oral and inhalation exposures to 1,2,3-TMB. Although a PBPK model exists for 1,2,4-TMB that allows for route-to-route extrapolation from inhalation to oral exposure, no such model exists for 1,2,3-TMB. However, similarities in blood:air and tissue:air partition coefficients and degree of absorption into the bloodstream between 1,2,4-TMB and 1,2,3-TMB support the conclusion that internal blood dose metrics for 1,2,3-TMB would be similar to those calculated for 1,2,4-TMB using that isomer's available PBPK model. Also, the qualitative metabolic profiles for the two isomers are similar, with dimethylbenzyl hippuric acids being the major terminal metabolite for both isomers, such that first-pass metabolism through the liver is not expected to differ greatly between 1,2,4-TMB and 1,2,3-TMB. **Therefore, given the similarities in chemical properties, toxicokinetics, and toxicity, the RfD derived for 1,2,4-TMB, 2×10^{-2} mg/kg-day was adopted as the RfD for 1,2,3-TMB.**

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

The uncertainties regarding adopting the RfD for 1,2,4-TMB as the RfD for 1,2,3-TMB encompass previous areas of uncertainty involved in the derivation of the RfC for 1,2,3-TMB and the RfD for 1,2,4-TMB (see Sections 2.1.6 and 2.2.6). Additionally, there is uncertainty in this adoption regarding the assumptions made about the similarity in toxicokinetics and toxicity between the two isomers. However, as discussed above in Sections 1.1.6 and 1.1.7 and in Appendix B (Section B.2), there is strong evidence that both isomers share multiple commonalities and similarities regarding their toxicokinetic and toxicological properties that support adopting one isomer's value for the other.

2.5.4. Confidence Statement for 1,2,3-TMB

The chronic RfD for 1,2,4-TMB was adopted as the RfD for 1,2,3-TMB; thus, confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium (see above). The inhalation database for 1,2,3-TMB includes acute, short-term, and subchronic studies in rats and mice. However, confidence in the database is low to medium because

it lacks chronic, multi-generation reproductive/developmental, developmental toxicity, or developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,2,3-TMB, the overall confidence in the RfD for 1,2,3-TMB is low.

2.6. Oral Reference Dose for Effects Other Than Cancer for 1,3,5-TMB

2.6.1. Identification of Studies and Effects for Dose-Response Analysis for 1,3,5-TMB

Only one subchronic study (Koch Industries, 1995b) investigating 1,3,5-TMB's toxicity was located that utilized the oral route of exposure. As this study was not located in the peer-reviewed literature (it was submitted to EPA under a TSCA 4(a) test rule), EPA sought an independent external peer review to assess the study's reliability and suitability for use as the basis of an RfD derivation (Versar, 2013). Ultimately, the results of the external peer review led EPA to conclude that this study was not suitable to serve as a principal study with which to derive human health reference doses (see Appendix F). The most critical shortcoming noted in the external peer review of the Koch Industries (1995b) study was its lack of investigation of neurotoxicity endpoints, as these effects (e.g., decreased pain sensitivity, altered cognitive ability) have been demonstrated to be the most sensitive endpoints following inhalation exposure to other TMB isomers. Given the conclusion that the Koch Industries (1995b) study is insufficient for use in RfD derivation, the available oral database for 1,3,5-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 thus this database is inadequate for the derivation of an RfD.

2.6.2. Methods of Analysis and Derivation of the Reference Dose for 1,3,5-TMB

The available oral database is inadequate to derive an RfD for 1,3,5-TMB. The only identified oral toxicity study was judged to be unsuitable for derivation of the RfD. However, as outlined in the RfC Derivation for 1,3,5-TMB, the chemical, 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 for 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 and 1,2,4-TMB are observed to elicit similar neurotoxic effects in rats in acute and short-term oral and inhalation 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. Further, no evidence exists to suggest that toxicity profiles would differ substantially between oral and inhalation exposures to 1,3,5-TMB. In fact, in acute oral studies in rats (Tomas et al., 1999a; Tomas et al., 1999b), the observed neurotoxic effects of exposure to 1,3,5-TMB (i.e., alterations in motor function) are similar to effects observed following short-term exposures via inhalation. 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 to 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 similarities in chemical properties, toxicokinetics, and toxicity, the RfD derived for 1,2,4-TMB of 2×10^{-2} mg/kg-day was adopted as the RfD for 1,3,5-TMB.**

2.6.3. Uncertainties in the Derivation of the Reference Dose 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 2.3.6 and 2.4.4). There is uncertainty regarding this adoption. However, as discussed above in Section 2.3.3, both isomers share multiple commonalities and similarities regarding their chemical, 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 2.4.3) apply to the derivation of the RfD for 1,3,5-TMB.

2.6.4. Confidence Statement for 1,3,5-TMB

The chronic RfD for 1,2,4-TMB was adopted as the RfD for 1,3,5-TMB; thus confidence in the study from which the critical effect was identified, Korsak and Rydzyński (1996), is low to medium (see above). The inhalation database for 1,3,5-TMB includes acute, short-term, and developmental toxicity studies in rats and mice. However, confidence in the database is low to medium because it lacks chronic, multi-generation reproductive/developmental and developmental neurotoxicity studies, and the studies supporting the critical effect predominantly come from the same research institute. Reflecting the confidence in the study and the database and the uncertainty surrounding the adoption of the RfD derived for 1,2,4-TMB as the RfD for 1,3,5-TMB, the overall confidence in the RfD for 1,3,5-TMB is low.

2.7. Cancer Risk Estimates for 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for 1,2,4-TMB, 1,2,3-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 via any route of exposure. Information available on which to base a quantitative cancer assessment is lacking, and thus, **no cancer risk estimates for either oral or inhalation exposure are derived**.

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