Air Toxics Hot Spots Program

Trimethylbenzenes

Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

October 2023

Air and Site Assessment and Climate Indicators Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Trimethylbenzenes Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

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Preface

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines (including technical methodologies, factors) for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section (HSC) 44360(b)(2)). Pursuant to this mandate, OEHHA developed a Technical Support Document (TSD; 2008) that includes the methodologies for deriving Reference Exposure Levels (RELs) and OEHHA develops RELs using these methods.

RELs are airborne concentrations of a chemical that are not anticipated to result in adverse noncancer health effects for specified exposure durations in the general population and sensitive subpopulations thereof. They explicitly account for possible differential effects on the health of infants, children, and other sensitive subpopulations in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 et seq.).

RELs are completed using the public process outlined in HSC section 44360(b)(2). This includes public comment, and review by the Scientific Review Panel (SRP) on Toxic Air Contaminants, an external committee of scientific experts. When finalized the RELs are adopted in Appendix D of the TSD.

The acute, chronic, and 8-hour RELs on trimethylbenzenes in this document were developed pursuant to the process described above. Information on participating in the public comment process is provided on OEHHA's website.

Trimethylbenzenes
Reference Exposure Levels

1,3,5-trimethylbenzene; 1,2,4-trimethylbenzene;
1,2,3-trimethylbenzene

CAS: 108-67-8 (1,3,5-Trimethylbenzene); 95-63-6 (1,2,4-Trimethylbenzene); 526-73-8 (1,2,3-Trimethylbenzene); 25551-13-7 (Trimethylbenzenes)

1. Summary

1.1 Trimethylbenzenes Acute REL

Reference exposure level	2400 µg/m^3 (490 ppb)
Critical effect(s)	Latency in visual discrimination tests (neurobehavioral)
Hazard index target(s)	Nervous system

1.2 Trimethylbenzenes Chronic REL

Reference exposure level	4 µg/m^3 (1 ppb)
Critical effect(s)	Pain sensitivity behavior
Hazard index target(s)	Nervous system

1.3 Trimethylbenzenes 8-Hour REL

Reference exposure level	8 µg/m^3 (2 ppb)
Critical effect(s)	Pain sensitivity behavior
Hazard index target(s)	Nervous system

Trimethylbenzenes (TMBs) have a number of commercial uses, as constituents of surface coatings, paints, printing inks, cleaning fluids, and hydraulic fracturing fluids. The 1,2,4-TMB isomer is also used as a chemical intermediate. TMBs are common

components of petroleum refinery distillation fractions, such as gasoline, high flash point naphthas, and white spirit. TMBs are emitted by steel-making facilities and coal-fired plants. In addition, all three (1,2,4; 1,3,5; and 1,2,3 TMB) TMB isomers are found as constituents of biogas.

Exposure to TMBs has been found to cause adverse effects on the respiratory, hematologic, and central nervous systems (CNS) in animals and humans. These effects include acute toxicity such as CNS effects and respiratory irritation. Chronic effects include neuromuscular, pulmonary, hematologic, and other organ/tissue toxicity. Several human exposure studies evaluated acute effects, such as sensory irritation, in healthy volunteers. Effects on the nervous system are seen in acute animal studies and these form the basis of the acute TMB REL. There is little information on the chronic toxic effects of TMBs in humans (no human-controlled studies or child-specific toxicity data in the toxicological literature). Occupational studies on TMBs suffer from a lack of good exposure data and are confounded by exposure to multiple solvents. No lifetime chronic animal TMB studies were identified in the literature. There are a number of subchronic studies in animals that show effects on the nervous system, including impairment of neuropsychological functions, as well as effects on clinical chemistry and organ weights, following exposure to TMBs. The most sensitive endpoint in the subchronic animal studies is neurotoxicity, and this forms the basis of the 8-hour and chronic RELs. Benchmark dose (BMD) modeling established points of departure (PODs) for the acute, 8-hour, and chronic RELs. For the chronic REL, because the Korsak and Rydzynski (1996) study on which the chronic REL is based is a 13-week study, the POD was adjusted for a continuous 24-hour exposure. For all TMB RELs, the intraspecies toxicodynamic component of the uncertainty factor (UF) was increased to 10 from the default factor of 3 because TMBs are neurotoxicants, and likely to impact infants and children disproportionately.

Literature summarized and referenced in this document covers the relevant published literature for TMBs through July 2023.

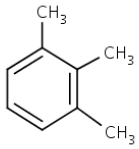
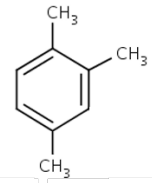
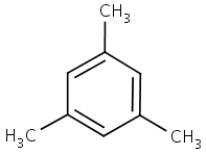
List of Abbreviations

AHH	Aryl Hydrocarbon Hydroxylase
ALB	Albumin
AMPH	Amphetamine
ANH	Aniline Hydroxylase
ANOVA	Analysis of Variance
AP	Acid Phosphatase
APD	Aminopyrine Demethylase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area Under the Curve
BALF	Bronchoalveolar lavage fluid
BMC_{1SD}	Benchmark Concentration, 1 SD change from the control mean
$BMCL_{1SD}$	Lower 95% confidence limit on BMC_{1SD}
BMD	Benchmark Dose
BMDS	Benchmark Dose Software
BMR	Benchmark Response
BUN	Blood Urea Nitrogen
BW	Body weight
CARB	California Air Resources Board
CAS	Chemical Abstracts Service
CHMS	Canadian Health Measures Survey
CI	Confidence Interval
CK	Creatine Kinase
CL	Chemiluminescence
CNS	Central Nervous System
CV	Constant Variance
CYT	Cytochrome
DCF	Dichlorofluorescein
DMHA	Dimethylhippuric Acid
EC_{50}	Effective Concentration required to have a biological effect in 50% of the cells or animals in a test
EEG	Electroencephalographic
ELISA	Enzyme-Linked Immunosorbent Assay
GD	Gestation Day
GLDH	Glutamate Dehydrogenase
GOT	Aspartate Aminotransferase
GPT	Alanine Aminotransferase
HC	Hydrocarbon
HEC	Human Equivalent Concentration
HPLC	High-Pressure Liquid Chromatography
HVS	High Voltage Spindles
IUR	Inhalation Unit Risk
KOC	Carbon-water Partition Coefficient
KOW	Octanol-water Partition Coefficient
LD_{50}	Lethal Dose required to kill 50% of the animals in a test
LOAEL	Lowest-Observed-Adverse-Effect Level
LP	Lymphocytes Percentage
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Reduced)
NCV	Non-constant Variance
NOAEL	No-Observed-Adverse-Effect Level
OEHHA	Office of Environmental Health Hazard Assessment
O_3	Ozone
OH	Hydroxyl Radical
OR	Odds Ratio
PC	Partition Coefficient
PSNG	Percentage of Segmented Neutrophilic Granulocytes
RBC	Red Blood Cell
RD_{50}	50% reduction in Respiratory Rate
REL	Reference Exposure Level
RfC	Reference Concentration
RGDR	Regional Gas Dose Ratio
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SAS	Statistical Analysis System
SD	Standard Deviation
SEM	Standard Error of the Mean
TAC	Toxic Air Contaminant
TLV	Threshold Limit Value
TMB	Trimethylbenzene
TSD	Technical Support Document
TSLP	Thymic Stromal Lymphopoietin
TWA	Time-weighted Average
UF	Uncertainty Factor
US EPA	United States Environmental Protection Agency
VOC	Volatile Organic Compounds
WBC	White Blood Cell

2. Physical & Chemical Properties

Trimethylbenzenes (TMBs) exist in three isomeric forms: 1,2,3-trimethylbenzene (hemimellitene), 1,2,4-trimethylbenzene (pseudocumene), and 1,3,5-trimethylbenzene (mesitylene). For simplicity, they will be abbreviated as 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB, respectively, throughout this document (Table 1, below). 1,3,5-TMB is also referred to as symmetrical trimethylbenzene because of the symmetrical arrangement of the three methyl groups on the benzene ring. The other two isomers, 1,2,3-TMB and 1,2,4-TMB, are o-xylenic dimethyls (that is, two of their methyl substituents are bonded to adjacent carbon atoms in the aromatic ring).

Table 1. Trimethylbenzene Isomers

Isomer	Abbreviation	CAS #	Chemical Structure
1,2,3-trimethylbenzene	1,2,3-TMB	526-73-8	
1,2,4-trimethylbenzene	1,2,4-TMB	95-63-6	
1,3,5-trimethylbenzene	1,3,5-TMB	108-67-8	

Abbreviations: CAS = Chemical Abstracts Service; TMB = Trimethylbenzene

Table 2. Trimethylbenzene isomer physical and chemical properties

Isomer	**1,2,3-TMB**	**1,3,5-TMB**	**1,2,4-TMB**
Description	Clear, colorless liquid		
Molecular formula	C_9H_{12}		
Molecular weight (g/mol)	120.19		
Melting point, °C	−25.4	−44.8	−43.8
Boiling point, °C @ 760 mm Hg	176.1	164.7	168.9
Vapor pressure, mm Hg @ 25°C	1.69	2.48	2.10
Density, g/mL at 20 °C	0.8944	0.8637	0.8758
Flashpoint, °C	44	50	44
Water solubility, mg/L at 25 °C	75.2	48.2	57
Other solubilities	Ethanol, acetone, benzene, petroleum ether	Alcohol, ether, benzene, acetone, oxygenated and aromatic solvents	Ethanol, benzene, ethyl ether, acetone, petroleum ether
Henry's law constant, atm m^3/mol	4.36×10^{-3}	8.77×10^{-3}	6.16×10^{-3}
Log K_{OW}	3.66	3.42	3.78
Log K_{OC}	2.80−3.04	2.70−3.13	2.73
Conversion factors	1 ppm = 4.92 mg/m^3 1 mg/m^3 = 0.2 ppm		

Adapted from US EPA (2016).
Abbreviations: CAS = Chemical Abstracts Service; K_{OC} = carbon-water partition coefficient; K_{OW} = octanol-water partition coefficient.

3. Occurrence and Major Uses

The three TMB isomers occur naturally in petroleum deposits and are common components of petroleum refinery distillation fractions such as white spirit, high flash point naphtha, and gasoline. The proportion of TMBs in these products has been reported to vary from about 5 to 50% (v/v) (Jarnberg et al., 1997a). TMBs are also emitted by steel-making facilities (Chang et al., 2010) and coal-fired plants (Shi et al., 2015). In the case of the latter, TMBs were among the largest contributors of the VOCs emitted in the exhaust gases from the stacks (36.3% of all volatile organic chemicals (VOCs)). TMBs are also found in surface coatings, printing inks, paint, cleaners, and as an additive in pesticides. Although all three isomers are typically present in paint solvents, the proportion of the individual isomers may vary considerably. The US Environmental Protection Agency (US EPA) lists TMB as an inert ingredient in 18 pesticides (US EPA, 1994b). 1,2,4-TMB is also used as a chemical intermediate.

General exposure to TMBs may result from the use of solvent and paint thinner preparations, gasoline and fuel spills, and air pollution emissions. According to Chowdhury and Brock (2001), “There are thousands of sites across the U.S. with fuel spills/underground plumes”. The 1,2,4-TMB and 1,3,5-TMB isomers are two of the contaminants of concern that comprise the standard JP-4 jet fuel mixture. Many of these sites are near occupied residential or commercial structures, and may potentially pose an inhalation risk (AFCEE, 1999; US EPA, 2002). At one facility in Utah, onsite soils exhibited high levels of volatile organic carbon compounds (VOCs), including TMB (2900 ppm).

The California Air Resources Board (CARB) provides stationary source (point and aggregated point) air emissions data for chemicals in the Hot Spots program. TMBs (combined) and data specific for the 1,2,4-TMB isomer are shown in Tables 3a and 3b for years 2011 to 2020 (2020 is the latest year for which data are available). The data represent emissions that have been reported to CARB for facilities by the local air districts (it does not necessarily represent every source of toxic air emissions in the state). Aggregated TMBs and 1,2,4-TMB are the only two CAS numbers for which emissions have been reported. Note that aggregated TMBs (CAS # 25551-13-7) may include, but are not limited to, the 1,2,4-TMB isomer. The data compiled for TMBs and 1,2,4,-TMB can be found using CARB’s public Facility Search Tool (https://ww2.arb.ca.gov/our-work/programs/ab-2588-air-toxics-hot-spots/facility-search-tool).

Table 3a. Point Source Emissions Reported to CARB (2011- 2020) for TMBs (CAS # 25551137)

Year	Number of Facilities	Emissions of TMBs (lbs/yr)		
		Total	Min	Max
2011	11	498.3	0.0576	158.4
2012	10	411.5	0.0576	142.4
2013	11	428.3	0.0576	142.4
2014	11	479.8	0.0056	145.4
2015	12	524.9	0.0056	145.4
2016	11	1251.3	0.0052	1145.4
2017	8	110.6	0.0035	68.4
2018	13	453.4	0.0169	292.0
2019	26	991.7	0.0039	292.0
2020	34	1141.1	0.0006	318.0

Abbreviations: CARB = California Air Resources Board; lbs = pounds; TMBs = trimethylbenzenes; yr = year.

Table 3b. Point Source Emissions Reported to CARB (2011- 2020) for 1,2,4-TMB (CAS # 95-63-6)

Year	Number of Facilities	Emissions of 1,2,4-TMB (lbs/yr)		
		Total	Min	Max
2011	516	134,411.8	1.0E-05	85,068.1
2012	455	128,952.0	1.7E-05	85,068.1
2013	472	120,164.5	1.7E-05	85,168.9
2014	192	34,194.7	1.7E-05	13,072.5
2015	241	51,885.1	6.4E-06	13,072.5
2016	194	36,622.1	6.7E-05	13,072.5
2017	268	34,307.1	5.4E-07	13,072.5
2018	220	34,690.3	1.4E-08	13,072.5
2019	377	68,640.0	1.4E-08	30,822.6
2020	485	55,839.5	5.0E-08	15,937.9

Abbreviations: CARB = California Air Resources Board; lbs = pounds; TMB = trimethylbenzene; yr = year.

In California, per CARB, the leading TMBs and 1,2,4-TMB emission sources include: petroleum refining, construction, cement, paving mixtures, asphalt and metal coatings, petro bulk stations, as well as other sources. Of the sites on U.S. EPA's National Priorities List that report TMB isomer contamination, 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 (US EPA, 2016). (The National Priorities List is a list of hazardous waste sites in the U.S. eligible for long-term remedial action, financed under the federal Superfund program). The three TMB isomers are on the US EPA list (US EPA, 2012) for chemicals used in hydraulic fracturing fluids. The isomers have been detected in flowback and produced waters ("hydraulic fracturing wastewater") from fracturing operations. 1,2,4 and 1,3,5-TMB have been detected in air emissions associated with shale gas development and production operations (Rich et al, 2014). All three TMB isomers are also found as constituents of biogas (OEHHA/CARB, 2013); the source of the TMBs is largely municipal landfills which capture/supply the biogas for use in energy applications.

3.1 Exposure Assessment

There are no studies available that uniquely evaluated exposure to TMB isomers. There are several studies that evaluated residential exposure to a number of different contaminants, including TMBs. Minoia et al. (1996) measured daily indoor/outdoor inhalation exposures to sixteen aromatic and aliphatic hydrocarbons (HCs), including all three TMB isomers, experienced by 3 groups of primary school children (1st and 2nd grade) living in three different urban areas in Italy (towns with 50,000 inhabitants or less). The largest sample (from Treviglio) included 165 children, followed by Valenza (*n* = 137) and Poggibonsi (*n* = 130). The parents of the children filled out questionnaires about smoking or other possible sources of HCs in the house. Simultaneous use of two personal samplers (radial diffusion passive mini-samplers) for each child, over one 24-hour period, determined both indoor (children's houses) and indoor + outdoor daily inhalation concentrations. One sampler was placed in the child's house at a height of 80 to 100 cm and used to determine indoor ambient air HC concentrations. The second sampler was worn by the child near his/her mouth and used to determine indoor + outdoor breathing zone HC concentrations. Additional samplers were used to determine HC background levels. Indoor mean environmental levels for the three towns ranged from 3.6 to 4.6 µg/m^3 for 1,2,3-TMB, 5.0 to 7.4 µg/m^3 for 1,2,4-TMB, and 2.8 to 4.2 µg/m^3 for 1,3,5-TMB for the period March-April 1995. In general, indoor + outdoor concentrations were somewhat higher. Table 4, below, shows the average indoor and indoor + outdoor environmental TMB concentrations that were determined from the personal samplers worn by the children. For the 1,2,3-TMB and 1,3,5-TMB isomers, in most cases the children's exposure was comparable. 1,2,4-TMB consistently had a somewhat higher profile across all towns. In some cases, indoor only concentrations were higher than indoor/outdoor concentrations.

Table 4. Trimethylbenzene Exposure Concentrations in 3 Urban Areas in Italy[1]

TMB Isomer	Treviglio (µg/m³)		Poggibonsi (µg/m³)		Valenza (µg/m³)	
	Indoor	Indoor + Outdoor	Indoor	Indoor + Outdoor	Indoor	Indoor + Outdoor
	Mean ± SD (number samples with detections)		Mean ± SD (number samples with detections)		Mean ± SD (number samples with detections)	
1,2,3-TMB	3.6 ± 1.5 (97)	4.2 ± 1.8 (106)	3.6 ± 1.8 (61)	2.8 ± 1.3 (56)	4.6 ± 2.2 (34)	5.3 ± 2.6 (34)
1,2,4-TMB	5.0 ± 2.6 (139)	6.2 ± 2.9 (144)	7.4 ± 2.8 (117)	6.9 ± 2.2 (117)	5.0 ± 2.3 (102)	6.8 ± 11.2 (128)
1,3,5-TMB	2.8 ± 2.1 (82)	2.9 ± 1.4 (104)	3.2 ± 1.9 (84)	2.6 ± 1.3 (90)	4.2 ± 3.6 (19)	4.2 ± 3.8 (20)

Adapted from Minoia et al. (1996).
Abbreviations: TMB = trimethylbenzene; SD = standard deviation
[1] each sample was taken over one 24-hour period between March–April of 1995.

Zhu et al. (2013) conducted a population-based survey of residential indoor air in Canada as part of the Canadian Health Measures Survey (CHMS). CHMS is an on-going survey designed to provide health measures data at the Canadian national level. Canadians aged 3 to 79 living in private households were included. Indoor air samples were collected from 5 geographic regions between 2009 to 2011. A total of 8520 households were selected, with 6465 households participating. A total of 4722 households reported to a mobile examination center where physical measures and heath examinations were performed. There was one indoor air sampler per household. Samples were collected using a passive sampler tube and had a sampling duration of 4 to 10 days. Indoor air quality from 3587 households were included in the analysis. Statistical analyses were performed using SAS version 9.2 and SUDAAN version 10 software. A total of 84 VOCs were measured in this survey, including 1,2,4- and 1,2,3-TMB. Forty-seven VOCs had detection frequencies above 50%, including the two TMB isomers. The percentage of detection frequency for 1,2,4- and 1,2,3-TMB were 98.96 and 99.74, respectively. The arithmetic and geometric means (with its respective 95% CI, µg/m^3), and 95th/99th percentile for the 1,2,4-TMB isomer were 1.37 (1.01 – 1.73), 0.51 (0.42 – 0.61), 5.18 (95th percentile), 14.96 (99th percentile). For the 1,2,3-TMB

isomer, the mean values were 4.33 (3.24 – 5.43), 1.58 (1.31 – 1.90), 18.65 (95th percentile), 61.61 (99th percentile). Upper range distribution values are useful for assessing upper boundary estimates of human exposure to these TMB isomers. A comparison of the indoor air arithmetic mean values for 12 of these VOCs, including 1,2,4- and 1,2,3-TMB, with the results of a prior Canada National Health Survey from 1992, showed a decrease in indoor air levels for 1,2,4-TMB (from 11.5 to 1.37 µg/m^3) (no results reported for 1,2,3-TMB). The authors attributed this to reductions in vehicle emissions through improved emissions technology in Canada.

McKenzie et al. (2012) conducted a health risk assessment to assess how proximity to air emissions from the development of unconventional (*e.g.*, directional drilling, hydraulic fracturing) natural gas resources impacts the health of the surrounding community. Residents in Garfield County, Colorado living < ½ mile from shale gas wells had greater risk of health impacts due to subchronic and chronic exposure to TMBs and other hydrocarbons than those living at a distance > ½ mile (subchronic defined as a 20-month exposure duration, and chronic as a 30-year exposure duration). Of the 78 compounds analyzed, trimethylbenzenes were the primary contributor to both the subchronic hazard index, and the chronic non-cancer hazard index, 46 and 45%, respectively. Median and maximum air concentrations for 1,2,3-, 1,2,4-, and 1,3,5-TMB were 0.11 and 0.85 µg/m^3, 0.18 and 3.1 µg/m^3, and 0.12 and 1.2 µg/m^3, respectively.

4. Toxicokinetics

There are some differences in metabolism between the three TMB isomers. At least one study reported that the methyl group configuration on the benzene ring of 1,2,3-TMB is not stable, and that oxidation or reduction of the methyl group at the C_2 position takes place more easily than for 1,3,5-TMB or 1,2,4-TMB (Tomas et al. 1999a). However, in both animals and humans, the three TMB isomers demonstrate similar qualitative metabolic profiles. All three isomers metabolize primarily to dimethylbenzoic and hippuric acids. Specifically, TMB isomers are metabolized via side-chain oxidation to alcohols and aromatic carboxylic/mercapturic acids, or by hydroxylation to form phenols, and are excreted as glucuronides and sulphate esters (Jarnberg et al., 1996; Huo et al., 1989; Milkuski and Wiglusz, 1975; US EPA, 2016). Currently, it is not known which CYP450 isozyme is most responsible for TMB metabolism.

In a recent *in vitro* study, Meausoone et al. (2021) evaluated toxicological responses of a human bronchial epithelial cell line, BEAS-2B, to a number of industrial solvents, including benzene, toluene, *m*-xylene and 1,3,5-TMB. The authors noted that, “the pulmonary epithelium covers the whole surface of the human respiratory tract and is in direct contact with inhaled xenobiotics”. Only the data that pertain to 1,3,5-TMB, are

discussed in this section. The bronchial cells were cloned from a healthy donor at autopsy, are metabolically active, and able to secrete cytokines. The cells were exposed to gaseous steams of 20 or 100 ppm (98 or 492 mg/m^3) 1,3,5-TMB using an air-liquid interface for either a single 1hour exposure, or to repeated exposures (two exposures of 1 hour/day for 3 or 5 days duration). Control cells were exposed to filtered air. Following exposure on days 1, 3 and 5, cells were harvested 5 hours after the end of exposure and preserved at -80°C. Gene expression of 8 enzymes involved in xenobiotic metabolism were evaluated for their response: the P450 enzymes CYP2E1, CYP1A1, CYP1B1, CYP2F1; the non-P450 enzyme EPHX1; DHDH, a dihydrodiol dehydrogenase; and the aldehyde dehydrogenases ALDH3B1 and ALDH2. Exposure to 1,3,5 -TMB significantly induced gene expression in cytochrome P450 enzymes CYP2E1 and CYP1A1, as well as EPHX1, DHDH, and ALDH3B1 and ALDH2. Repeated exposure to either 20 or 100 ppm 1,3,5-TMB significantly induced the gene expression of the cytochrome P450s CYP2E1 and CYP1A1; CYP1A1 was only overexpressed following exposure to the 100 ppm 1,3,5-TMB concentration. Expression of the CYP2E1 gene increased with time from day 1 to 5. No significant gene induction of CYP1B1 or CYP2F1 was observed following cell exposure. Repeated exposure to 100 ppm 1,3,5-TMB resulted in significant gene induction of EPHX1 on day 5. The aldehyde dehydrogenases ALDH2 and ALDH3B1 were also significantly overexpressed at the higher concentration on day 5; ALDH3B1 was also significantly overexpressed at the 20-ppm concentration on days 3 and 5. DHDH was significantly induced after BEAS-2B cell exposure on day 3. No significant variation was observed in the expression of GSTM1 and NQO1 genes following cell exposure. These two genes participate both in the biotransformation of many xenobiotics as well as the regulation of oxidative stress. The authors stated that its possible these two genes are not very active in the BEAS-2B cell line. The CYP2E1 enzyme is abundant in lung cells and is known to be induced after human exposure to organic compounds (Pohl and Scinicariello, 2011).

There are some quantitative metabolism differences between humans and other animal species (Jarnberg et al., 1996; Milkulski and Wiglusz, 1975). When the human data are compared with the animal metabolism studies, it appears humans are less likely to eliminate TMBs via glycine conjugation (*i.e.,* dimethylhippuric acids, DHMAs). In 1,3,5-TMB metabolism studies in animals, 78% of the dose was eliminated as 3,5-DMHA or 3,5-dimethylbenzoic acid in rabbits (Laham and Potvin, 1980) and rats (Mikulski and Wiglusz, 1975).

In humans, the following routes of metabolism appear to contribute substantially to the elimination of TMBs: excretion of unconjugated (free) dimethylbenzoic acids, formation of glucuronides, and benzyl alcohols conjugated with glucuronic acid or sulfates (Jarnberg et al., 1997a). Figures 1, 2, and 3 below (from US EPA, 2016) show the proposed metabolic scheme in mammals for the three TMBs. Additionally, in humans at least, exhalation of the unchanged parent compound is an important route of

elimination, accounting for 20 to 37% of the absorbed amount depending on the specific TMB isomer. In comparison, urinary excretion of unchanged TMBs is very low, <0.002% (Jarnberg et al., 1996).

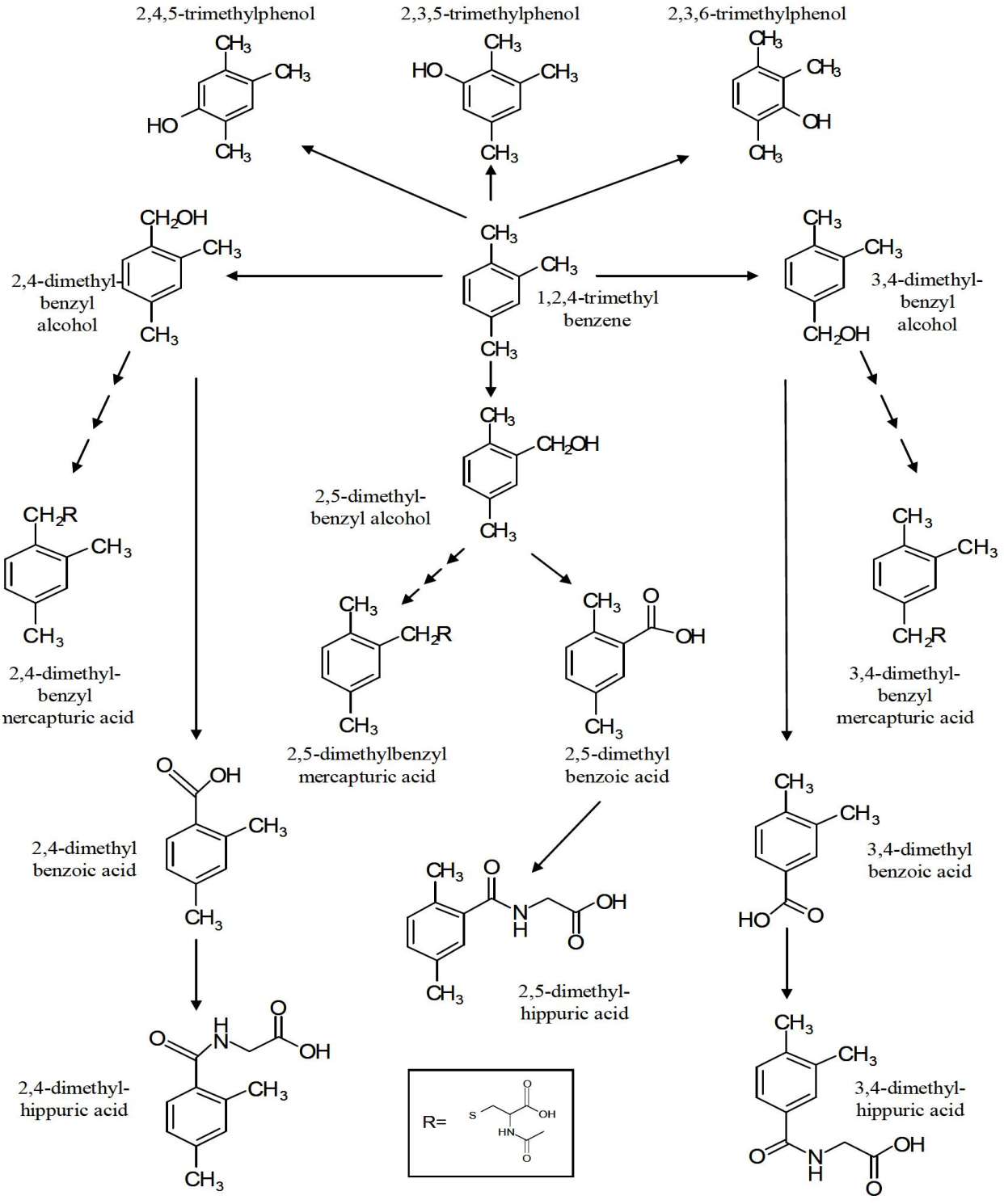


Figure 1. Metabolic Scheme for 1,2,4-Trimethylbenzene in mammals. Broken arrows indicate a postulated pathway (e.g., mercapturic acid formation via side-chain oxidation of alcohols) (US EPA, 2016).

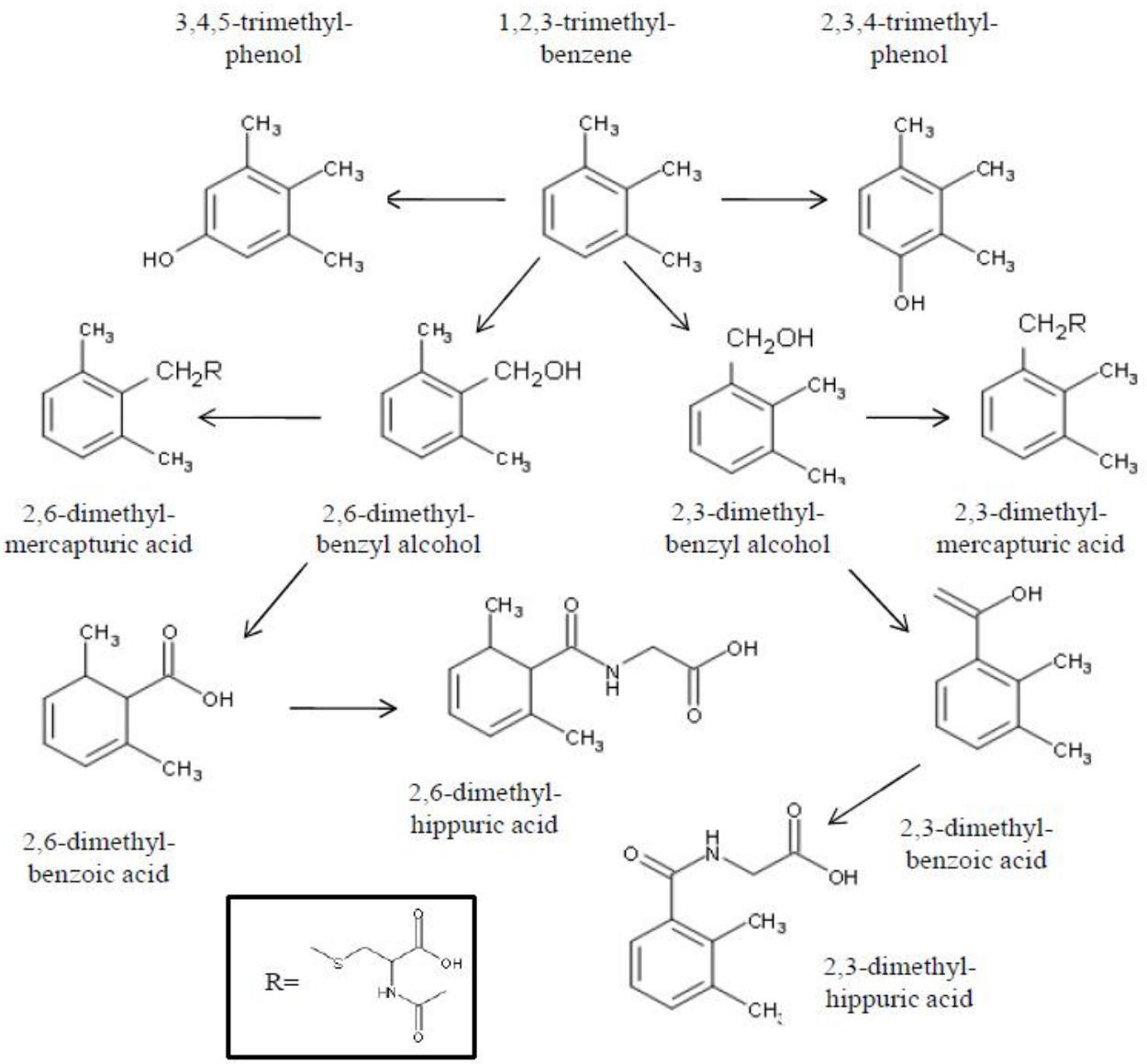


Figure 2. Metabolic Scheme for 1,2,3,-Trimethylbenzene in mammals (US EPA, 2016).

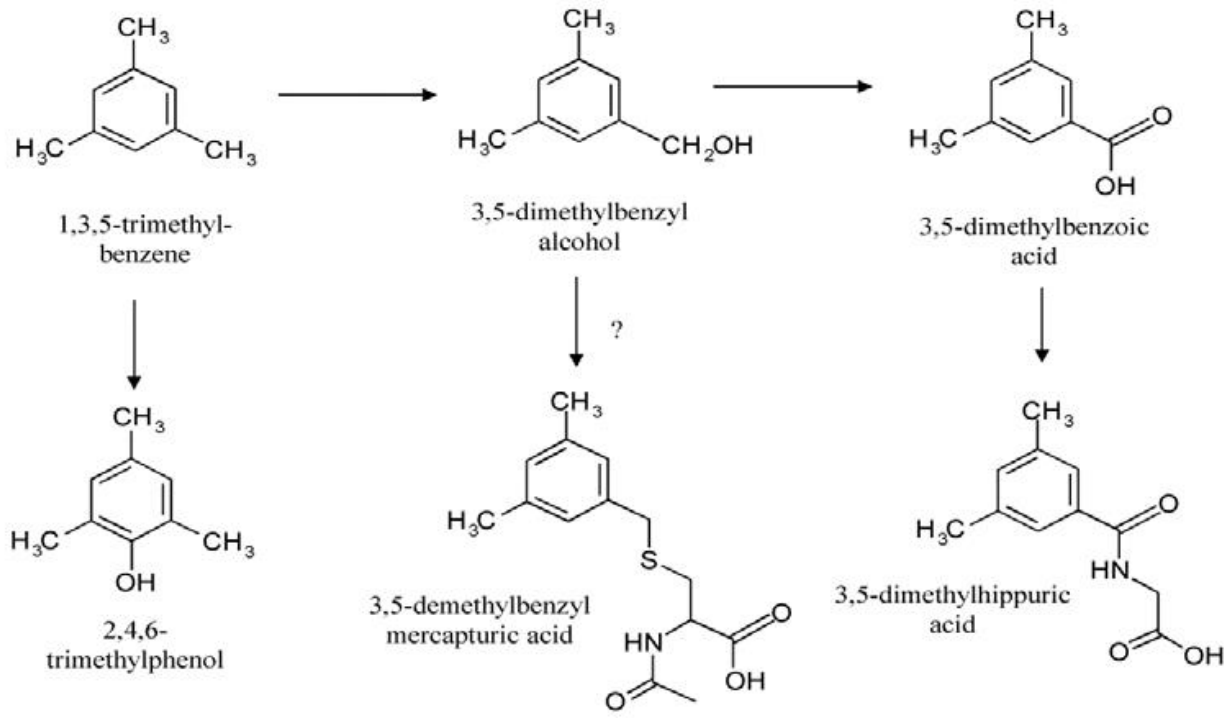


Figure 3. Metabolic Scheme for 1,3,5-Trimethylbenzene in mammals (US EPA, 2016).

4.1 Toxicokinetic Studies in Humans

A number of *in vitro* and *in vivo* studies designed to elucidate the distribution and metabolism of TMB isomers in humans have been conducted. Liquid/air partition coefficients (PCs) for the three TMB isomers were determined *in vitro* using physiological saline, adult human blood, and olive oil (Jarnberg and Johanson, 1995). The blood/air, water/air, oil/air and oil/blood PCs are shown in Table 5. While the oil/blood PC are in the order of 1,2,3-TMB < 1,2,4-TMB < 1,3,5-TMB, the other PCs (i.e., blood/air, water/air, and oil/air) increased in the order of 1,3,5-TMB < 1,2,4-TMB < 1,2,3-TMB. The TMBs have high blood/air and oil/air PCs; their affinity for water is limited. From these results, high respiratory uptake and accumulation of TMBs in adipose tissue is expected (Jarnberg and Johanson 1995). Of the three isomers, 1,3,5-TMB has the highest oil/blood PC, indicating it has a somewhat greater affinity for adipose tissue than 1,2,4-TMB and 1,2,3-TMB.

Note that Hissink et al. (2007), in an industry study designed to predict a no-effect level for acute CNS depression in humans from white spirit, a complex hydrocarbon solvent, used a different set of blood/air PC values for the 1,2,4-TMB isomer in humans and rats: 148 and 85, respectively. The Hissink study only evaluated the 1,2,4-TMB isomer (where it is a component of white spirit). OEHHA was not able to obtain the unpublished

proprietary report that derived the 1,2,4-TMB liquid/air PCs that Hissink used to develop their blood/air PC values in its study (Leenheers et al., 1996). It was therefore not possible to validate how the PC values for the 1,2,4-TMB isomer were generated.

Table 5. Partition Coefficients of Trimethylbenzenes in Human Blood, Water, and Oil (37 °C)

	Measured Values			Calculated Values
Isomer	Blood/Air (*n*=39)	Water/Air (*n*=42)	Oil/Air (*n*=25)	Oil/Blood*
1,3,5-TMB	43 (40.0–45.2)	1.23 (1.11–1.35)	9880 (9620–10,140)	230
1,2,4-TMB	59.1 (56.9–61.3)	1.61 (1.47–1.75)	10,200 (9900–10,400)	173
1,2,3-TMB	66.5 (63.7–69.3)	2.73 (2.54–2.92)	10,900 (10,500–11,300)	164

Adapted from Jarnberg and Johanson (1995). *Oil/Blood is calculated by dividing oil/air by blood/air.
Abbreviations: *n* = number of samples; blood samples taken from 10 “healthy” volunteers (5 men, 5 women); TMB = trimethylbenzene.

For comparison purposes, Table 6 shows the human and animal blood:air partition coefficients for the three TMB isomers, as determined by Meulenberg and Vijverberg (2000). In this study, reported values of partition coefficients were compiled from the scientific literature and then modeled by the authors; the human values used for the TMB isomers were initially derived from the Jarnberg and Johanson (1995) *in vitro* study, and the rat values were calculated by the authors.

Table 6. Human and Rat Blood:Air Trimethylbenzene Isomer Partition Coefficients

TMB Isomer	Human	Rat
1,3,5-TMB	43.0	55.7
1,2,4-TMB	59.1	57.7
1,2,3-TMB	66.5	62.6

Source: Meulenberg and Vijverberg (2000). TMB = trimethylbenzene

A number of experimental inhalation studies in humans, using both volunteers and workers, have been carried out to obtain toxicokinetic data on the absorption and elimination of TMBs and their metabolites (Table 7). A few of these studies also

evaluated toxicological endpoints and those findings are discussed in Section 5.1, Acute Toxicity to Adult Humans.

Table 7. Trimethylbenzene Metabolic Inhalation Studies in Humans

Study	Study Type	TMB Isomer(s)	# Subjects (sex)	Exposure (time/concentration)
Ichiba et al. (1992)	O	1,2,4-TMB, 1,2,3-TMB, 1,3,5-TMB	7 (sex not specified)	8 hours/3–8 ppm (15–40 mg/m^3)
Fukaya et al. (1994)	O	1,2,4-TMB, 1,2,3-TMB, 1,3,5-TMB	6 (M)	8 hours/25 ppm (123 mg/m^3)
Kostrewski and Wiaderna-Brycht (1995)	C	1,3,5-TMB	5 (sex not specified)	8 hours/2–30 ppm (10–150 mg/m^3)
Ashley and Prah (1997)	C	1,2,4-TMB	5 (4 M,1 F)	4 or 6 hours/VOC mixture containing 1,2,4-TMB, 3.2 or 6.4 ppm (16 or 32 mg/m^3)
Jarnberg et al. (1997b)	C	1,2,4-TMB, 1,2,3-TMB, 1,3,5-TMB	10 (M)	2 hours/25 ppm (123 mg/m^3)
Jarnberg et al. (1997a)	C	1,2,4-TMB	9 (M)	2 hours/2 ppm (9 mg/m^3)
Kostrzewski et al. (1997)	C	1,2,4-TMB, 1,2,3-TMB, 1,3,5-TMB	8 (sex not specified)	4 or 8 hours/1–30 ppm (5–150 mg/m^3)
Jones et al. (2006)	C/O	1,2,4-TMB, 1,2,3-TMB, 1,3,5-TMB	4 (2M,2 F)/12 workers (sex not specified)	4 hours/25 ppm (123 mg/m^3) 1,3,5-TMB; separate occupational hygiene study (1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB)

Abbreviations: C = chamber exposure study; F = female; M = male; mg/m^3 = milligram per cubic meter; O = occupational exposure study; ppm = parts per million; TMB = trimethylbenzene; VOC = volatile organic compound.

Ichiba et al. (1992), in a biological monitoring study of Japanese transfer printing workers, reported that the correlation between the urinary excretion level of the 1,2,4-

TMB metabolite 3,4-DMHA and 1,2,4-TMB exposure was statistically significant at low exposures, 3 to 8 ppm (15 to 39 mg/m^3). The print workers were exposed to a solvent mixture containing all 3 TMBs and, in addition, toluene and ethyltoluene. Urine samples were collected from two workers during the work period, and from additional workers at the end of shift (n = 7; gender not specified), and analyzed for metabolites by high-pressure liquid chromatography (HPLC). The authors did not quantify TMB metabolites other than 3,4-DMHA, and did not specify why they only quantified 3,4-DMHA. Urinary 3,4-DMHA concentrations in the samples ranged from 14 to 96 mg/g creatinine (Cr). The urinary 3,4-DMHA concentration showed a positive correlation with the level of exposure to 1,2,4-TMB (r = 0.72). In the first urine of the next day, the concentration of 3,4,-DMHA was below the detection limit of 0.5 mg/L. The metabolite was not detected in the urine of nonexposed workers.

In another study that evaluated Japanese printing workers, occupational exposure to TMBs at the TLV of 25 ppm (123 mg/m^3) resulted in a mean urinary concentration of 410 mg 3,4-DMHA/g Cr (r = 0.897, p < 0.001) (Fukaya et al., 1994). Urine samples from six male workers, with a mean age of 51.0 ± 4.8 years and an average duration of exposure to TMBs of 20.8 ± 9.0 years, were collected at the start and end of the workshift for 5 days. Urinary samples were analyzed for the 3,4-DMHA metabolite using HPLC. Analysis of airborne TMBs showed that 1,2,4-TMB comprised approximately 70% of the total. The other isomers, 1,3,5-TMB and 1,2,3-TMB, contributed 20% and 10%, respectively. A small amount of xylene was detected in some samples. According to the study authors, the urinary concentration of 3,4-DMHA was low at the start of each shift and high at the end (mean 3,4-DMHA concentrations were between 0 and 100 mg/g Cr at the start, whereas mean concentrations at shift end ranged between 250 and 500 mg/g Cr). Total TMB air levels (mean ± SD) over the 5 days ranged from a low of 18.4 ± 3.03 to a high of 38.3 ± 3.94 ppm; mean 1,2,4-TMB air concentrations were consistently higher than the other two isomers.

In humans, statistically significant differences in respiratory uptake between the various isomers have been observed (Jarnberg et al., 1996). Ten healthy male volunteers were exposed to 25 ppm (123 mg/m^3) 1,2,4-, 1,2,3- or 1,3,5-TMB vapors in a 20 m^3 exposure chamber on four different occasions for 2 hours, at a constant workload of 50 watts (as displayed on a bicycle ergometer). Twenty-five ppm corresponds to the occupational exposure limit in Sweden for all three TMB isomers. Volunteers were also exposed to 2 ppm (10 mg/m^3) 1,2,4-TMB for the same length of time. Urine was collected at the onset of exposure (0) and at 2, 4, 11, and 20 hours postexposure, and analyzed via HPLC for all six possible DMHA isomers. Following a 2-hour exposure to 25 ppm (123 mg/m^3) of 1,3,5-TMB, 1,2,3-TMB, or 1,2,4-TMB during light physical exercise, relative respiratory uptake for 1,2,3-TMB was significantly lower (56%) than 1,2,4-TMB (64%) and 1,3,5-TMB (62%). The average concentration of 1,2,3-TMB in arterial blood was significantly

higher than the corresponding concentrations of 1,2,4-TMB and 1,3,5-TMB. The authors concluded that the slower metabolic rate of 1,2,3-TMB explains the lower respiratory uptake and higher blood levels (despite its higher blood:air partition coefficient) that are seen for 1,2,3-TMB, compared with the two other isomers. Calculations of area under the curve (integral or AUC) during exposure, which references the total amount of the chemical absorbed by the body over time, gave a significantly greater value for 1,2,3-TMB than for 1,3,5-TMB. 1,3,5-TMB had a higher total blood clearance (significantly different than 1,2,4-TMB and 1,2,3-TMB) and higher metabolic clearance than 1,2,4-TMB and 1,2,3-TMB. Significantly less (20% to 25%) 1,2,4-TMB and 1,3,5-TMB was exhaled unchanged compared to 1,2,3-TMB (as much as 37% of the absorbed 1,2,3-TMB was exhaled unchanged). Thus, respiratory excretion makes an important contribution to total clearance, especially for 1,2,3-TMB. Twenty-two percent of the inhaled 1,2,4-TMB was excreted as DHMA acids within 24 hours, mainly as 3,4-DMHA. The twenty-four hour recovery of 1,2,3-TMB was 11%, mainly as 2,3-DMHA. Only 3% of the absorbed amount of 1,3,5-TMB was excreted as 3,5-DMHA. The excretion of unconjugated dimethylbenzoic acids in urine was approximately 3% of the dose of TMBs. The study authors stated that the short (*i.e.,*1 day) follow-up period resulted in an underestimation of the more slowly excreted 3,5-DMHA, as compared with the other DMHA isomers (3,5-DMHA isomer has the longest half-time, 16 hours, of the six DMHAs).

Two different studies by the same research group (Kostrewski and Wiaderna-Brycht, 1995; Kostrewski et al., 1997) studied the elimination kinetics of 1,3,5-TMB and 1,2,4-TMB, respectively, following human inhalation exposure. In the first study (Kostrewski and Wiaderna-Brycht, 1995), five volunteers (gender not specified) were exposed in a closed chamber to 1,3,5-TMB concentrations ranging from 10 to 150 mg/m^3 (2 to 30 ppm) for 8 hours. 73% of the absorbed 1,3,5-TMB dose was metabolized to 3,5-DMHA or 3,5-dimethylbenzoic acid. Pulmonary ventilation in the volunteers ranged from 0.56 to 0.99 m^3/hr. During the first minute of 1,3,5-TMB exposure at 20 ppm (100 mg/m^3), retention was as high as 77%. After 2 hours exposure, the retention stabilized at 67%.

Kostrzewski et al. (1997), using human volunteers, reported that pulmonary retention appears to be comparable for the three TMB isomers. Five subjects (gender not specified) aged 20 to 39 years with no history of exposure to TMBs inhaled 1,2,4-TMB, 1,3,5-TMB, or 1,2,3-TMB at concentrations ranging from 1 to 30 ppm (5 to 150 mg/m^3) of air for 4 or 8 hours in an exposure chamber (the two exposures were spaced at “3–4 week” intervals). Exhaled air, blood, and urine samples were collected before, during and after all exposures. Blood samples were collected just prior to exposure, at the last minute of exposure, and 15 to 20 minutes following exposure termination. Urine samples were collected just prior to exposure, during the exposure and 2, 4, 6, and 8 hours from exposure onset. Urine was also collected at 2, 4, 6, 8, 15, 19, 23, 27, 31, 39,

43, 47, 51, 55, 63, 67, 71, 75, 79, 83, 87, and 95 hours post-exposure. During experiments involving exposure to 20 or 30 ppm (100 or 150 mg/m^3) TMB for 4 hours, determinations of lung ventilation and absorption were also carried out. The kinetics of 1,2,4- and 1,3,5-TMB absorption and elimination from capillary blood at a concentration of 30 ppm (150 mg/m^3), and 1,2,3-TMB at 20 ppm (100 mg/m^3), was evaluated at the time of exposure and "several hours" after exposure termination. Dimethylbenzoic acid concentrations were determined using gas chromatography. Pulmonary ventilation in the volunteers ranged from 0.56 to 1.0 m^3/hour. The retention of 1,2,4-TMB, 1,3,5-TMB, and 1,2,3-TMB in the lungs was 68%, 67%, and 71%, respectively (preliminary experiments on TMB inhalation found that constant pulmonary vapor retention was reached after 3 to 4 hours of continuous exposure). Blood TMB concentrations were higher for all 3 isomers at 8 hours than at 4 hours exposure.

In one human subject study, TMB blood concentrations were shown to be directly related to TMB air exposure concentrations. Ashley and Prah (1997) exposed human subjects in a controlled inhalation chamber to a mixture of 21 VOCs, including 1,2,4-TMB, to determine the kinetics of VOC blood uptake and elimination. Five adult subjects, 4 men and 1 woman (ages not provided), were exposed for either 4 hours on each of two occasions (*n* = 1 subject) or for 6 hours on one occasion (*n* = 4 subjects) to either 3.2 or 6.4 ppm (12 or 24 mg/m^3) toluene-equivalent VOC mixture. The authors were unclear as to the definition of the term "toluene equivalent", but it appears that VOC mixture ppm values were generated from VOC mixture mg/m^3 values using the molecular weight of toluene. 1,2,4-TMB concentrations in the 3.2 and 6.4 ppm VOC mixtures were 0.0079 ppm (0.039 mg/m^3) and 0.0158 ppm (0.078 mg/m^3), respectively. Blood concentrations were measured before, during, and after exposure. Mean plateau blood 1,2,4-TMB concentrations were 0.14 $\pm$ 0.03 ppb and 0.21 $\pm$ 0.2 ppb (0.14 $\pm$ 0.03 and 0.21 $\pm$ 0.2 µg/L) for the two 4-hour exposures to the 3.2 and 6.4 ppm VOC mixtures respectively. Mean 1,2,4-TMB blood concentration elevations associated with 2-, 4-, and 6-hour exposures to an air concentration of 0.0158 ppm 1,2,4-TMB (air concentration for the 6.4 ppm exposure), were 0.082, 0.15, and 0.40 ppb (0.4, 0.74, and 2 µg/m^3) 1,2,4-TMB, respectively.

Jarnberg et al. (1997b) exposed ten male volunteers to approximately 25 ppm (123 mg/m^3) of each TMB isomer for 2 hours duration on four separate occasions and to 2 ppm (11 mg/m^3) 1,2,4-TMB for 2 hours on four separate occasions in a 20 m^3 exposure chamber while exercising on a bicycle ergometer at a constant workload of 50 watts. About 22% of the inhaled amount of 1,2,4-TMB was excreted as dimethylhippuric acids (DMHAs) within 24 hours, mainly as 3,4-DMHA. The 24-hour recovery of 1,2,3-TMB as DMHAs was 11%; only 3% of the absorbed amount of 1,3,5-TMB was excreted as 3,4-DMHA. Of the 6 DHMAs, the 3,5-DMHA isomer had the longest half-life. In addition, the recovery of 1,3,5-TMB as 3,5-DMHA was much lower than that of the other two TMB

isomers. No significant difference was seen in the excretion pattern of DMHA isomers between the 2- and 25-ppm exposures to 1,2,4-TMB.

In a related study, the inhalation toxicokinetics of exposure to the 1,2,4-TMB isomer in white spirit and to 1,2,4-TMB alone were compared (Jarnberg et al., 1997a). White spirit is comprised of a complex mixture of hydrocarbons (*e.g.,* nonane, decane, undecane, xylene, TMBs, aliphatic compounds, and other aromatic compounds). Healthy male volunteers (*n* = 9, average age 36) were exposed in an inhalation chamber for 2 hours to 2 ppm (10 mg/m^3) 1,2,4-TMB or (300 mg/m^3) white spirit, while doing light physical work. One year elapsed between the 1,2,4-TMB and white spirit exposures; the same subjects were used for both experiments. The proportion of 1,2,4-TMB in the white spirit was 3.8% (w/w), corresponding to 2.3 ppm (11.3 mg/m^3) 1,2,4-TMB. Exhaled air was collected at 30, 60, 90 and 120 minutes during the exposure, and after the exposure was terminated. Blood and urine samples were taken at the onset, during, and post-exposure. 1,2,4-TMB was analyzed in blood and exhaled air by gas chromatography. The authors stated that, “neither the absolute nor relative uptake differed between the two experimental conditions” (data not shown). Table 8, below, summarizes some of the toxicokinetic results. Blood levels of 1,2,4-TMB were significantly higher during and after exposure to white spirit compared to 1,2,4-TMB alone (data not shown). The area under the curve (AUC) was significantly higher (p<0.0001) after exposure to white spirit compared to 1,2,4-TMB alone. Both the urinary excretion rate, as well as the cumulative excretion of 3,4-DMHA, were significantly higher after exposure to white spirit. Respiratory excretion rates did not differ significantly. The authors concluded that, “…results indicate that exposure to white spirit alters the relation between internal (blood levels) and external exposure (air levels) to 1,2,4-TMB”. The authors further stated that “urinary excretion of the 3,4-DHMA metabolite seems to reflect internal (blood levels) but not external exposure (air levels) to 1,2,4-TMB.”

Table 8. Experimental Results from Inhalation Exposure of Human Volunteers to 1,2,4-Trimethylbenzene alone, or in White Spirit (mean values + 95% CI)

Exposure	1,2,4-TMB	1,2,4-TMB in White Spirit	*p*-value
Net Respiratory Uptake (mmol)	0.15 ± 0.01	0.14 ± 0.02	0.5[a]
AUC (µM × min), 0–3 h	53 ± 4	86 ± 9	<0.0001[a]
Half life of 3,4-DMHA (h)	3.7 ± 0.4[b]	3.0 ± 0.7	0.2[c]
Excretion of 3,4-DMHA (%)[d], 0–6 h	11 ± 2	18 ± 3	0.007[c]

Adapted from Jarnberg et al., 1997a (9 male volunteers, 2 h exposure).
Abbreviations: AUC = area under the curve; CI = confidence intervals; DMHA = dimethylhippuric acid, metabolite of 1,2,4-TMB; h= hour; µM = micromolar; mmol = millimoles; TMB = trimethylbenzene.
[a] Student's paired t-test
[b] recalculated from 9 subjects from a 120 mg/m^3 (24 ppm) exposure to 1,2,4-TMB
[c] Analysis of Variance
[d] % of net respiratory uptake

Jones et al. (2006) conducted chamber and occupational exposure TMB biological monitoring studies. For the chamber study, two male and two female volunteers were exposed to 25 ppm (123 mg/m^3) 1,3,5-TMB in an inhalation chamber for 4 hours. Urine, blood, and expired air samples were collected before, during, and after exposure. Blood and exhaled air samples showed 1,3,5-TMB was readily absorbed, reaching a mean level in the bloodstream of 0.85 µmol/L (0.85 ppb). Steady state was reached within one hour of exposure onset. In urine, the TMB metabolites dimethylbenzoic acids were used as biomarkers. The peak urinary dimethylbenzoic acid level for a 4 hour exposure to 25 ppm 1,3,5-TMB was 42 mmol/mol Cr (range 30 to 58 mmol/mol Cr). Exhaled air 1,3,5-TMB levels peaked within an hour of exposure and averaged 137 nmol/L (0.137 ppb, 0.674 µg/m^3) during the exposure. The majority of the absorbed dose was excreted in the first 50 hours post-exposure; however, levels of the urinary metabolite 3,5-dimethylbenzoic acid were still detected after 160 hours post-exposure.

For the occupational exposure component of the study (Jones et al., 2006), 12 workers (gender not specified) at a screen-printing company underwent biological and environmental monitoring. Urine, blood, and expired air samples were collected before, during, and after exposure. Air monitoring showed that all three isomers of TMB were present, with 1,2,4-TMB predominating (70% of the TMB detected; percentages of the other TMB isomers were not reported. Total TMB levels ranged from none to 25.3 ppm (124 mg/m^3) (8-hour TWA); two air sampling results marginally exceeded the occupational exposure standard of 25 ppm. All of the workers showed some detectable

dimethylbenzoic acid levels in their pre-shift samples (sample type not specified by authors). Urinary dimethylbenzoic acid levels correlated very well with airborne TMB personal air samples. According to the authors, the regression equation from this study indicated that an 8-hour exposure to 25 ppm TMB would result in a urinary dimethylbenzoic acid level of 206 mmol/mol Cr.

In summary, TMBs are readily absorbed in humans via inhalation (*i.e.,* high respiratory uptake); some significant differences in respiratory uptake have been reported between the different TMB isomers (Jarnberg et al., 1996). Based on their high blood/air and oil/air PCs, accumulation in adipose tissue is expected (the 1,3,5-TMB isomer has the highest oil/blood PC). Toxicokinetic findings (*i.e.,* elimination rate in blood and concentration of dimethylbenzoic acids in urine) indicate that TMBs accumulate in the body (Kostrewski et al., 1997). TMBs are metabolized primarily to dimethylbenzoic and hippuric acids. In the Kostrewski and Wiaderna-Brycht 1995 study, 73% of the absorbed 1,3,5-TMB dose was metabolized to 3,5-DMHA or 3,5-dimethylbenzoic acid. In at least one study, 3,5-DMHA had the longest half-life of the six DMHA metabolites, 16 hours (Jarnberg et al., 1997b). Respiratory excretion appears to represent an important contribution to total clearance, especially for the 1,2,3-TMB isomer. Respiratory excretion accounts for 20% to 37% of the absorbed amount, depending on the specific isomer (Jarnberg et al., 1996). The 1,3,5-TMB isomer appears to have a significantly higher total blood clearance and a higher metabolic clearance than 1,2,3- and 1,2,4-TMB.

4.2 Toxicokinetic Studies in Animals

4.2.1 Inhalation

In adult female Sprague-Dawley rats (*n* = 5/group) exposed to air concentrations of 120, 180, 400 or 720 ppm (590, 885, 1966 or 3539 mg/m^3) 1,3,5-TMB for 2 hours in a dynamic glass chamber (air flow 1.25 L/minute), the corresponding blood concentrations (mean $\pm$ standard error of the mean [SEM] from 5 rats/group) were 15.7 $\pm$ 2.2, 19.6 $\pm$ 3.2, 75.8 $\pm$ 2.1, and 143.5 $\pm$ 4.3 µmol/L, respectively (Freundt et al., 1989); there was no control group. Exposure concentrations during the inhalation period were monitored repeatedly. Blood was collected from the retro-orbital plexus immediately after cessation of exposure. Co-administration of varying amounts of ethyl acetate (an ester commonly found in paints, coatings and printing inks along with TMBs) lowered the blood level of inhaled 1,3,5-TMB at all exposure levels in a dose-dependent manner. However, the reduction was not statistically significant at any exposure level. The authors' conclusion was that interactions between aromatic HCs and esters can be expected because of a common pathway of biotransformation.

TMBs have been shown to cross the blood:brain barrier following inhalation exposure. Zahlsen et al. (1990) exposed male Sprague-Dawley rats (number unspecified, maximum number = 24) to 1000 ppm (4916 mg/m^3) 1,2,4-TMB 12 hours/day for 14 consecutive days in a dynamic inhalation chamber. The absorption, distribution, and accumulation of 1,2,4-TMB were assessed by measuring the concentration of 1,2,4-TMB in blood, brain, and perirenal fat immediately after the 12 hour exposure on days 1, 3, 7, 10 and 14 of the exposure period. The concentration of 1,2,4-TMB in the inhalation chambers was monitored by on-line gas chromatography, measured in 15 minute intervals. The concentration of 1,2,4-TMB in biological material was determined using head space gas chromatography. The highest concentration of 1,2,4-TMB in blood and other tissues was found after the first day of exposure. Those concentrations in blood, brain and perirenal fat were 537 $\pm$ 100 µmol/L, 998 $\pm$ 250 µmol/kg, and 49,190 $\pm$ 12,840 µmol/kg 1,2,4-TMB, respectively. For all tissues/organs, the 1,2,4-TMB concentration measured on day 1 was significantly higher (p <0.05) than those measured from days 3 to 14. The fat/brain distribution ratio for 1,2,4-TMB was 31.5 calculated at steady state. The authors suggest that the pattern of decreasing biological concentrations with time is likely a result of metabolic enzyme induction.

Lam et al. (1992) measured brain concentrations of all three TMB isomers in rats following a three-week inhalation exposure to white spirit. Male Wistar rats, 3 months of age, were exposed in inhalation chambers to 0, 400, or 800 ppm white spirit vapor 6 hr/day, 5 days/week, for 3 weeks. The concentration of the TMB isomers in the solvent was not reported. The mean whole rat brain concentration of the 1,3,5-TMB isomer (n = 5) was 0.10 $\pm$ 0.09 and 0.08 $\pm$ 0.08 mg/kg wet weight for 400 and 800 ppm white spirit, respectively. The 1,2,4-TMB and 1,2,3-TMB isomers were not detected at the 400 ppm exposure concentration. At 800 ppm white spirit (n = 5), the 1,2,4-TMB and 1,2,3-TMB mean whole brain concentrations were 0.40 $\pm$ 0.03 and 0.07 $\pm$ 0.10 mg/kg wet weight, respectively.

In male rats (4/group) exposed to concentrations of 25, 100 or 250 ppm (0, 123, 492, 1230 mg/m^3) 1,2,4-TMB for 6 hours for 4 weeks (6 hours/day, 5 d/week) in a dynamic inhalation chamber, lung and brain concentrations were similar after single and repeated exposures of similar magnitude (Swiercz et al., 2003). 1,2,4-TMB concentration levels were lower in liver at the 100 and 250 ppm exposure levels after repeated exposure than after a single exposure. Concentration levels were measured by head space gas chromatography. Much lower concentrations of 1,2,4-TMB were found in arterial blood than venous blood; the venous blood/arterial blood distribution after repeated exposure at 25, 100, and 250 ppm 1,2,4,-TMB was 1.7, 2.6 and 1.8, respectively (this may be an artifact and due to “possible mixing of arterial blood with other body fluids during decapitation of the rat”). Amongst the brain structures evaluated in exposed animals (*e.g.,* brainstem, temporal cortex, hippocampus, cerebellum), a

significantly higher concentration (p <0.05) of 1,2,4-TMB was found in brainstem. The authors stated that "The higher concentrations of pseudocumene [1,2,4-TMB] in brainstem can be associated with higher fat affinity of this structure as compared to the other structures under study."

Swiercz et al. (2016) exposed male Wistar rats (5/group) in a dynamic inhalation chamber to 25, 100, or 250 ppm (123, 492, 1229 mg/m^3)1,2,3-TMB vapors for either a single 6-hour exposure, or for 6 h/day, 5 days/week for 4 weeks duration. The highest 1,2,3-TMB levels were found in kidneys, following either single or repeated exposures. Following repeated exposure to the lowest concentration of 25 ppm, significantly higher levels of 1,2,3-TMB were found in kidneys and lung tissues compared to those after single inhalation exposure (p <0.01 for lung and p <0.001 for kidneys). Significantly lower concentrations of 1,2,3-TMB were detected in blood (at 25 and 250 ppm, p <0.01 and p <0.001, respectively) and liver tissue (at 100 and 250 ppm, p <0.05 and p<0.01, respectively) after repeated inhalation exposures, compared to the single, 6-hour exposure. The major metabolites formed were the two dimethylbenzoic acids, 2,3,-dimethylbenzoic acid and 2,6-dimethylbenzoic acid. The authors concluded that the significantly higher urinary excretion of the 2,3-dimethylbenzoic acid metabolite after repeated exposure was indicative of enzymatic induction by 1,2,3-TMB. In this study, liver induction appears to have occurred in rats somewhere between 25 and 100 ppm 1,2,3-TMB.

Other authors have similarly reported that at high exposure levels (1,2,4-TMB levels of 25 to 1000 ppm) the alkyl benzenes induce their own metabolism, increasing elimination rates. McKee et al. (2010) conducted a number of neurobehavioral tests in rats following acute exposure to 1,2,4-TMB. Male Wistar rats (8/group) were exposed via whole body inhalation for periods "up to 8 hours/day" on 3 consecutive days to 1,2,4-TMB. Animals were observed for up to 1 day post exposure. Target TMB exposure concentrations were 125, 1250 and 5000 mg/m^3 (25, 250, 1000 ppm) 1,2,4-TMB. The mean analytically determined concentrations were 128, 1255, and 4980 mg/m^3 1,2,4-TMB. 1,2,4-TMB was rapidly taken up into the blood and brain. At 2, 4, and 8 hours exposure, and including after 3 consecutive days of 8-hour exposure, brain concentrations were 2 to 3 times the corresponding blood concentrations at all exposure levels. After 3 consecutive days of exposure, concentrations of 1,2,4-TMB in the blood and brain of rats from the high exposure group (5000 mg/m^3) were approximately half the concentrations seen in animals after a single 8-hour exposure; the blood levels for the single 8-hour exposure and the 3 consecutive day's exposure were 65,000 ± 3786 and 36,167 ± 1590 ng/mL, respectively, and brain 1,2,4-TMB levels were 160,000 versus 93,333 ± 2048 ng/g for the single and consecutive day exposures, respectively. The authors stated this implied that TMB induced its own metabolism at the highest exposure concentration.

4.2.2 Oral

Following oral administration of TMBs, some marked differences in kinetics have been noted between isomers. When isomeric TMBs were administered individually to male Wistar rats (9/group) at a dose of 1.2 g/kg, approximately 59%, 37%, and 33% of the dose of 1,3,5-TMB, 1,2,4-TMB, and 1,2,3-TMB, respectively, were excreted in urine within 48 hours (Mikulski and Wiglusz, 1975). Approximately 78% of the 1,3,5-TMB was excreted as 3,5-DMHA; an additional 7.6% and 8.2% were excreted as glucuronic and sulphate conjugates. The investigators were not able to isolate any metabolites from urine following administration of 1,2,4-TMB or 1,2,3-TMB (the 48 hour time period was inadequate to assess total urinary excretion). Using kinetic data developed for the various TMB isomers (i.e. excretion half-life, the velocity constant, maximal excretion time), the authors calculated that approximately 57% of the dose of 1,2,3-TMB is excreted within 8 to 15 days.

The authors of this study reported that the primary urinary metabolite of 1,2,4-TMB was 3,4-dimethylbenzoic acid. The corresponding urinary excretion of the glycine, glucuronic, and sulphate conjugates for the 1,2,4-TMB isomer were 43.2%, 6.6%, and 12.9%, respectively, and 17.3%, 19.4%, and 19.9% for the 1,2,3-TMB isomer, respectively. The kinetic data for the excretion of conjugates were found to be similar for the 1,3,5-TMB and 1,2,4-TMB isomers whereas the half-life of excretion for the glycine and glucuronic acid conjugates of 1,2,3-TMB was four times greater. The half-life values for the sulphate conjugates were similar for all three compounds. The authors suggested that the qualitative and quantitative differences in the metabolism of TMBs may be due to differences in the rate of aromatic hydroxylation, oxidation of the methyl substituent, or conjugation.

The effects of 1,3,5-TMB exposure on liver, kidney and lung microsomal enzyme activity was investigated in rats (Pyykko, 1980). Male Sprague-Dawley rats (n = 8 to 10/group) were given 1,3,5-TMB in corn oil by gastric tube once per day for 3 successive days at a dose of 10 mmol/kg-day (1200 mg/kg-day). The control rats were given only the corn oil vehicle (n = 6 to 10/group). All rats survived through the treatment period. All animals lost weight (according to the authors, this was likely due to fasting periods during the experiments). Weight loss in the 1,3,5-TMB-treated group differed significantly (p <0.05) from controls (27.0 $\pm$ 1.9 g *vs.* 6.4 $\pm$ 1.5 g). 1,3,5-TMB significantly increased mean absolute liver weight (9.28 $\pm$ 0.25 g vs. 7.54 $\pm$ 0.28 g in controls). Lung and kidney absolute weights of the 1,3,5-TMB-treated group were decreased, but not significantly, from control animals. The effect of 1,3,5-TMB treatment on microsomal enzymes is summarized in Table 9, below. Values are mean $\pm$ standard error (SE) for 8 to 10 animals for the 1,3,5-TMB-exposed group. 1,3,5-TMB-treatment significantly increased cytochrome P-450 in liver and kidney and NADPH-cytochrome *c* reductase in liver.

(Cytochrome content in lungs was not determined). 1,3,5-TMB-treatment affected most of the cytochrome P-450 dependent monooxygenase enzymes. Significant increases were seen in liver and lung for aminopyrine demethylase and aryl hydrocarbon hydroxylase, and in liver and kidney for aniline hydroxylase. Aniline hydroxylase activities in kidney showed the most significant effect, a greater than 3-fold increase compared to control kidneys. In contrast, 1,3,5-TMB treatment significantly decreased aniline hydroxylase activity in the lungs.

Table 9. Effects of 10 mmol/kg-day 1,3,5-Trimethylbenzene by oral gavage for three days on microsomal enzyme induction in rat liver, kidney and lung (from Pyykko, 1980)

Organ	Cytochrome (CYT) content (percentage of control)			Enzyme activity (percentage of control)		
	CYT P-450	CYT b_5	NADPH-CYT-C reductase[1]	APD	AHH[2]	ANH
Liver	123 ± 4***	160 ± 4***	161 ± 9**	165 ± 7***	154 ± 7***	125 ± 8*
Kidneys	132 ± 9**	127 ± 7*	90 ± 9	93 ± 15	74 ± 11	199 ± 29**
Lungs	Not determined		102 ± 1	152 ± 17*	183 ± 13**	48 ± 3**

[+]Values in the table represent the mean ± SEM for 8 to 10 animals, expressed as percentages of the values of individual control groups in *each* treatment (6 to 10 animals).
*p<0.05; ** p<0.01; *** p<0.001 indicates values are significantly different from controls.
Means ± SEM of all the controls (14 to 29 animals) are as follows: cytochrome *P*-450 in liver, 0.616 + 0.022, and in kidneys, 0.096 ± 0.007 nmol/mg microsomal protein; cytochrome b_5, 0.247 ± 0.012 in liver and 0.078 ± 0.003 nmol/mg microsomal protein in kidneys. Control values of enzyme activities in liver, kidneys, and lungs are: NADPH-cytochrome *c* reductase 187 ± 13, 82 ± 8, and 68 ± 5 nmol of cytochrome *c* reduced/minute/mg protein, respectively; APD 9.6 ± 1.2, 0.65 ± 0.04, and 0.64 ± 0.06 nmol of formaldehyde formed/min/mg protein, respectively; AHH 357 ± 55, 20 ± 5, 16 ± 3 fluorescent units/min/mg protein, respectively; and ANH 0.627 ± 0.036, 0.022 ± 0.005, and 0.012 ± 0.001 nmol *p*-aminophenol formed/min/mg protein, respectively.
Abbreviations: AHH = aryl hydrocarbon hydrolase (CYP1A1); ANH = aniline hydroxylase; APD = aminopyrine demethylase; CYT = cytochrome; NADPH = Nicotinamide adenine dinucleotide phosphate.
[1] nmol/min-mg protein
[2] fluorescent units/min-mg protein

5. Acute Toxicity of Trimethylbenzene

5.1 Acute Toxicity to Adult Humans

Table 10 lists the controlled acute human experimental exposure studies that entail inhalation exposure to one or more of the TMB isomers. A number of these studies were described previously in the section on toxicokinetics and are, additionally, included here for information on potential toxic effects.

Table 10. Acute Human Trimethylbenzene Inhalation Chamber Studies

Study	TMB Isomer(s)	Gender (#)	Exposure	Toxic Effects
Jarnberg et al., 1996	1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB	Male (10)	2 hours to 2 or 25 ppm (10 or 123 mg/m^3) 1,2,4-TMB, or 25 ppm (123 mg/m^3) 1,3,5-TMB or 1,2,3-TMB	None reported
Jarnberg et al., 1997b	1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB	M (10)	Four 2-hr exposures to 25 ppm (123 mg/m^3) PM, 1,2,3-TMB, or 1,3,5-TMB	None reported
Jarnberg et al.,1997a	1,2,4-TMB	Male (9)	2 hour exposure to 2 ppm (10 mg/m^3)	None reported
Jarnberg et al., 1998	1,2,4-TMB	Male (9)	2 hour exposure to 2 ppm (10 mg/m^3); concomitant exposure to white spirit	None reported
Jones et al., 2006	1,3,5-TMB	M (2) F (2)	4 hour exposure to 25 ppm (123 mg/m^3)	“Little to no” sensory irritation of the eyes, nose, throat

Abbreviations: F = female; M = male; # = number of subjects; ppm = parts per million; TMB = trimethylbenzene.

In a series of inhalation studies using human volunteers exposed to approximately 25 ppm (123 mg/m^3) of 1,3,5-TMB, 1,2,3-TMB, or 1,2,4-TMB for 2 hours, no evidence of respiratory irritation, CNS toxicity or other toxic effects were reported (Jarnberg et al., 1996; Jarnberg et al., 1997a and 1997b; Jarnberg et al., 1998). These studies are described in greater detail, below.

In two short-term inhalation exposure studies (Jarnberg et al.,1996; Jarnberg et al., 1997b) ten healthy male volunteers were exposed in an inhalation chamber for 2 hours duration on four occasions while doing light physical work to 25 ppm (123 mg/m^3) 1,2,4-TMB, 1,3,5-TMB, or 1,2,3-TMB or 2 ppm 1,2,4-TMB. There was a time interval of at least 2 weeks between successive exposures. The relative respiratory uptake rate was in the range of 56% to 64%. The occurrence of acute effects was studied by means of a questionnaire. Subjects rated the degree of irritation and CNS effects using a visual analog scale. Symptoms were graded from “no effect at all” to “unbearable”. Subjective ratings were analyzed in terms of time vs. exposure condition, and well as interaction (time × substance and time × exposure level). According to the authors, no acute symptoms were reported in either study; subjects reported “no effect” or “hardly any effect or discomfort”. Statistically significant differences in smell, however, were seen at all exposure conditions in the Jarnberg et al. (1996) study (*p* values were not provided). Ratings of smell were also statistically significantly higher at the 25 ppm (123 mg/m^3) 1,2,4-TMB exposure than the 2 ppm (10 mg/m^3) exposure (*p* values were not provided by the study authors).

In a related study, Jarnberg et al. (1997a) exposed healthy male volunteers (*n* = 9, average age 36) in an inhalation chamber for 2 hours to 2 ppm (11 mg/m^3) 1,2,4-TMB while doing light physical work. The subjects did not have occupational exposure to solvents. Exhaled air, urine, and blood samples were collected at exposure onset, during, and after exposure termination. Subjects were asked to rate symptoms of irritation and CNS-related effects before, during and after exposure: irritation of eyes, nose, throat; headache, fatigue, nausea, dizziness, intoxication, difficulty breathing, and solvent odor. Symptoms were graded from “no effect at all” to “unbearable” (visual analog scale). Only toxicity results are described here. The authors stated that, “No significant irritation or central nervous system effects were detected at these exposure conditions. All mean values of rating (except those of smell) corresponded verbally to something from ‘no effect’ to ‘hardly any effect or discomfort’”. In a follow-up study which also used healthy male volunteers (*n* = 9), exposure to white spirit (3.8% w/w 1,2,4-TMB, corresponding to 11.3 mg/m^3 or 2 ppm) under the same exposure conditions did not result in any toxic effects (Jarnberg et al., 1998).

Jones et al. (2006) conducted a chamber and occupational TMB toxicokinetic study using human volunteers for the chamber study, and printing workers for the

occupational study. For the chamber study, the volunteers (two males and two females) were exposed in a laboratory-controlled atmosphere to 25 ppm (123 mg/m^3) 1,3,5-TMB for 4 hours. Urine, blood, and expired air samples were collected before, during, and after exposure. Before and during exposure, volunteers completed a detailed questionnaire for recording subjective experiences of eye, nose, or throat irritation. Only the sensory irritation findings are reported here. According to the study authors, "Almost no sensory irritation [of the eyes, nose and throat] was reported by the volunteers". No further details regarding the sensory irritation effects were provided. Solvent odor was apparent.

For the occupational hygiene portion of the study (study details described in Section 4.1), there was no indication from the study description that the workers were surveyed for health effects.

5.2 Acute Toxicity to Infants and Children

Exposure of children to TMBs is a concern due to anatomical and other developmental differences that make them more susceptible to inhaled toxicants (*e.g.,* higher rate of respiration than adults) (OEHHA, 2008). There are no acute TMB toxicity studies pertaining specifically to infants and/or children.

5.3 Acute Toxicity to Experimental Animals

Most of the acute TMB studies in animals are inhalation studies. There are, in addition, a few oral studies. Acute exposure to TMB causes primarily respiratory and neurotoxic effects. Table 11 provides a summary of the acute inhalation toxicity studies in animals; the inhalation exposure studies largely investigated neurobehavioral endpoints.

There are some notable differences in toxicity amongst the three TMB isomers. In many instances only one, or sometimes two of the three isomers, were evaluated in a toxicity study. Therefore, it is not known what effect(s) the other isomers may have had under the conditions of a particular study. There are some differences in metabolism between the isomers, and this can result in differences in toxicity.

Koch Industries Inc., (1995) exposed male and female Sprague-Dawley rats (n = 10/group/sex) via gavage to 60, 150, or 600 mg/kg-day 1,3,5-TMB (20, 50 and 200 ppm), consecutively, for 14 days. There were 10 vehicle controls/sex. Data were analyzed by Analysis of Variance (ANOVA), followed by Dunnett's multiple-range comparison using Systat software (Systat, Inc., Evanston, IL, version 5). A significant increase in relative liver weights was seen in mid- and high-dose female rats ($p \leq 0.05$) compared to vehicle controls. In addition, increased cholesterol levels were seen in mid- and high-dose females. Mean monocyte counts were significantly increased in mid-dose

females. In male rats, there were a number of significant effects on clinical chemistry parameters at the lowest exposure dose. Statistically significantly affected parameters in treated animals compared to vehicle controls included: creatine kinase (CK), increased in 60 mg/kg-day males; urea nitrogen (BUN), decreased in 60 and 150 mg/kg-day males; albumin (ALB), decreased in 60 mg/kg-day males. According to the authors, all changes in clinical chemistry parameters, except for cholesterol, were not considered treatment related (largely because they were not seen in high-dose males). In high-dose males, treatment-related effects included increased white blood cell counts, with corresponding increases in neutrophils and lymphocytes, statistically significant increases in relative and absolute liver weights, and relative adrenal weights ($p \leq 0.05$). Centrilobular hepatocyte hypertrophy was observed in 10/10 males and 3/10 females in the 600 mg/kg-day group at termination of treatment.

5.3.1 Mortality Studies

Acute 4-hour inhalation LC_{50} values of 3662 ppm (18,000 mg/m^3) and 4882 ppm (24,000 mg/m^3) have been reported in rats for the 1,2,4-TMB and 1,3,5-TMB isomers, respectively (Firth, 2008). Differences in median oral lethal dose (LD_{50}) toxicity between the TMB isomers have been observed: 1,2,3-TMB appears to be the most acutely toxic, and 1,2,4-TMB the least *(i.e.,* 1,2,3-TMB>1,3,5-TMB>1,2,4-TMB) (Janik-Spiechowicz et al., 1998). Male/female mice (4 mice/dose group) were injected (single intraperitoneal injection) with 5 different doses of each TMB isomer. The LD_{50} values were found to be 3670 mg/kg (male) and 2700 mg/kg (female) for 1,2,3-TMB; 4500 mg/kg (male) and 3700 mg/kg (female) for 1,3,5-TMB; and 5000 mg/kg (male) and 4100 mg/kg (female) for 1,2,4-TMB. Age, weight, and breed of the mice were not provided but, across all three TMB isomers, females appeared more sensitive than males in this lethality assay.

Table 11. Acute and Subacute Trimethylbenzene Inhalation Toxicity Studies in Animals

Study	Sex/Species	TMB Isomer(s)	Exposure	Results
Wiglusz et al., 1975a	M/ Wistar Rats	1,3,5-TMB	6 hour inhalation, 0.3, 1.5, 3.0 mg/L (300, 1500, 3000 mg/m^3; 61, 305, 610 ppm)	Significant ↑ in serum alkaline phosphatase levels at 3.0 mg/L (3000 mg/m^3)
Wiglusz et al., 1975b	M/ Wistar Rats	1,3,5-TMB	6 hour inhalation, 0, 1.5, 3.0 or 6.0 mg/L (0, 1500, 3000, and 6000 mg/m^3)	Significant ↑ in PSNG with corresponding ↓ in LP at highest concentration, 6.0 mg/L (6000 mg/m^3)
Frantik et al., 1974	M/ Albino SPF Rats and F/ H strain mice	1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB	Inhalation; varying concentrations for either 2 (mice) or 4 (rats) hours	Neurotoxicity: for rats, doses evoking a 37% seizure discharge depression were 440, 489, and 636 ppm (2163, 2404, and 3126 mg/m^3) for 1,3,5-TMB, 1,2,3-TMB, and 1,2,4-TMB, respectively; in mice, doses evoking a 30% seizure discharge depression were 611, 416, 391 ppm (3000, 2045, 1922 mg/m^3) for 1,3,5-TMB, 1,2,3-TMB, and 1,2,4-TMB, respectively

Abbreviations: F = female; LP = lymphocytes percentage; M = male; mmol = millimole; ppm = parts per million; PSNG = percentage of segmented neutrophilic granulocytes; TMB = trimethylbenzene; ↑ = increase; ↓ = decrease.

Table 11. Acute and Subacute Trimethylbenzene Inhalation Toxicity Studies in Animals (continued)

Study	Sex/Species	TMB Isomer(s)	Exposure	Results
Korsak et al., 1995	M/ Balb/C Mice, Wistar Rats	1,2,4-TMB	Rats: 4-hours inhalation, 250–2000 ppm (1230–9840 mg/m^3)[a]; Mice:12 minutes to same range of concentrations	Neurotoxicity in rats (rotarod performance, pain sensitivity)/ Respiratory Irritation in mice (↓ respiratory rate)
Korsak and Rydzynski, 1996	M/ Wistar Rats	1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB	4-hour inhalation, 250–2000 ppm (1230–9840 mg/m^3)[a]	Neurotoxicity (rotarod performance, pain sensitivity)
Korsak et al., 1997	M/ Balb/C Mice	1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB	6-minute inhalation to varying air concentrations	Respiratory Irritation (↓respiratory rate)
McKee et al., 2010	M/ Wistar Rats	1,2,4-TMB	up to 8-hour inhalation, 0, 125, 1250 or 5000 mg/m^3 (25, 250, or 1000 ppm) on 3 consecutive days (tested after each daily exposure)	Neurotoxicity (↓ forelimb grip strength at 125 mg/m^3, visual discrimination latencies at ≥ 5000 mg/m^3 after 1st exposure; ↓ motor activity at 5000 mg/m^3 after 3rd exposure)

Abbreviations: F = female; LP = lymphocytes percentage; M = male; mmol = millimole; ppm = parts per million; PSNG = percentage of segmented neutrophilic granulocytes; TMB = trimethylbenzene; ↓ = statistically significant decrease relative to controls.

[a] Individual concentrations not specified.

5.3.2 Inhalation Studies in Animals

Wiglusz et al. (1975a) examined the effect of TMB toxicity on blood serum enzymes (elevated liver enzymes are indicative of inflammation or damage to liver cells). Male Wistar rats (*n* = 6/group) were exposed via inhalation to a concentration of 0.3, 1.5, 3.0 mg/L (300, 1500, 3000 mg/m^3; 61, 305, 610 ppm) 1,3,5-TMB for a single 6-hour exposure. There were 6 controls. Blood samples were collected from the caudal vein 3 days prior to exposure and at 2, 7, 14 and 28 days post-exposure. A significant increase in serum alkaline phosphatase (AP) levels ($p < 0.05$) was seen at the highest concentration of 3.0 mg/L (3000 mg/m^3). The value, 58.7 mg/l, was 57% higher than the baseline value of 37.3 mg/l at 7 days post-exposure (the authors noted that the study does not rule out the possibility of the appearance of the maximum value between the 2nd and 7th day post-exposure window). After 14 days, AP enzyme activity was only slightly higher than initial values. No significant changes in aspartate amino transferase (GOT) or alanine amino transferase (GPT) enzyme activity at 2, 7, 14, and 28 days post-exposure was found. No glutamate dehydrogenase (GLDH) activity was detected in blood serum.

In a related study, Wiglusz et al. (1975b) evaluated the hematological/immunological effects of 1,3,5-TMB on the peripheral blood of rats. Only the acute portion of the study is described here. Male Wistar rats were exposed (*n* = 5-8/concentration) to 1.5, 3.0 or 6.0 mg/L (1500, 3000, and 6000 mg/m^3, respectively) of 1,3,5-TMB in an inhalation chamber for 6 hours. Blood samples were collected from control and exposed animals 3 days prior to the start of the experiments, and then on days 1, 7, 14 and 28 after completion of the exposure. No changes in erythrocyte or leukocyte counts were seen in any 1,3,5-TMB-exposed groups. Exposed rats showed increases in the percentage of segmented neutrophilic granulocytes (PSNG) in relation to dose (*i.e.,* neutrophilia). A significant increase ($p < 0.02$) in PSNG, with a corresponding decrease in lymphocytes percentage (LP), was seen in rats exposed to the highest 1,3,5-TMB concentration, 6.0 mg/L (6000 mg/m^3). These changes usually appeared on the first day of exposure and remained for the period of 7 to 14 days. Neutrophils are an essential part of the immune system and one of the first responders of inflammatory cells to migrate to the site of infection (as a result of bacterial or environmental exposure). They express and release cytokines, which in turn amplify inflammatory reactions by several other cell types. There are significant differences between neonatal and adult neutrophils (Lawrence et al., 2018).

All three TMB isomers were evaluated to determine their ability to induce seizure discharge depression in an acute neurotoxicity inhalation study by Frantik et al. (1994). In rodents, inhibition of the generation, propagation, and maintenance of the seizure discharge is considered to be a sensitive measure of neurotoxicity. Male albino SPF

rats, 6 months to 1 year of age (*n* = 4/group) and female H strain mice, 2 to 4 months of age (*n* = 16/group) were exposed in inhalation chambers to varying air concentrations of the TMB isomers for either 2 (mice) or 4 (rats) hours. The authors did not specifically report the exposure concentrations used for each TMB. Ranges of effective air concentrations were estimated in preliminary experiments; three concentrations were selected in the linear part of the effective-concentration curve (ECC, btw 25% to 75% of the maximum effect).

Each TMB isomer was tested in at least 2 independent experiments on two samples of animals (*i.e.,* most animals went through several exposures to various solvent concentrations; the intervals between exposures were a minimum of 3 weeks). All animals were given three control tests at weekly intervals before the first exposure. Following removal from the inhalation chamber, a short electrical impulse (0.2 seconds, 50 Hz, 180 V in rats and 90 V in mice) was applied through ear electrodes to elicit a seizure. The duration of tonic extension (shortening the duration of seizures) of hindlimbs was investigated in rats exposed to each TMB isomer singly for 4 hours. The velocity of tonic extension (slowing the propagation of a seizure) was determined in female mice exposed for 2 hours. According to the authors, "the duration of tonic extension of hindlimbs in the rats and the velocity of tonic extension in mice were the most sensitive and reproducible response measures". Inhibition of propagation and maintenance of the electrically-evoked seizure discharge was used as the criterion for the acute neurotoxic effect. All data were analyzed using linear regression analysis. Isoeffective air concentrations, corresponding to the critical level of effect (*i.e.,* the effect in the lower third of the *linear* part of the dose-response function was chosen as the critical level), were interpolated on all regression lines as 37% and 30% of the maximum effect in rat and mice, respectively. The study authors considered this level of effect (approximately one third of the maximum possible effect) physiologically significant. Per the authors, high correlation between ECC values in rats and mice support a broader biological significance of the estimates.

For male rats, the air concentrations evoking a 37% seizure discharge depression were 440, 489, and 636 ppm (2163, 2404, and 3126 mg/m^3) for 1,3,5-TMB, 1,2,3-TMB, and 1,2,4-TMB, respectively. In female mice, the air concentrations evoking a 30% seizure discharge depression were 611, 416, 391 ppm (3000, 2045, 1922 mg/m^3) for 1,3,5-TMB, 1,2,3-TMB, and 1,2,4-TMB, respectively. According to the authors, the chemically-induced changes in seizure activity are "perhaps a better model of chemical effects on complex psychological functions than other behavioral endpoints".

Korsak et al. (1995) evaluated the toxic effects of acute inhalation exposure to 1,2,4-TMB in experimental animals (mice and rats). Male Wistar rats (*n*=10/group) were exposed in a dynamic inhalation chamber for 4 hours to concentrations of 250 – 2000

ppm (1230 to 9840 mg/m^3) 1,2,4-TMB. Individual concentrations were not specified. Male Balb/C mice (*n* = 8 to 10/group) were exposed to "various concentrations of 1,2,4-TMB" (ranging from 250 to 2000 ppm) for 6 minutes to determine the concentration depressing the respiratory rate of exposed animals to 50% of the pre-exposure respiratory rate (RD_{50}).

Mice were placed in a plethysmograph attached to a small dynamic inhalation chamber. The respiratory rate was recorded continuously before the exposure, during the 6 minute exposure, and for 6 minutes following termination of exposure. In rats, rotarod performance and pain sensitivity behavior were tested before exposure and immediately after termination of exposure. Probit analysis was used to determine the median effective concentration (EC_{50}) in the rotarod performance test. The EC_{50} is the concentration required to have a biological effect in 50% of the animals in a test. The concentration depressing the respiratory rate in mice, and the latency of paw-lick response (pain sensitivity behavior) to 50% over control (EC_{50}) were calculated from least squares regression lines of concentration-effect relationship.

In rats, 1,2,4-TMB caused a concentration-dependent disturbance in rotarod performance. Results were expressed as the probit of failures × concentration. The rotarod performance behavior disturbance EC value with 95% confidence intervals (CI) was 4693 mg/m^3 (3891 to 5493 mg/m^3). This is equivalent to 954 ppm 1,2,4-TMB (791 to 1113 ppm). 1,2,4-TMB decreased sensitivity to pain (measured latency of the paw-lick response) in a concentration–dependent manner. Latency results were expressed as a percentage, the mean value of separate measurements of latency over the control (*n* = 10). The EC value with its 95% CI was 5682 mg/m^3 (2715 to 7596 mg/m^3) 1,2,4-TMB. This was equivalent to 1115 ppm (552 to 1544 ppm) 1,2,4-TMB. 1,2,4-TMB caused a concentration-dependent decrease in respiratory rate in mice. The maximum decrease was observed in the first 1 to 2 minutes of exposure. After an exposure recovery period of 6 minutes duration, the respiratory rate in the mice recovered. The RD_{50} with its 95% CI was 578 ppm (311 to 793) 1,2,4-TMB. The authors stated that the RD rate in mice correlates well with the extent of eye and respiratory irritation seen in humans.

In a subsequent study, Korsak et al. (1997) evaluated the acute respiratory toxicity in mice of the other two TMB isomers, 1,2,3-TMB and 1,3,5-TMB. Adult male Balb/C mice (8 to 10/group) were exposed for 4 hours in a dynamic inhalation chamber. The RD_{50} concentrations were 519 and 541 ppm (2551 and 2659 mg/m^3) for 1,3,5-TMB and 1,2,3-TMB, respectively. This can be compared to the RD_{50} of 578 ppm (2841 mg/m^3) for 1,2,4-TMB reported in Korsak et al. (1995).

Korsak and Rydzynski (1996) evaluated both acute and subchronic exposure to the three TMB isomers in rats; only the acute findings are discussed here. In the acute study, adult male Wistar rats (*n* = 10/group) were exposed to 250 to 2000 ppm (1230 to 9830 mg/m^3) 1,3,5-TMB, 1,2,3-TMB, or 1,2,4-TMB for 4 hours in an inhalation chamber.

Neurotoxicity was evaluated via the rotarod performance test and the hot plate behavior test (the latter measures a decrease in sensitivity to pain). All rats in all experiments survived the exposures. Clinical observations were unremarkable. Exposure to TMB isomers caused a concentration-dependent disturbance in rotarod performance and pain sensitivity, but because control animal data for the acute experiments were not shown, it was not possible to determine if the changes were statistically significant. For rotarod performance, EC_{50} values were 954 ppm (4693 mg/m^3), 963 ppm (4738 mg/m^3) and 768 ppm 3779 mg/m^3) for 1,2,4-TMB, 1,3,5-TMB, and 1,2,3-TMB, respectively. EC_{50} values for the hot-plate test were 1155 ppm 5682 mg/m^3), 1212 ppm (5963 mg/m^3), and 848 ppm (4172 mg/m^3) for 1,2,4-TMB, 1,3,5-TMB, and 1,2,3-TMB, respectively. The neurotoxic effects of 1,3,5-TMB and 1,2,4-TMB were similar, and the effect of 1,2,3-TMB was more pronounced; 1,2,3-TMB elicited the lowest EC_{50} values in both neurotoxicity tests.

In a related study (Korsak et al.,1997), 1,3,5-TMB and 1,2,4-TMB appeared to have comparable airway irritation activity in mice. The exposure concentration producing a 50% reduction in respiratory rate (RD_{50}) was 519 and 578 ppm (2551 and 2841 mg/m^3), respectively. The 1,2,3-TMB isomer was not evaluated in this study.

McKee et al. (2010) conducted a number of neurobehavioral tests in rats following acute exposure to 1,2,4-TMB. Male Wistar rats (8/group) were exposed via whole body inhalation for periods “up to 8 hours/day” on 3 consecutive days. Animals were observed for up to 1 day post exposure. Target TMB exposure concentrations were 25, 250, and 1000 ppm (125, 1250 and 5000 mg/m^3) 1,2,4-TMB. The mean analytically determined concentrations were 128, 1255, and 4980 mg/m^3 1,2,4-TMB. Probability values of $p < 0.05$ were considered significant. Continuous variables from the functional observational battery were analyzed using repeated measures of ANOVA. ANOVA was performed at each test time point if a significant effect of treatment, or a significant treatment-by-time interaction, was indicated. Group comparisons were made using Dunnett’s multiple comparison tests. No data were reported or shown for the second day’s experimental exposure.

Prior to visual discrimination performance testing, rats were evaluated for 2-choice visual discrimination performance. The testing apparatus consisted of a number of operant chambers equipped with 2 levers, 2 stimulus lights, and a water dipper for delivering water reinforcement, connected to recording and programming equipment.

Water-deprived rats were trained to obtain water reinforcements and to press a lever. Rats subsequently received four weeks of training, 5 days/week, on a discrete-trial light/dark visual discrimination task to stabilize baseline response (*i.e.,* if the rat presses the illuminated lever under the stimulus light, it gets a water reward). A given trial remained in effect until the correct lever was pressed; trials were separated by an inter-trial interval of 10 seconds. Rats were tested on the day prior to the first exposure, and on each day of exposure immediately after the exposure period.

Significant increases in drink response latency, latency > 6 seconds, trial response latency, and discrimination ratio were seen after a single 8-hr exposure in the 5000 mg/m^3 exposure group. Drink response latency was also statistically significant in the 1250 mg/m^3 exposure group compared to controls following the first 8-hour exposure. However, the 1250 mg/m^3 exposure group rats also showed a statistically significant increase in drink response latency compared to controls when tested one day prior to exposure. Because of this observation, the authors considered the increase in drink response latency shown in the 1250 mg/m^3 exposure group after the first 8-hour exposure to not be treatment-related. For all these effects, elucidated in Table 12, there was a relationship (*e.g.,* reduction in movement, latency) to exposure level. Significantly reduced latencies in neurobehavioral tests have been seen in several other studies following exposure to TMB (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997; Wiaderna et al., 2002).

Also in this study, a significantly reduced mean grip strength was observed in the low exposure group (only) after the first 8-hr exposure period (p <0.05). The authors state that this finding is not treatment related as there was no dose-response observed. A statistically significant increase (p <0.05) in forelimb grip strength was seen following the third exposure in the high exposure group. The authors believed that it was implausible that TMB exposure would increase forelimb grip strength, and therefore, this response was spurious. Significant reductions in motor activity (*e.g.,* total distance traveled, number of movements) were seen at the highest exposure concentration following the third exposure. The data show a clear trend in reduction of movements and distance traveled at each exposure concentration from the first through the third exposures. There were some treatment-related effects on the visual discrimination performance tests. These data are summarized in Table 12.

Table 12. Treatment-Related Neurobehavioral Test Results in Rats Following Inhalation Exposure to 1,2,4-Trimethylbenzene (from McKee et al., 2010)

Visual Discrimination Test (concentration in mg/m^3)	One Day Pre-Exposure	First 8–Hour Exposure	Third 8-Hour Exposure	One Day Post-Exposure
Discrimination Ratio[1]				
Control	0.81 ± 0.84	0.86 ± 0.02	0.89 ± 0.02	0.87 ± 0.03
125	0.84 ± 0.03	0.91 ± 0.03	0.88 ± 0.03	0.89 ± 0.03
1250	0.83 ± 0.02	0.91 ± 0.01	0.94 ± 0.01	0.92 ± 0.02
5000	0.83 ± 0.03	**0.95 ± 0.01**	0.95 ± 0.02	0.88 ± 0.03
Repetitive Inter-Trial Responses[2]				
Control	3.63 ± 1.02	6.13 ± 1.73	7.25 ± 1.24	6.63 ± 1.94
125	5.88 ± 1.33	3.88 ± 1.22	3.25 ± 0.88	2.88 ± 0.83
1250	7.25 ± 1.93	5.63 ± 1.97	**2.25 ± 1.52**	5.13 ± 1.54
5000	3.25 ± 1.35	8.38 ± 2.50	**1.63 ± 0.98**	2.63 ± 0.68
Trial Response Latency[3]				
Control	1.83 ± 0.18	1.70 ± 0.18	1.91 ± 0.23	1.68 ± 0.16
125	2.25 ± 0.55	2.38 ± 0.43	2.69 ± 0.69	2.70 ± 0.60
1250	2.06 ± 0.40	2.52 ± 0.40	2.75 ± 0.94	2.18 ± 0.73
5000	2.28 ± 0.43	**3.91 ± 0.73**	1.82 ± 0.13	1.45 ± 0.06
Latency > 6 Seconds[4]				
Control	3.38 ± 0.71	3.88 ± 0.58	4.25 ± 0.98	2.13 ± 0.67
125	5.38 ± 1.48	5.00 ± 1.69	5.63 ± 2.44	6.00 ± 1.68
1250	4.63 ± 1.15	6.00 ± 1.34	5.63 ± 1.92	3.38 ± 1.40
5000	4.00 ± 1.05	**10.63 ± 1.80**	3.13 ± 0.61	1.88 ± 0.35
Drink Response Latency[5]				
Control	0.29 ± 0.01	0.26 ± 0.01	0.30 ± 0.02	0.27 ± 0.01
125	0.32 ± 0.02	0.30 ± 0.02	0.32 ± 0.03	0.34 ± 0.03
1250	**0.38 ± 0.03**	**0.43 ± 0.03**	0.37 ± 0.02	0.36 ± 0.03
5000	0.33 ± 0.02	**0.49 ± 0.03**	0.34 ± 0.03	0.30 ± 0.02

n = 8 for all groups. Bolded values indicate statistical significance at the $p < 0.05$ level. Data sets are included if they show a clear observed dose-response in reduction of movement/distance traveled or latencies in the first or third 8-hour exposure. Abbreviation: SD = standard deviation.

[1] number of *correct* trial responses divided by the number of trial responses.

[2] total number of inter-trial intervals (ITI) responses following an initial ITI response.

[3] the latency (seconds) to make a correct trial response.

[4] the number of responses taking more than 6 seconds.

[5] the mean latency (seconds) to obtain reinforcements.

5.3.3 Oral Studies in Animals

Neurotoxic effects have also been observed following acute oral exposure to TMBs. Tomas et al. (1999a) measured changes in electrocortical arousal following acute oral exposure to the 3 TMB isomers in rats. Male WAG/Rij rats (*n*= 18/group) had electroencephalographic (EEG) recording electrodes implanted surgically 14 days prior to the start of the experiment in the fronto-parietal cortex. The rats were exposed to 0, 0.002, 0.008, or 0.032 mol/kg (240, 960, 3800 mg/kg) 1,2,3-TMB, 1,3,5-TMB or 1,2,4-TMB via gavage (6 rats/group). For each solvent, at all doses applied, the number and duration of high-voltage spindles (HVS) episodes decreased. (HVS represent bursts in spike-wave discharges). 1,2,3-TMB was more potent than 1,2,4-TMB or 1,3,5-TMB in the inhibition of HVS activity. At the highest doses, 0.008 and 0.032 mol/kg (960 and 3800 mg/kg), 1,2,3-TMB completely eliminated HVS activity. 1,2,4-TMB had the least marked effect on HVS activity. The efficacy of 1,3,5-TMB and 1,2,3-TMB was similar. The authors stated that, “the EEG records support a hypothesis that the solvents examined exert a depressive effect on CNS functions”.

Tomas et al. (1999b) also evaluated the acute effects of the three TMB isomers on spontaneous locomotor activity as assessed using the open-field test. Male WAG/Rij strain rats were exposed by gavage to 1,2,3-TMB, 1,2,4-TMB and 1,3,5-TMB at doses of 0, 0.008, 0.016, and 0.032 mol/kg (961,1923, and 3846 mg/kg, respectively) (*n* = 10 and 30 for controls and TMB treatment groups, respectively). Of the 3 TMB isomers, only 1,3,5-TMB caused a stimulating effect on locomotor activity at the two highest doses, 0.016 and 0.032 mol/kg. Other toxic effects were also noted. At the two lowest doses, moderate piloerection, and enhanced locomotor activity were seen; this behavior was dose-related. Rats given TMB at a dose of 0.032 mol/kg showed an increase in mobility 15 minutes following exposure, and their gait was disturbed (paresis of the hind legs). Rats at this dose also showed tachypnea, tremor, piloerection, and blood contaminated secretion from the upper airways, especially in the 1,2,3-TMB and 1,2,4-TMB groups. Ninety minutes post-exposure, the rats were motionless. Mortality was noted in all high-dose TMB treatment groups 24 hours post-exposure (3 dead animals in the 1,2,4-TMB group, 4 in the 1,2,3-TMB group, and 2 in the 1,3,5-TMB group). No mortality was noted in the low and mid-dose groups (observed up to 5 days post exposure).

Myhre and Fonnum (2001) determined that 1,2,4-TMB stimulates the formation of free radical reactive oxygen species (ROS) and reactive nitrogen species (RNS) in rat brain synaptosomes. Male Wistar rats were used as a source of synaptosomes. The rat brain synaptosome fraction was exposed to different concentrations of 1,2,4-TMB (320, 640, and 1280 µM), and the production of ROS and RNS was measured as the formation of 2’,7’-dichlorofluorescein (DCF) from non-fluorescent H_2DCF. The concentrations of

1,2,4-TMB chosen for the study were comparable to rat brain 1,2,4-TMB concentrations after inhalation of white spirit. TMB increased fluorescence in a concentration-dependent manner and there was a statistically significant difference between the controls and 1,2,4-TMB at all three concentrations. The highest concentration,1280 µM 1,2,4-TMB, showed the strongest stimulatory effect on ROS/RNS production (approximately 150% of control).

5.3.4 Dermal Studies in Animals

Several of the TMB isomers, 1,3,5-TMB and 1,2,4-TMB, have been shown to induce thymic stromal lymphopoietin (TSLP). TSLP is a cytokine similar to interleukin-7 which is produced by epithelial cells and mast cells, stimulates Th2-type immune responses, and plays a role in the initiation of allergic dermal inflammation in mice (Satou et al., 2012). A number of organic solvents, including 1,3,5-TMB and 1,2,4-TMB, were painted (separately) on the earlobes of male BALB/c mice (20 µl each of a 0.04 µg/µl TPA solution). The earlobe tissue was excised 24 h after the painting and the level of TSLP in the tissue homogenate was determined by enzyme-linked immunosorbent assay (ELISA). Both 1,3,5-TMB and 1,2,4-TMB significantly induced the production of TSLP. The mean level of TSLP $\pm$ SEM (n = 6 to 10 mice) was 0 $\pm$ 74, 1531 $\pm$ 184 (p <0.01), and 2178 $\pm$ 279 (p <0.01) pg/ml for the no solvent-exposed, 1,3,5-TMB, and 1,2,4-TMB groups, respectively. Thus, in this study, the 1,2,4-TMB isomer elicited a greater cytokine response than 1,3,5-TMB. Because TSLP production has been shown to be increased in patients with asthma, atopic dermatitis, and allergic rhinitis, the authors suggest that inhalation of these solvents may also induce TSLP in respiratory tissue as well.

6. Chronic Toxicity of Trimethylbenzene

6.1 Chronic Toxicity to Adult Humans

There is little information on the chronic toxic effects of TMBs in humans overall. OEHHA has not found either human controlled studies or child-specific toxicity data in the TMB scientific literature. In general, the occupational studies suffer from a lack of good exposure data and are confounded by exposure to multiple organic solvents. In an occupational study translated by OEHHA from German to English, CNS effects, including nervousness, anxiety, tension, as well as anemia and bronchitis, were found in a significant number of male workers (n = 27) who were exposed over several years to a paint thinner containing more than 50% 1,2,4-TMB, 30+ % 1,3,5-TMB, and traces of 1,2,3-TMB (Battig et al., 1956). The presence of benzene could not be excluded. Examination of the peripheral blood showed a tendency toward hyperchromic anemia,

and abnormal blood coagulation. Airborne concentrations of hydrocarbon vapors in the workplace ranged from 10 to 60 ppm (50 to 295 mg/m^3).

A number of other studies that evaluated low-level, prolonged exposure to white spirit in painters have reported behavioral and physiological changes (Arlien-Sobog et al., 1992; Mikkelsen et al., 1998; Triebig et al., 1992). The toxic effects of white spirit are hypothesized to depend on aromatic compounds (Myhre and Fonnum, 2001). In most of these studies, the percentage of TMB in the solvent mixture is not specified.

In one case study, a 55-year old female worked with liquid scintillation counting solutions in a laboratory for 2 ½ years (Kenndler et al., 1989). 1,2,4-TMB is often used as a solvent for liquid scintillation counting solutions. A serum sample taken 2 hours after handling of the scintillation cocktail showed the presence of 1,2,4-TMB; the 1,2,4-TMB serum concentration was 200 ppb. Twenty-four hours post-exposure, the 1,2,4-TMB concentration in the serum sample was below the detection limit of 10 ppb. According to the study authors, the patient experienced “euphoric calmness after handling the solutions, and then agony two or three days later, especially in the morning. Additionally, she felt lassitude and stiffness in the back. Liver toxicity, chronic gastritis, and conjunctivitis occurred. The EEG was “diffusely abnormal”. The concentration of the solvent vapor in the workplace air was not determined.

Sulkowski et al. (2002) evaluated the effects of occupational exposure to a mixture of solvents, including the three TMB isomers, on the inner ear. The study consisted of 61 men who worked in a factory in Poland that produced paints and varnishes. The control group consisted of 40 non-exposed healthy male workers in the same factory. Exposed subjects had a mean age of 39.8 ± 11.2 years (range 22 to 58); they were selected following a survey questionnaire and otolaryngological examinations. Those with prior ear pathologies and/or head injuries, diabetes, hypertension, or alcohol abuse were excluded from the study. Duration of employment and exposure to the solvent mixture in the study group ranged from 2 to 34 years (mean 15.8 ± 9.1). The control group mean age was 39.2 ± 10.5 (range 25 to 56 years) and they had no exposure to solvents. Individual dosimeters were used for environmental sampling, and biological monitoring included blood and urine samples. Several different solvents were identified in the breathing zone of the workers: 1,2,4-TMB, 1,3,5-TMB, 1,2,3-TMB, xylene, styrene, and toluene. TMB isomers made up approximately 42% of the solvent mixture. Measured levels of noise associated with the production processes in the factory were low, below the permissible hygiene standard of 85 decibels (dBA).

Exposed subjects were divided into three categories of cumulative exposure: ≤10, 10 to 20, and > 20; categories represent the product of exposure duration (in years) multiplied by total exposure rate. Biological monitoring involved analysis of blood levels and

urinary metabolites (gas chromatography). Blood samples were collected within 15 to 20 minutes after working shifts and urine samples were taken during the last 4 hours of the shift. Clinical examinations were carried out using a mobile audiological vehicle. Audiological tests consisted of air and bone pure tone audiometry, acoustic reflex threshold measurement, and otoacoustic emissions (transiently-evoked and distortion product optoacoustic emissions). Electronystagmographic testing included a battery of tests: saccadic and eye-tracking test, spontaneous and positional nystagmus optokinetic tests, and rotary and bithermal caloric test. Statistical analysis comprised the Student t-test, calculation of means, and linear regression analysis.

The results of biological monitoring showed that blood concentrations of 1,2,4- and 1,2,3-TMB ranged from 0.8 – 53.12 and 0.6 – 48.24 μg/dm^3, respectively, in exposed subjects. Electronystagmographic tests showed a significant prevalence of vestibular disorders of mild and advanced degree in as many as 47.5% of the workers, versus 5% of controls. The authors stated this represented a significant prevalence of vestibular abnormalities but did not provide *p* values for the significance. This was accompanied by high frequency (above 1 kHz) sensorineural hearing loss with reduced amplitudes of optoacoustic emissions in 42% of exposed subjects, versus 3% of controls. The authors found that frequency and progression of the pathologies closely corresponded with cumulative dose of exposure, defined as exposure duration (years) × (calculated) exposure rate for the solvent mixture; the higher the dose, the more lowered amplitudes and the highest thresholds were observed. The authors stated that complaints of vertigo were reported by 26.1 percent of the study subjects but did not report vertigo incidence data for the control group. Per the study authors, the most significant relationships between audiological findings (*i.e.,* distortion product otoacoustic emission amplitudes) and exposure, “were found in the subjects’ breathing zone, in which trimethylbenzene isomers predominated as the main constituents of the solvent mixture”. The authors concluded that, “the exposure to trimethylbenzene isomers…contributed most significantly to the development of clinically detectable inner ear disorders in the workers…”.

One epidemiological study that examined the relationship between indoor air pollution and respiratory health in France reported that exposure to 1,2,4-TMB is significantly associated with asthma (OR = 2.10; 95% CI:1.21–3.65) (Billionnet et al., 2011). Data were collected from a self-administered survey that was carried out by the Indoor Air Quality Observatory between October 2003 and December 2005. A representative cross-sectional population-based sample of residences was drawn from an entire sample of all principal residences in mainland France (24 million). Municipalities with more than 100,000 residences were selected for inclusion; the final sample was composed of 567 dwellings, comprising 1,612 individuals > 15 years of age, distributed among 74 municipalities over 19 regions. Surveys consisted of standardized

questionnaires and air quality measurements taken over a 1-week period. Questions were derived from the European Community Respiratory Health Survey. A weekly diary about activities and time spent at home was completed by the inhabitants. The survey focused on asthma and rhinitis and was completed by inhabitants > 15 years of age.

The air pollutants measured were selected by a panel of experts on the basis of their potential impact on air quality, toxicity, and ubiquitous nature. The pollutants included 20 volatile organic compounds, 4 common allergens, carbon monoxide, carbon dioxide, relative humidity, particulate matter (PM), and radon. VOCs were measured using passive samplers in the bedroom of the reference person of the household. Adsorbed VOCs were extracted through thermodesorption and analyzed using gas chromatography and/or mass spectrometry.

Selected confounders were gender, age, smoking habit, relative humidity, time of survey, pets, mold, highest educational level among household, and outdoor sources of pollution (*e.g.,* highways, rail, airports, industrial and sewage treatment plants within 500 meter radius of dwelling). Correlations between pollutants were checked with Spearman's rank correlation coefficient. Relationships between air pollutants and health outcomes were analyzed using the generalized estimating equation approach with exchangeable covariance matrix to adjust for correlation within persons living in the same residence (who tend to exhibit the same habits, etc.). Exposure to each VOC was categorized as a binary variable, as low versus high exposure, using the 3rd quartile value of the distribution as a threshold value. To take into account multiple exposure to pollutants and correlation between VOCs, a global VOC score variable was created. The global VOC score was equivalent to the number of air pollutants in each dwelling for which elevated (> 3rd quartile) concentrations were found. All variables associated with asthma or rhinitis with a p<0.3 were retained. Age, sex, and smoking status were included in all models. Survey respondents were 47.9% male; 52.1% female. The median age of the respondents was 44 years (range 15-89). The employed population represented 47.9%, retired 21.1%, students 12.2%, unemployed 5.2%, and housewives/husbands 4.9%. 8.6% of respondents reported asthma; 38.3% rhinitis.

VOC concentrations were differently distributed in the 450 dwellings. The highest median and 3rd quartile concentrations were found for formaldehyde. Of the 20 chemicals assessed in the study, 1,2,4-TMB was one of only two VOCs significantly associated with asthma (8.6%), N-undecane being the other. The median 1,2,4-TMB concentration in the dwellings (n = 490) was 4.0 µg/m^3 (0.8 ppb). The maximum concentration was 111.7 µg/m^3 (23 ppb) 1,2,4-TMB.

6.2 Chronic Toxicity to Children

OEHHA was not able to locate any scientifically adequate subchronic or chronic TMB toxicity studies pertaining specifically to infants and/or children.

6.3 Chronic Toxicity to Experimental Animals

No lifetime chronic inhalation animal toxicity studies were identified for any of the three TMB isomers. There is one chronic oral exposure study that evaluated the carcinogenicity of the 1,2,4-TMB isomer (Maltoni et al., 1997). Some effects on survival (mortality) of treated animals were reported; however, this study used a single dose, and it lacked any discussion of histopathological analyses or tests of statistical significance, and therefore was not considered any further.

There are a number of subchronic TMB studies in animals. Table 13, below, provides a summary of adverse effects reported in these subchronic TMB toxicity studies. Table 14 comprises studies that entail subchronic exposure to mixtures containing one or more TMB isomers. More detailed descriptions of the studies in Tables 13 and 14 follow below.

Table 13. Summary of Effects from Subchronic Inhalation Trimethylbenzene Studies in Animals

Study	Sex/Species	TMB Isomer(s)	Exposure	Results
Battig et al., 1958	M/ Rats (*n* = 8, age/ strain not specified)	1,2,4-TMB	1700 ppm (8357 mg/m^3) 8 h/day, 5 d/week for 4 months (neurotoxicity, kidney effects, blood changes)	↑ PSNG and ↓ in LP
Koch industries, Inc, 1995	M/F Spraque-Dawley Rats (*n* = 10/group, 8 weeks old)	1,3,5-TMB	0, 60, 150, 600 mg/kg-day (0, 12, 30, or 122 ppm) for 14 consecutive days	↑ organ weights (liver, kidney, adrenal) ≥ 150 mg/kg-d; ↑ monocyte counts at 150 mg/kg-d; ↑ clinical chemistry effects (CK, BUN, ALB) ≥ 60 mg/kg-d; Centrilobular hepatocyte hypertrophy and ↑ WBC at 600 mg/kg-d
Korsak and Rydzynski, 1996	M Wistar rats (1,2,4-TMB: *n*=9–10/group; 1,2,3-TMB: *n*=10=30/group)	1,2,4-TMB, 1,2,3-TMB	6 h/day, 5 d/week for 3 months to 0, 25, 100, 250 ppm (0, 123, 492, 1230 mg/m^3)	Significant ↓ in pain sensitivity (latency) at ≥ 100 ppm (492 mg/m^3) 1,2,4-TMB, and ≥ 25 ppm (123 mg/m^3) 1,2,3-TMB; significant ↑ in rotarod disturbances at 250 ppm (1230 mg/m^3) 1,2,4-TMB and ≥ 100 ppm (492 mg/m^3) 1,2,3-TMB
Korsak et al.,1997	M/ Wistar Rats (*n* = 6–7/group, age not specified)	1,2,4-TMB	6 h/day, 5 d/week for 90 days to 0, 25, 100, 250 ppm (0, 123, 492, 1230 mg/m^3)	↑ cell macrophages, polymorphonuclear leukocytes, lymphocytes; ↑ LDH & AP in BALF, total protein content ≥25 ppm (123 mg/m^3)
Gralewicz et al., 1997	M/ Wistar Rats (*n* = 60, 5 months old)	1,2,4-TMB	6 h/day, 5 d/week, for 4 weeks to 0, 25, 100, 250 ppm (0, 123, 492, 1230 mg/m^3)	Persistent impairment of neuropsychological functions ≥ 100 ppm (492 mg/m^3)

Abbreviations: ALB = albumin; AP = alkaline phosphatase; BALF = bronchoalveolar lavage fluid; BUN = blood, urea, nitrogen; CK = creatinine kinase; CNS = central nervous system; F = female; LDH = lactate dehydrogenase; LP = lymphocytes percentage; M = male; PSNG = percentage of segmented neutrophilic granulocytes; WBC = white blood cells; ↑ = increase; ↓ = decrease

Table 13. Summary of Effects from Subchronic Inhalation Trimethylbenzene Studies in Animals (continued)

Study	Sex/Species	TMB Isomer(s)	Exposure	Results
Wiaderna et al., 1998	Male Wistar Rat (*n* = 13–14/group, 5 months old)	1,2,3-TMB	0, 25, 100, or 250 ppm (0, 123, 492, 1230 mg/m^3) for 4 weeks	↑ CNS effects (impaired learning) at 25 and 100 ppm (123 and 492 mg/m^3)
Korsak et al., 2000a	M/F Wistar Rats (*n* = 10 M/F/group, 3 months old)	1,2,3-TMB	6 h/day, 5 d/week, for 90 days to 0, 123, 492, 1230 mg/m^3 (0, 25, 100, 250 ppm)	Treatment-related respiratory effects at all concentrations: ↑ goblet cells, ↓ peribronchial lymphatic tissue at ≥ 100 ppm (492 mg/m^3); ↑ interstitial lung infiltration at 250 ppm (1230 mg/m^3); lymphoepithelium formation at 25 and 100 ppm (123 and 492 mg/m^3)
Korsak et al., 2000b	M/F Wistar Rats (*n* = 10 M/F/group, 3 months old)	1,2,4-TMB	6 hr/day, 5 d/week, for 90 days to 0, 123, 492, or 1230 mg/m^3 (0, 25, 100, 250 ppm)	↑ # animals w/ peribronchial, lung parenchymal, and perivascular lymphocytic infiltrations ≥ 100 ppm (492 mg/m^3)
Gralewicz and Wiaderna, 2001	M Wistar Rats (*n*= 11/group for exposed and 10/group for control animals, 5 months old)	1,2,3-TMB, 1,2,4-TMB, 1,3,5-TMB	6 hr/day, 5 d/week, for 4 weeks to 0 or 100 (±10) ppm TMB (492 mg/m^3)	↑ behavioral alterations/functional changes in CNS (1,2,4- and 1,3,5-TMB-exposed rats showed a significantly higher locomotor activity, impaired passive avoidance learning, and longer paw-lick latencies 24 hrs after footshock. Acquisition of the 2-way active avoidance response was significantly impaired in all TMB-exposed groups)

Abbreviations: ALB = albumin; AP = alkaline phosphatase; BALF = bronchoalveolar lavage fluid; BUN = blood, urea, nitrogen; CK = creatinine kinase; CNS = central nervous system; F = female; LDH = lactate dehydrogenase; LP = lymphocytes percentage; M = male; PSNG = percentage of segmented neutrophilic granulocytes; WBC = white blood cells; ↑ = increase; ↓ = decrease.

Table 13. Summary of Effects from Subchronic Inhalation Trimethylbenzene Studies in Animals (continued)

Study	Sex/Species	TMB Isomer(s)	Exposure	Results
Wiaderna et al., 2002	Male LOD:WIST Rats (*n* = 12/group, 5 months old)	1,3,5-TMB	0, 25, 100, 250 ppm (123, 492,1230 mg/m^3) for 4 weeks	↑ functional changes in CNS. Passive and active avoidance ≥ 25 ppm (123 mg/m^3); paw lick latency at 100 ppm (492 mg/m^3)
Lutz et al., 2010	M/ Wistar Rat (*n* = 6–8 animals/group, adult)	1,2,3-TMB, 1,2,4-TMB	0, 25,100, or 250 ppm (0, 123, 492, 1230 mg/m^3) 6 hr/day, 5 days/week for 4 weeks	↑ behavioral alterations (locomotor and sensitization to the psychostimulant amphetamine) with 1,2,3-TMB and ↓ behavioral alterations with1,2,4-TMB
Adenuga et al., 2014	M/F Sprague-Dawley Rats (*n* = 60 M/F, 6 weeks of age)	1,3,5-TMB	0, 50, 200, 600 mg/kg-d for 90 days	↑ monocyte counts > 50 mg/kg-d; ↑ phosphorus levels, and ↓ in kidney weights at 600 mg/kg-d

Abbreviations: ALB = albumin; AP = alkaline phosphatase; BALF = bronchoalveolar lavage fluid; BUN = blood, urea, nitrogen; CK = creatinine kinase; CNS = central nervous system; F = female; LDH = lactate dehydrogenase; LP = lymphocytes percentage; M = male; PSNG = percentage of segmented neutrophilic granulocytes; WBC = white blood cells; ↑ = increase; ↓ = decrease.

Table 14. Summary of Effects from Subchronic Inhalation Trimethylbenzene Mixture Studies in Animals

Study	Sex/Species	Mixture/TMB isomer(s)	Exposure	Results
Nau and Neal, 1966	Rats/species not specified; Rhesus Monkeys	C_9-C_{10} aromatic fraction/specific TMB isomers not specified*	Rats: 50 and 200 ppm (246 and 983 mg/m^3) 8h/day, 5 days/week for 90 and 93 days, respectively (*n*=18/group); 616 (*n*=60) and 1000 (*n*=38) ppm (3028 and 4916 mg/m^3) 18 h/day, 7 day/week for 108 and 73 days, respectively. Rhesus monkeys (*n*=3): 50 or 200 ppm (246 and 983 mg/m^3) 7 h/day, 5 d/week, for 90 days	Hematological effects (rats/monkeys), skin irritation (monkeys) at 50 ppm; "pronounced" CNS effects (tremors, sedation) in monkeys at 200 ppm
Lam et al., 1992	M/Wistar Rats (*n* = 5), 3 months of age	white spirit/1,2,4-TMB, 1,2,3-TMB, 1,3,5-TMB	0, 400, 800 ppm (0, 1966, and 3932 mg/m^3) 6 hr/day, 5 days/week, for 3 weeks	↑ levels of the CNS neurotransmitters noradrenaline, dopamine, and 5-hydroxytryptamine ≥ 400 ppm (1966 mg/m^3)

Abbreviations: CNS = central nervous system; M = male; TMB = trimethylbenzene; ↑ = increase.
*TMBs are produced during petroleum refining as a component of the C9 fraction; individual TMB isomers make up approximately 45% to 55% (US EPA, 1994a).

6.3.1 Inhalation Studies

6.3.1.1 Neurological Effects

A number of studies have reported neurobehavioral effects in rats and mice following inhalation exposure to TMBs (Frantik et al., 1994; Gralewicz et al., 1997; Gralewicz and Wiaderna, 2001; Korsak et al., 1995; Korsak and Rydzynski, 1996; Korsak et al., 1997; Wiaderna et al., 1998; Wiaderna et al., 2002). Sensorimotor impairment (a deficit in rotarod performance and a decrease in pain sensitivity) was found in rats within 24

hours following subchronic exposure to 1,2,4-TMB at concentrations of 100 to 250 ppm (Korsak and Rydzynski,1996).

The data regarding the neurotoxic potency of the individual TMB isomers (vis a vis each other) are mixed; some subchronic inhalation studies with rats have shown that the various TMB isomers produce similar effects but that 1,2,4-TMB and 1,3,5-TMB have a higher neurotoxic potential than 1,2,3-TMB (Korsak et al., 2000a,b; Gralewicz and Widerna, 2001). Other findings (some from the same laboratory) report that 1,2,3-TMB is the most toxic isomer with regard to neurotoxic effects (Korsak and Rydzynski, 1996). In this latter study, in both the acute and subchronic investigations, 1,2,3-TMB was the most neurotoxic of the three isomers. In one study, 1,2,3-TMB was shown to have a much greater ability than 1,2,4-TMB to induce behavioral sensitization and/or to increase the susceptibility of animals to psychostimulant (amphetamine) treatment (Lutz et al., 2010).

Nau and Neal (1966) exposed rats, mice, rabbits and monkeys via inhalation (whole-body exposure), skin application, and subcutaneous injection to various concentrations of C_9-C_{12} petroleum fractions for varying lengths of time. The authors did not describe the percent content of mixed TMBs in the C_9-C_{10} fraction, but commercial C_9 aromatic hydrocarbon solvents typically include TMBs (20% to 45%), ethyltoluene (25% to 40%), isopropylbenzene (<4%), and mixed xylenes (<5%) (Firth, 2008). Only the inhalation data for the C_9-C_{10} aromatic fraction are discussed here. No data in mice, and very little data in rabbits, were reported by the study authors. Rats (sex not specified) were exposed to 50 (*n* = 18), 200 (*n*=18), 616 (*n*=60), or 1000 ppm (*n*=38) (246, 983, 3028, or 4916 mg/m^3) C_9-C_{10} for varying lengths of time; maximum days of exposure were 90, 93, 150, and 78 days, respectively. At the 50 and 200 ppm concentrations, rats were exposed 8 hours/day, 5 days/week. At the 616 and 1000 ppm concentration levels, rats were exposed up to 18 hours/day, 7 days/week. Animals were assessed for appearance/behavior, body weight gain, organ weights, hematological findings, bone marrow changes, and pathological changes.

Rats exposed for 18 hours/day, seven days per week “for as long as 2,424 hours” to 616 ppm C_9-C_{10} showed a significant decrease (no *p* value provided) in body weight and total white blood cell (WBC) count. Lungs, liver, kidneys, spleen, and omental tissues showed evidence of congestion and hemorrhagic changes. After 54 hours of exposure, focal, inflammatory changes were seen in the lungs, as well as fatty changes in the liver. Seventy percent of the rats developed bilateral cataracts (two months post-exposure). No cataracts were observed in unexposed rats.

Rhesus monkeys (4 lbs; 1.8 kg) (*n* = 3/group) exposed by inhalation to either 50 or 200 ppm C_9-C_{10} aromatic fraction 7 hours/day, 5 days/week for a total of 90 exposures

developed a number of adverse effects at both exposure levels: at the 50 ppm concentration, there was an increase in hematocrit readings and a shift in the polymorphonuclear-lymphocyte ratio and, at 200 ppm, there was a decrease in WBC counts, reversal of the polymorphonuclear-lymphocyte ratio, evidence of skin irritation with loss of hair, and formation of dry, leathery skin. At the higher exposure level, monkeys exhibited a "noticeable tremor" during the first week of exposure and appeared "groggy and sedated while being exposed".

Lam et al. (1992) evaluated neurological effects of white spirit (containing the TMB isomers) in rats following a three week inhalation exposure. Male Wistar rats, 3 months of age, were exposed in inhalation chambers to 0, 400, or 800 ppm white spirit vapor 6 hr/day, 5 days/week, for 3 weeks. The concentration of the TMB isomers in the solvent was not reported. Exposure to white spirit did not induce changes in brain weight, protein concentration, or esterase activities (acetylcholinesterase and butyrylacetylcholinesterase). White spirit exposure significantly increased levels of the CNS neurotransmitters noradrenaline, dopamine, and 5-hydroxytryptamine. Because this study entailed exposure to a mix of chemicals, it cannot be concluded from the study data that TMBs increase CNS neurotransmitter levels.

Korsak and Rydzynski (1996) evaluated both acute and subchronic exposure to the three TMB isomers in rats; only the subchronic findings are discussed here. In the subchronic study, adult male Wistar rats were exposed to 0, 25, 100 or 250 ppm 1,2,4-TMB (n= 10/group) or 1,2,3-TMB (n = 10 to 30/group) 6 h/day, 5 days/week for 3 months in an inhalation chamber (equivalent to 0, 123, 492, or 1230 mg/m^3). Neurotoxicity was evaluated via the rotarod performance test and the hot plate behavior test (the latter measures a decrease in sensitivity to pain). All rats in all experiments survived the exposures. Clinical observations were unremarkable. According to the authors, no significant differences in bodyweight were observed between exposed groups and controls (data not shown). Exposure to TMB isomers caused concentration-dependent disturbances in rotarod performance and a decrease in pain sensitivity. Changes in pain sensitivity (latency of the paw lick response) were statistically significant at all exposure concentrations $\geq$ 25 ppm 1,2,3-TMB ($p \leq$ 0.05 and $p \leq$ 0.01), and at $\geq$ 100 ppm 1,2,4-TMB ($p \leq$0.01). Recovery in hot-plate behavior was observed two weeks post exposure (for both isomers). Disturbances in rotarod performance were statistically significant for 1,2,3-TMB exposure $\geq$ 100 ppm, and for 1,2,4-TMB at 250 ppm (p <0.005). Recovery in rotarod performance was not observed two weeks post-exposure. It is not clear from the study description whether the same cohort of animals was used for testing in both the rotarod and pain sensitivity experiments.

Gralewicz et al. (1997) also observed persistent behavioral disturbances following a 4-week inhalation exposure to 1,2,4-TMB in rats. Male Wistar rats (n = 15/group) were

exposed to 0, 25, 100 or 250 ppm (0, 123, 492, or 1230 mg/m^3) 1,2,4-TMB in an exposure chamber 6 hr/day, 5 days/week for 4 weeks duration. Behavioral tests were performed between 21 to 54 days after the last exposure. According to the study authors, there were no overt signs of toxicity. No differences were found between any of the groups in the radical maze test (tests short-term spatial memory). Exposed animals demonstrated increased motor activity at the 100 ppm (492 mg/m^3) concentration and a decreased ability to learn passive avoidance response at 100 and 250 ppm (492 and 1230 mg/m^3) 1,2,4-TMB. Two-way active avoidance learning was slightly retarded in rats exposed to 250 ppm. Rats exposed to 100 and 250 ppm (492 and 1230 mg/m^3) 1,2,4-TMB appeared more fearful on the hot plate on the second day of testing (reflects shock-induced fear response). The authors concluded that, “inhalation exposure to TMB may lead to long-lasting disturbances in CNS function”.

Under the same exposure conditions, and using the same concentrations as for 1,2,4-TMB, above, similar findings of neurotoxicity were seen in a parallel study with 1,2,3-TMB (Wiaderna et al., 1998). Male Wistar rats (*n* = 13 to 14/group) were exposed to concentrations of 0, 25, 100, or 250 ppm (0, 123, 492, 1230 mg/m^3) 1,2,3-TMB 6 hr/day, 5 days/week for 4 weeks in an inhalation chamber. The effects of radial maze performance, open-field activity, passive- and active-avoidance learning, and shock-induced changes in latency of the paw-lick response to heat were investigated. In rats exposed to 25 or 100 ppm (123 or 492 mg/m^3), but not 250 ppm (1230 mg/m^3), passive avoidance learning *(i.e.,* refraining from stepping off a platform where the animal received an electric shock) was significantly impaired. In rats exposed to 100 ppm (123 mg/m^3), but not 250 ppm (492 mg/m^3), an increase in latency of the paw lick response (following a foot shock), as well as retarded acquisition of the two-way active avoidance response, persisted longer than in control animals. The non-linear concentration effect relationship could not be explained by the authors, but as it occurred in both studies, it is not considered to be an artifact. The authors concluded (as previously in studies with 1,2,4-TMB) that, “low-level exposure to 1,2,3-TMB may lead to long-lasting disturbances in the CNS functions”.

Gralewicz and Wiaderna (2001) evaluated the behavioral effects of all three TMB isomers in rats. Male Wistar rats (10 to 11/group) were exposed 6 hours/day, 5 days/week for 4 weeks to 100 ppm (492 mg/m^3) of either 1,2,3-TMB, 1,2,4-TMB or 1,3,5-TMB in an inhalation chamber. Controls were sham-exposed. Two weeks post-exposure, behavior was assessed in a series of tests: radial maze performance, spontaneous activity in an open field, learning and retention of passive and active avoidance response, and heat-induced paw licking before and after footshock. Rats in the 1,3,5-TMB and 1,2,4-TMB groups, but not the 1,2,3-TMB group, showed significantly higher locomotor activity, an impaired passive avoidance learning, and significantly longer paw-lick latencies than controls. Acquisition, but not retention, of the two-way active avoidance learning was significantly impaired in all TMB-exposed

groups. In this study, the 1,2,3-TMB isomer was less active than the 1,2,4-TMB and 1,3,5-TMB isomers.

Low-level inhalation exposure to the TMB isomers 1,2,3-TMB and 1,2,4-TMB appears to induce behavioral sensitization and/or increase the susceptibility of male rats to psychostimulants (amphetamine (AMPH)) (Lutz et al., 2010). The behavioral effects are long-lasting. Male Wistar rats (*n* = 6 to 8 animals/group) were exposed independently to 0, 25, 100, or 250 ppm (0, 123, 492, 1230 mg/m^3) 1,2,3-TMB or 1,2,4-TMB in an inhalation chamber, 6 h/day, 5 days/week for 4 weeks. Rats were then administered 0.5 mg/kg AMPH *i.p*. (intraperitoneal). Motoric behavior in an open field was tested 2- and 3- weeks after inhalation exposure to the TMBs. Afterwards, the rats were subjected to a sensitization procedure, which consisted of repeated *i.p.* administrations of AMPH at 2.5 mg/kg bw; one injection per day for five consecutive days. TMB exposure resulted in an alteration in the rat's behavioral sensitivity to an AMPH challenge and susceptibility to become sensitized by a repeated AMPH treatment. For each isomer, the concentration-effect was non-linear. The authors stated that, "nonlinearity in the concentration-dose response relationship is common in the case of the acute effects of solvents – psychomotor arousal at low and depression at high concentrations". The behavioral alterations were most pronounced in rats exposed to 100 ppm (123 mg/m^3) 1,2,3-TMB or 1,2,4-TMB. In 1,2,3-TMB-exposed rats, the augmented behavioral response was significantly more evident than in controls. In 1,2,4-TMB-exposed rats, the augmenting was significantly less evident than in controls. Prior experiments by these same authors have not found qualitative differences in other behavioral effects between the TMB isomers (Gralewicz et al., 1997; Wiaderna et al., 1998). The authors concluded that, "AMPH sensitization found in the present experiments may confirm the suggestion that TMB exposures produce long-lasting changes in the functional state of the dopaminergic system". AMPH is an indirect dopaminergic agonist, and the dopaminergic system plays a key role in the sensitization to psychostimulants (Berman et al., 2009).

The same authors (Wiaderna et al., 2002) investigated neurotoxic effects of subchronic exposure to 1,3,5-TMB. Male rats (*n*= 12/group), 5 months old, were exposed to the solvent at concentrations of 0, 25, 100 or 250 ppm (0, 123, 492, 1230 mg/m^3) 6 hr/day, 5 days/week for 4 weeks in an inhalation chamber. The five behavioral responses tested included: ability to find water in a radial maze (14 to 19 days post exposure), open field locomotor activity (25 days post exposure), step-down passive avoidance test (50 to 51 days post exposure), sensitivity to pain (50 to 51 days post exposure), and acquiring 2-way active avoidance (54 to 60 days post-exposure). No long-term effect was seen on short-term spatial memory (radial maze test) between groups, nor were treatment-related effects seen on open-field behavior (assay of spontaneous motor activity). In the step-down passive avoidance test (assay of long-term memory), groups

exposed to ≥ 25 ppm (123 mg/m^3) 1,3,5-TMB remained on the platform significantly less time than the control group (differences were significant for all groups). Findings were similar for the active avoidance test (tests ability to learn and memorize). In the hot plate test, an assay of sensitivity to pain and pain-related stress level, the latency of the reaction to the thermal stimulus was significantly longer in the group exposed to 100 ppm (492 mg/m^3) 1,3,5-TMB than the 25 ppm or control groups. There was no statistically significant difference between the 100 and 250 ppm (492 and 1230 mg/m^3) exposed groups. There was, however, no dose-response relationship for any of the endpoints studied. The authors' conclusions were that concentrations close to 20 ppm (98 mg/m^3) for trimethylbenzene may produce long-term functional changes in the rat central nervous system.

6.3.1.2 Respiratory Effects

TMB exposure via inhalation causes respiratory irritation and inflammation in animals. Korsak et al. (1997) conducted several studies to evaluate the respiratory toxicity of the TMBs in both rats and mice. In a subchronic study, adult male Wistar rats (10/group) were exposed via inhalation to 25, 100 or 250 ppm (123, 492, 1230 mg/m^3) 1,2,4-TMB 6 hr/day, 5 days/week for 90 days. Inhalation exposure to 1,2,4-TMB significantly increased the total number of cells obtained by bronchoalveolar lavage at all three test concentrations compared with controls: p <0.001, p <0.01, and p <0.05, respectively, at the 25, 100, and 250 ppm (123, 492, 1230 mg/m^3) concentrations. A statistically significant increase in macrophages was seen at concentrations ≥ 100 ppm (492 mg/m^3) 1,2,4-TMB (p <0.01). Total protein and lactate dehydrogenase enzyme activity were significantly increased at the 25 (p ≥0.001) and 100 ppm (p ≤0.05) (123 and 492 mg/m^3) concentrations (levels were increased versus controls at the highest concentration, but the results were not significant). Mucoproteins were significantly decreased at the 25 ppm (123 mg/m^3) concentration compared to controls (p ≤0.05); at higher concentration levels, mucoproteins continued to decrease, but the observed changes showed little progression of effects, even if the exposure was 10 times higher. Finally, acid phosphatase enzyme levels were significantly increased at all concentration levels (p <0.05), and a trend test for dose-response was statistically significant.

Korsak et al. (2000a) exposed male and female Wistar rats to concentrations of 25 ppm (123 mg/m^3), 100 ppm (492 mg/m^3) or 250 ppm (1230 mg/m^3) 1,2,3-TMB 6 hr/day, 5 days/week for 3 months. There were 10 male and 10 female rats per group except for the highest exposure concentration group, which had 20 male and 20 female rats. High exposure group rats were observed for an additional month after termination of exposure. All animals were necropsied at 3 months. No differences in body weight gain were found between groups. Treatment-related respiratory effects were observed at all

3 exposure concentrations. An increased number of goblet cells were noted in the bronchi of female rats at the mid- and high concentrations. Trend analysis indicated that the number of goblet cells present was related to the concentration of 1,2,3-TMB (p = 0.001). In addition, a decreased amount of peribronchial lymphatic tissue was noted in female rats at mid- and high exposure concentrations. In male rats, a significant increase in the intensity of interstitial lung infiltration was seen at the highest concentration (p <0.01); this was also related to 1,2,3-TMB concentrations (trend significance at p = 0.006). Lymphoepithelium formation (loss of bronchial epithelium cuboidal character, forming lymphoepithelium) was observed in male rats at the lowest and mid concentrations.

Korsak et al. (2000b) exposed male and female Wistar rats to concentrations of 25 ppm (123 mg/m^3), 100 ppm (492 mg/m^3) or 250 ppm (1230 mg/m^3) 1,2,4-TMB, 6 hr/day, 5 days/week for 3 months. Hematological, clinical chemistry, and respiratory effects were observed in animals of both sexes. There were 10 male and 10 female rats per group except for the highest exposure concentration group, which had 20 male and 20 female rats. High exposure group rats were observed for an additional month after the termination of exposure. All animals were necropsied at 3 months. In both male and female treated rats, an increased number of animals with peribronchial, lung parenchymal, and perivascular lymphocytic infiltrations were observed. Trend analysis indicated that the number of alveolar macrophages was concentration-dependent in both male and female rats (p = 0.002 and 0.03, respectively). Based on a cumulative score of all pathological lung changes in male and female rats, the authors concluded that 1,2,4-TMB has a toxic effect on the respiratory system at a concentration of 100 ppm (492 mg/m^3).

6.3.1.3 Organ Effects

A number of experimental animal studies have reported liver and kidney effects following both inhalation and oral exposure to TMBs (Adenuga et al., 2010; Clark et al., 1989; Koch Industries, 1995; Korsak et al., 2000a,b; Pykko, 1980, Wiglusz et al., 1975a). Liver and kidney effects have also been found following inhalation exposure to organic solvent mixtures containing TMBs.

Wiglusz et al. (1975a) examined the effect of chronic TMB toxicity on blood serum enzymes: GOT, GPT, AP and GLDH. Elevated liver enzymes are indicative of inflammation or damage to liver cells. Male Wistar rats (n = 6) were exposed in an inhalation chamber to 3.0 mg/L (610 ppm, 3000 mg/m^3) 1,3,5-TMB 6 hr/day, 6 days/week for 5 weeks. There were 6 control animals. Blood samples were collected from the caudal vein 3 days prior to exposure and at 2, 7, 14, and 28 days post-exposure. Considerable changes were seen in GOT activity: an initial decrease followed

by a steady increase throughout the first week. The highest enzyme activity, statistically significant at $p < 0.05$, was seen at 14 days post-exposure; the value was 38% higher than the baseline value. The level did not remain elevated throughout the exposure period. AP activity was considerably (but not significantly) decreased after the first few days of treatment compared to control animals. AP activity increased "considerably" on day 7 of treatment. After 14 days, AP activity was only slightly higher than initial values. GPT activity did not show any noticeable changes. No activity of the GLDH enzyme was detected in blood serum (the authors stated this may indicate that the mitochondrial cell structure was left intact under the conditions of the study). No changes in GOT or GPT activity were seen in control animals.

Clark et al. (1989) exposed groups of 50 male and female Wistar rats to 0 (control), 92 ppm (450 mg/m^3), 183 ppm (900 mg/m^3), or 366 ppm (1800 mg/m^3) high aromatic naphtha (a 50:50 blend of Shellsol A/Solvesso 100) 6 hr/day, 5 days/week for up to 12 months. Analysis of the test substance showed it was comprised of predominantly C9 isomers (75%), primarily TMBs (44.8%). Ten males/females were killed after 6 months of exposure, 25 males/females after 12 months of exposure, and 15 males/females 4 months after exposure termination. Examination included hematology, clinical chemistry, urinalysis, and pathology; only the organ effects are discussed here. Other toxicological findings are discussed in their respective sections. Five animals died during the study (3 controls, and two males exposed at the 183 ppm (900 mg/m^3) concentration). No further mortality information was given. Seven rats were removed from the study during the exposure period. The authors said the removals were distributed across all groups. At 6 and 12 months necropsy, high exposure males had increased liver and kidney weights (significant increase at 12 months; $p < 0.05$) Females in the four month recovery groups showed significant decreases in kidney weights at all exposure levels. Mineralization of the cortico-medullary junction was seen in almost all females (including control animals), but none of the males.

Significant increases in liver weight and accompanying changes in liver biochemistry, indicative of liver damage, were seen in rats exposed to 1,2,3-TMB (Korsak et al., 2000a) and 1,2,4-TMB (Korsak et al., 2000b). Korsak et al. (2000a,b) exposed male rats to vapors of 1,2,4-TMB or 1,2,3-TMB at concentrations of 0, 25, 100, and 250 ppm (0, 123, 492, and 1230 mg/m^3) 6 hours/day, 5 days/week for 90 days. Statistically significant ($p < 0.05$) increases in relative liver weight and sorbitol dehydrogenase levels (a sensitive biomarker of liver damage seen in studies with other hepatotoxic chemicals), were seen in males at the highest 1,2,3-TMB exposure concentration. A decrease in red blood cell counts was observed in male and female rats at the highest concentration; the change was statistically significant in male rats. An increase in white blood cells was observed in highly-exposed males, and females of all exposure groups. The authors concluded that the "macrocytic anemia and increased sorbitol

dehydrogenase activity in male rats might be due to hepatotoxic 1,2,3-TMB effects" despite the absence of histopathological changes in liver.

In the Korsak et al. (2000b) 1,2,4-TMB study, female spleen and kidney absolute weights were significantly decreased at the 100 ppm (492 mg/m^3) concentration relative to controls (p <0.05). In males, spleen weights were significantly increased at the lowest exposure concentration, 25 ppm (123 mg/m^3) 1,2,4-TMB, and lung weights significantly increased at the mid concentration, 100 ppm (492 mg/m^3) 1,2,4-TMB, relative to controls (p <0.05). These organ weight changes were not concentration-dependent, and no apparent histopathological analyses were mentioned. Other adverse effects observed in these studies (*i.e.,* respiratory, hematological, clinical chemistry) are discussed in their respective sections.

6.3.1.4 Hematological and Clinical Chemistry Effects

TMBs have been shown to be hematotoxic in a number of animal studies (Adenuga et al., 2014; Battig et al., 1958; Clark et al., 1989; Korsak et al., 2000a,b; Wiglusz et al., 1975a). Battig et al. (1958) exposed male rats (n = 8/dose) 8 hours/day for 5 weeks to 0, 200, 500, and 1700 ppm (0, 954, 2460 and 8357 mg/m^3) of a solvent, Fleet-X DV 99, comprised of > 50% 1,2,4-TMB and > 30% 1,3,5-TMB (the presence of benzene cannot be ruled out in this study). The solvent mixture also potentially contained 1,2,3-TMB and numerous methylbenzenes. The authors implied that dose was not consistent throughout the experiment. Animals were evaluated for effects on growth (measured by BW), behavior, food consumption, red blood cell count, hemoglobin concentration and various histological parameters (study in German, translated by US EPA). Behavioral effects were assessed qualitatively. A statistically significant decrease in lymphocytes was seen in TMB-exposed rats at the highest dose, 1700 ppm (8364 mg/m^3) (p = 0.5). A significant increase in urinary phenol excretion (free and total) was seen at the 4-day exposure mark in rats exposed to the 1700 ppm (8364 mg/m^3) dose, at day 8 in 500 ppm (2460 mg/m^3) exposed rats, and at day 10 in 200 ppm (954 mg/m^3) exposed rats. No further information was translated. Hematological effects in painters (blood coagulativity and decreased erythrocytes and thrombocytes) have been reported by the same investigators following long-term exposure to Fleet-X DV 99 (Battig et al., 1956). These findings are in agreement with other authors that studied the effects of TMBs on peripheral blood in rats (Wiglusz et al, 1975b).

Wiglusz et al. (1975b) evaluated the effect of 1,3,5-TMB on the peripheral blood of rats. Only the subchronic portion of the study is described here. Male Wistar rats (n= 6) were exposed to a concentration of 3.0 mg/L (610 ppm, 3000 mg/m^3) 1,3,5-TMB 6 hr/day, 6 days/week for 5 weeks. Blood samples were collected from control and exposed animals 3 days prior to the start of the experiments, and then on days 1, 7, 14 and 28

after completion of exposure. In the 5 week study, no changes in erythrocyte or leukocyte counts were seen in the 1,3,5-TMB-exposed group. A slight increase in the percentage of segmented neutrophilic granulocytes (PSNG) and a decrease in the lymphocytes percentage (LP) were seen after 14 days of exposure only. A few animals in the 5 week study showed a slight increase in the percentage of monocytes after 14- and 28 days exposure.

A number of statistically significant hematological effects were seen in male and female rats following subchronic inhalation exposure to 1,2,3-TMB and 1,2,4-TMB (Korsak et al., 2000a,b). In male/female rats exposed 6 hr/day, 5 days/week for 3 months in an inhalation chamber to 0, 25, 100, or 250 ppm 1,2,3-TMB (0, 128, 492 or 1230 mg/m^3), observations included significant decreases in red blood cell (RBC) counts in high-concentration male, a decrease in segmented neutrophil counts in both sexes at the high concentration, and an increase in reticulocyte counts in high-exposed males and females of all exposure groups (Korsak et al., 2000a). Clinical chemistry observations include a statistically significant increase in sorbitol dehydrogenase in high-exposure males and a significant increase in AP in mid- and high-exposure females. Respiratory findings from this study are discussed in the respective section. The authors identified the lowest concentration tested, 25 ppm (123 mg/m^3) 1,2,3-TMB, as a NOEL in both male and female rats.

In the Korsak et al. (2000b) study, which was the same study design as Korsak et al. (2000a) but evaluated the 1,2,4-TMB isomer, hematological, clinical chemistry, and respiratory effects were observed in animals of both sexes. Only hematological findings are discussed here. In males, a decrease in RBCs and an increase in WBCs were seen. A trend analysis showed that the changes were concentration-dependent and were statistically significant at a concentration of 250 ppm (1230 mg/m^3) 1,2,4-TMB. The WBC count was similar to controls but RBC counts remained depressed at 2 weeks post-exposure. In females, hematological effects included decreased reticulocyte counts in all treated groups; changes were significantly different from controls at the 250 ppm (1230 mg/m^3) concentration. Clotting time decreased in male/female rats in all treated groups; changes were statistically significant in female rats at the mid- and high-concentrations. A two-fold increase in both reticulocyte counts and clotting time was found in male and female rats two weeks post-exposure, compared to control animals.

7. Developmental and Reproductive Effects

TMBs have been detected in the cord blood of infants (Cooper et al., 2001; Dowty and Laseter,1976) and in rat fetal blood (Ungvary et al., 1983, Ungvary and Tatrai, 1985) following exposure to TMBs. Ungvary et al. (1983) and Ungvary and Tatrai (1985)

demonstrated that all TMB isomers can pass the placental barrier in rats, and are found in fetal blood and amniotic fluid. TMB reproductive/developmental toxicity data are limited to one developmental toxicity study in pregnant rats exposed on gestational day (GD) 6 to 20 (Saillenfait et al., 2005). No chronic, multi-generation reproductive or developmental toxicity studies have been conducted. No developmental toxicity study to date has evaluated neurological/behavioral endpoints. All of the TMB isomers have been shown to have neurotoxic effects in adult animals. All studies are described in detail, below.

7.1 Human

Dowty and Lasseter (1976) analyzed eleven paired cord blood-maternal blood samples from gravid women at term admitted to the Charity Hospital in New Orleans, LA. Blood samples were analyzed using GC-MS. All neonates had Apgar scores $\geq$ 7 (Apgar is a measure of the physical condition of a newborn infant; a score of 10 represents the best possible condition). Physical examination at birth was normal except for one infant, who had a lumbosacral meningomyelocele ("abnormally high" concentrations of acetone, the food preservative BHT, and other components were found in this infant's cord blood). According to the authors, all infants were an appropriate weight for the number of weeks of gestation (38 to 42 weeks). TMB was one of a number of chemical constituents isolated from the cord-maternal blood samples. Neither the number of paired samples TMB was found in, nor the concentration of TMB in the samples, were provided. The significance of the study findings are limited since the TMB concentrations are unknown.

Cooper et al. (2001) tested maternal urine, cord blood, and placental samples of pregnant farmworkers (*n*=9) in Weslaco, TX for different analytes. Seven of the women worked in the fields, and two helped spouses and family members in the fields. All nine women worked or participated in activities in or near farm fields during their pregnancies for periods ranging from 2 to 8 months. Overall, the women worked for an average of 5.8 months during their pregnancy. Seven of 51 analytes were found in the biological samples: DDE, DDT, dichlorobenzene, toluene, TMB, and endosulfan sulfate were detected in cord blood samples of one or more of the women. The only compound detected in the maternal urine samples was 2,4-D. No analytes were detected in placental samples, and apparently only cord blood was tested for TMB. TMB was detected in cord whole blood in 7 of the 9 subjects, and the range of values was 1.2 - 3.9 ppb. TMB is used as an additive in pesticides (US EPA, 1994b).

7.2 Animal

In the sole developmental toxicity study concerning exposure uniquely to TMB isomers, pregnant Sprague-Dawley rats (*n* = 17 to 24) were exposed whole body via inhalation to

either 0, 100, 300, 600 or 900 ppm (0, 492, 1475, 2949, or 4424 mg/m^3) 1,2,4-TMB, or 0, 100, 300, 600 or 1200 ppm (0, 492, 1475, 2949, or 5899 mg/m^3) 1,3,5-TMB for 6 hours/day on GD 6 to 20 (Saillenfait et al., 2005). Actual air concentrations were measured once a day and were essentially the same as target concentrations. Data were presented as mean ± SD. The number of corpora lutea, implantation sites, live fetuses, and body weights were analyzed by one-way ANOVA, followed by Dunnett's test if differences were found. Frequency of post-implantation loss, dead fetuses, resorptions, and alterations among litters were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney test, where appropriate. Rates of pregnancy and incidences of fetal alterations per dose were analyzed using Fisher's test. The reported level of statistical significance was p <0.05.

Significant decreases in maternal body weight and food consumption were seen at concentrations of 300 and 600 ppm (1475 and 2949 mg/m^3) 1,3,5-TMB and 1,2,4-TMB, respectively. No other clinical signs of maternal toxicity were noted. There was no evidence of embryolethality or teratogenic effects following inhalation exposure to either of these TMB isomers. Of note: dams were exposed on GD 6 to 20 (*i.e.,* embryonic and fetal periods) which is not inclusive of implantation and potential early embryolethality. Dams were more sensitive to the 1,3,5-TMB isomer than 1,2,4-TMB.

The incidence of fetuses with incomplete sternebral ossification was slightly, but not significantly, elevated at 1200 ppm (5899 mg/m^3) 1,3,5-TMB. Both isomers were shown to cause developmental toxicity (fetal growth retardation). A dose-dependent decrease in fetal body weights occurred that was significantly different from controls at concentrations of 600 (p <0.05) and 900 ppm (p <0.01) 1,2,4-TMB (5% and 11% reductions, respectively) and at 600 (p <0.05) and 1200 ppm (p <0.01) 1,3,5-TMB (5% and 12%, respectively). This effect was only seen at maternally-toxic concentrations. However, the US EPA Guidelines for Risk Assessment for Developmental Toxicity (1996) state that adverse developmental effects produced only at doses that result in minimal maternal toxicity are still considered to represent developmental toxicity. OEHHA also considers the TMB-induced reduction in fetal body weights observed by Saillenfait et al. (2005) to be evidence of treatment-related developmental toxicity.

8. Derivation of Reference Exposure Levels

8.1 Trimethylbenzenes Acute Reference Exposure Level

RELs are based on the most sensitive and relevant health effects reported in the medical and toxicological literature. Acute RELs are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (OEHHA, 2008).

Study	McKee et al., 2010
Study population	Male Wistar rats (8/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 125, 1250, or 5000 mg/m^3 (0, 25, 250, 1000 ppm) 1,2,4-TMB
Exposure continuity	3 consecutive days, acute REL based on exposure day 1 data
Exposure duration	8 hours/day
Critical effects	CNS effects (visual discrimination performance)
LOAEL	5000 mg/m^3 (1000 ppm)
NOAEL	1250 mg/m^3 (250 ppm)
Benchmark Concentration	709 mg/m^3 (144 ppm)
Time-adjusted exposure	1417 mg/m^3 (288 ppm) ($C^n \times T = k$, where $n = 3$)
Human equivalent concentration	1417 mg/m^3 (288 ppm) (calculated RGDR = 0.98, rounded to 1)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	600
Reference Exposure Level	2400 µg/m^3 (490 ppb)

There are a number of acute inhalation exposure studies, both in humans and laboratory animals that provide information about the effects of short-term exposure to TMBs. These studies are enumerated in Tables 10 and 11 in the document and discussed in detail in their respective sections.

In humans, all of the acute studies comprise chamber inhalation studies that largely evaluated sensory irritation to TMBs and found no significant induction of sensory irritation. No TMB-specific human studies have examined the potential for neurological effects in sensitive populations. Occupational studies involving inhalation exposure to TMB-containing VOC mixtures show a number of adverse neurotoxic effects: neuropsychological changes (El Hamid Hassan et al., 2013; Chen et al., 1999), deficits in short-term memory and reduced motor speed/coordination (Lee et al., 2005), nervousness, anxiety, and/or vertigo (Battig et al.,1956), and visual dysfunction (Gong et al., 2002; Pratt et al., 2000), but it is not known whether these effects are attributable specifically to TMB and/or whether they result from acute or long-term exposure. Acute effects seen in laboratory animal studies include neurotoxicity, respiratory irritation, and reproductive/developmental toxicity following exposure to TMBs.

The human chamber TMB studies typically included only one exposure concentration, included only a few test subjects, were largely tested in healthy adult males, and provided limited or vague reporting of health effects (most were not designed as toxicity studies per se, but as toxicokinetic studies). Subjects filled out a questionnaire, reporting levels of severity of respiratory irritation and, in some cases, CNS effects. In each of the acute human inhalation studies, the lowest exposure concentration, ranging from 2 to 25 ppm (10 to 123 mg/m^3), was the NOAEL, regardless of TMB isomer tested (Table 15). No LOAELs were identified. Thus, in accordance with OEHHA's Noncancer REL TSD Guidance document (OEHHA, 2008) which notes that, "OEHHA may use a NOAEL without an associated LOAEL identified in the same study (a free standing NOAEL), but only if there are no other suitable studies, and so long as the overall health hazard data for that substance are consistent with the NOAEL study", the human chamber exposure studies were not relied upon to derive the acute REL.

There are two TMB rodent studies that can be used to develop a POD for the derivation of the acute REL: the McKee et al. (2010) neurotoxicity study, and the Saillenfait et al. (2005) reproductive/developmental study. Several additional neurotoxicity studies in mice and rats provide evidence for concentration-dependent acute CNS disturbances (decrease in pain sensitivity and rotarod performance) following a single 4-hour exposure to TMBs (Korsak et al., 1995; Korsak and Rydzynski, 1996). However, NOAEL/LOAEL values could not be determined from these studies for use in REL development because specific concentrations at which altered neurobehavioral changes occurred were not provided. The same is true of the Korsak et al. (1997) acute respiratory irritation study in mice.

In the Saillenfait et al. (2005) developmental study in Sprague-Dawley rats, significant decreases in maternal body weight and food consumption were seen at concentrations of 300 and 600 ppm 1,3,5-TMB and 1,2,4-TMB, respectively. Dams were exposed to concentrations of 0 to 900 ppm 1,2,4-TMB (0 to 4424 mg/m^3) and 0 to 1200 ppm 1,3,5-

TMB (0 to 5899 mg/m^3). There was no significant maternal mortality. There was no evidence of embryolethality or teratogenic effects following inhalation exposure to either of these TMB isomers. Of note: dams were exposed on GD 6 to 20 (*i.e.,* embryonic and fetal periods) which is not inclusive of implantation and potential early embryolethality. Dams were more sensitive to the 1,3,5-TMB isomer than 1,2,4-TMB. The only treatment-related developmental effect, fetal-growth retardation, was statistically significant at 600 and 1200 ppm (2949 and 5899 mg/m^3) 1,3,5-TMB and at 600 and 900 ppm (2949 and 4424 mg/m^3) 1,2,4-TMB. The NOAEL for maternal toxicity was 100 ppm (492 mg/m^3) for 1,3,5-TMB and 300 ppm (1475 mg/m^3) for 1,2,4–TMB and the NOAELs for developmental toxicity were 300 ppm (1475 mg/m^3) for both TMB isomers. While a number of datasets from this study showed a positive trend (*e.g.,* for 1,3,5-TMB, female fetal body weight, male/female fetal body weight combined), only those datasets that yielded viable BMDS results are included in Table 15. BMR modeling results for all Table 15 datasets follow in Table 16.

Table 15. Developmental Toxicity of 1,2,4- and 1,3,5 Trimethylbenzene in Rats (Reduced Maternal and Fetal Bodyweight)

TMB Isomer (Rat Model)	Endpoint	Exposure Concentration[a]				
		0 ppm	**100 ppm (492 mg/m^3)**	**300 ppm (1475 mg/m^3)**	**600 ppm (2950 mg/m^3)**	**1200 ppm (5900 mg/m^3)**
1,3,5-TMB (Dams)	Number Treated	24	24	24	24	24
	Body Weight (g) (GD 13–21)	110 ± 14	109 ± 10	95 ± 21*	80 ± 20**	63 ± 26**
1,3,5-TMB (Male Fetuses)	Number Exposed	21	22	21	17	18
	Body Weight (g)	5.8 ± 0.41	5.76 ± 0.27	5.5 ± 0.31	5.39 ± 0.55*	5.1 ± 0.57**
TMB Isomer (Rat Model)	**Endpoint**	**0 ppm**	**100 ppm (492 mg/m^3)**	**300 ppm (1475 mg/m^3)**	**600 ppm (2950 mg/m^3)**	**900 ppm (4424 mg/m^3)**
1,2,4-TMB (Dams)	Number Treated	25	24	24	24	24
	Body Weight (g) (GD 6–13)	27 ± 8	27 ± 6	26 ± 6	19 ± 8**	14 ± 12**
1,2,4-TMB (All Fetuses)	Number Exposed	23	22	22	22	24
	Body Weight (g)	5.71 ± 0.34	5.64 ± 0.31	5.56 ± 0.47	5.4 ± 0.39*	5.06 ± 0.40**
1,2,4-TMB (Male Fetuses)	Number Exposed	23	22	22	22	24
	Body Weight (g)	5.86 ± 0.34	5.79 ± 0.30	5.72 ± 0.49	5.55 ± 0.48*	5.2 ± 0.42**
1,2,4-TMB (Female Fetuses)	Number Exposed	23	22	22	22	24
	Body Weight (g)	5.57 ± 0.33	5.51 ± 0.31	5.4 ± 0.45	5.28 ± 0.40*	4.92 ± 0.4**

Saillenfait et al. (2005). Animals were exposed 6 hours/day, GD 6 to 20.
[a] Values are expressed as mean ± 1 SD.
*, ** Significant difference from control, p <0.05 and p<0.01, respectively.
Abbreviations: g = grams; GD = gestational day; TMB = Trimethylbenzene.

Table 16. BMR Modeling Results for Saillenfait et al. (2005) 1,2,4- and 1,3,5-Trimethylbenzene Developmental Toxicity Study

TMB Isomer	Endpoint	BMD Model	BMC_{1SD} ppm (mg/m^3)	$BMCL_{1SD}$ ppm (mg/m^3)	Goodness-of-fit *p*-value	Scaled Residual for Dose Group near BMD
1,3,5-TMB	Maternal BW (GD 13–21)	Exponential #2 (NCV)	259.14 (1274)	198.59 (976)	0.26	-0.23
	Fetal BW (Male)	Exponential #3 (NCV)	514.92 (2531)	362.6 (1782)	0.51	-0.09
1,2,4-TMB	Maternal BW (GD 6–13)	Exponential #3 (NCV)	578.57 (2844)	441.84 (2172)	0.60	-0.67
	Fetal BW (All)	Polynomial #4 (CV)	687.35 (3379)	475.20 (2336)	0.99	0.09
	Fetal BW (Female)	Polynomial #4 (CV)	691.75 (3400)	480.86 (2364)	0.97	0.27
	Fetal BW (male)	Polynomial #4 (CV)	714.24 (3510)	497.98 (2448)	0.99	0.048

Abbreviations: AIC = Akaike Information Criterion; BMC_{1SD} = benchmark concentration, 1 SD change from control mean; $BMCL_{1SD}$ = lower 95% confidence limit on the benchmark concentration, 1 SD change from control mean; BMD = benchmark dose; BW = bodyweight; CV = constant variance; GD = gestational days; NCV = non-constant (modeled) variance; PPM = parts per million; SD = standard deviation; TMB = trimethylbenzene
BMRs represent continuous data model runs (mean ± SD), and one SD change from control mean. Each dataset only had one viable model result thus AIC values were not included.

In this study, both maternal and fetal parameters were more sensitive to the 1,3,5-TMB isomer than the 1,2,4-TMB isomer (*i.e.,* yield the lowest BMRs). The lowest BMR for all datasets (maternal and fetal) is for maternal bodyweight decrease on GD 13 to 21 for the 1,3,5-TMB isomer, 259 ppm (1274 mg/m^3). Fetal bodyweights were not reported on a per litter basis, which is the typical unit of analysis, but were instead pooled (*i.e.,* male fetuses, female fetuses, all fetuses). Comparing fetal bodyweight datasets for the 1,2,4-TMB isomer, female offspring appear to be slightly more sensitive than males, with a BMD value of 692 versus 714 ppm (3400 and 3510 mg/m^3), respectively. For the 1,3,5-TMB isomer, only the male fetal bodyweight data could be modeled using BMDS.

The acute rat inhalation study by McKee et al. (2010), which exposed the animals to the 1,2,4-TMB isomer on 3 consecutive days, at concentrations ranging from 25 to 1000 ppm (125 to 5000 mg/m^3), is the best choice for the development of the acute TMB REL because it yields the lowest BMD values of the two studies (*i.e.*, McKee et al., 2010 and

Saillenfait et al., 2005).The McKee study evaluates a well-known and sensitive endpoint for TMBs, neurotoxicity. As stated by the study authors, “acute CNS effects are sensitive indicators of toxicological effects” and, “CNS effects are the most sensitive indicators of effects for most hydrocarbon solvents…”. US EPA in its 2016 Toxicological Review of Trimethylbenzenes document stated, “Neurotoxicity is the most consistently observed endpoint in the database for TMBs”. And, “the weight of evidence for TMB-induced neurotoxicity is coherent across species [*i.e.,* human, mouse, rat], coherent across isomers, and consistent across multiple exposure durations [*i.e.,* acute, short-term, and subchronic]”. OEHHA concurs with these conclusions.

In the McKee acute exposure study, fourteen-week old male rats (*n* = 8/group) were exposed “up to 8 hours/day” for 3 consecutive days to 0, 25, 250, and 1000 ppm (0, 125, 1250, or 5000 mg/m^3, respectively) 1,2,4-TMB, and evaluated for clinical and neurobehavioral endpoints (*e.g.,* motor activity, functional observations, and visual discrimination performance) on exposure days one and three, and 1-day post-exposure. Because the acute REL is meant to protect against an infrequent, 1-hour exposure, data evaluated from the McKee et al. (2010) study for the POD comprise the responses for visual discrimination tests, following the first 8-hour exposure only**.** A clear dose-response was observed for several visual discrimination performance testing endpoints after the first 8-hour exposure: discrimination ratio, trial response latency, latency > 6 seconds, and drink response latency. These results are summarized in Table 17, below.

These visual discrimination endpoints in the McKee et al. (2010) study were significantly different compared to controls at an exposure level of 1000 ppm (5000 mg/m^3) 1,2,4-TMB. Thus, the LOAELs for these endpoints are 1000 ppm (5000 mg/m^3) and the NOAELs are 250 ppm (1250 mg/m^3) 1,2,4-TMB. The drink response latency endpoint was also significantly different compared to controls at the 250 ppm (1250 mg/m^3) exposure level. However, McKee et al. (2010) reported that the 250 ppm (1250 mg/m^3) test group animals also demonstrated a significant drink response latency test response compared to controls in the pre-exposure period (zero exposure) and concluded that “the significance of the effect of exposure in this group is questionable”. OEHHA agrees with this assessment and does not believe that the increase in drink response latency observed in the 250 ppm (1250 mg/m^3) test group animals constitutes a LOAEL.

Table 17. Treatment-Related Neurobehavioral Test Results in Rats Following a Single Eight-Hour Inhalation Exposure to 1,2,4-Trimethylbenzene (McKee et al., 2010)

	Visual Discrimination Tests (mean ± SD)			
Concentration ppm (mg/m^3) *n* = 8/group	**Discrimination Ratio[1]**	**Trial Response Latency[2]**	**Latency > 6 seconds[3]**	**Drink Response Latency[4]**
0	0.86 ± 0.02	1.70 ± 0.18	**3.88 ± 0.58**	0.26 ± 0.01
25 (125)	0.91 ± 0.03	2.38 ± 0.43	**5.00 ± 1.69**	0.30 ± 0.02
250 (1250)	0.91 ± 0.01	2.52 ± 0.40	**6.00 ± 1.34**	0.43 ± 0.03[a]
1000 (5000)	0.95 ± 0.01[a]	3.91 ± 0.73[a]	**10.63 ± 1.80[a]**	0.49 ± 0.03[a]

Data sets are included if they show a clear observed dose-response in trial responses or latency. Bolded values in the table indicate the data that were used to derive the Acute REL.
Abbreviation: SD = standard deviation.
[a] p <0.05;
[1] number of correct trial responses divided by the number of trial responses.
[2] the latency (seconds) to make a correct trial response.
[3] the number of responses taking more than 6 seconds.
[4] the mean latency (seconds) to obtain reinforcement.

Several neurological endpoints from the McKee et al. (2010) study can be modeled using BMR methodology (visual discrimination Latency > 6 seconds, Latency < 2 Seconds, Drink Response Latency). Because the acute REL is meant to protect against an infrequent, 1 hour exposure, the data sets chosen for BMD analysis comprise those responses seen after the first 8 hours of exposure. Benchmark dose analysis was performed using BMDS version 3.2 (US EPA, 2020). A BMR equal to one standard deviation change in the model-estimated control mean for neurotoxic effects ($BMDL_{1SD}$) was used. As described above, exposed animals at the 250 ppm (1250 mg/m^3) concentration for the drink response latency endpoint were significantly different than control animals in the preexposure analysis; thus, this data set was not used for deriving the POD. While there was not a clear dose response for the Latency < 2 seconds test, this visual discrimination endpoint was significantly different compared to controls at the 1000 ppm (5000 mg/m^3) exposure level. It can be modeled using BMDS but yields a BMR that is higher than the result for the Latency > 6 seconds endpoint, so it was not considered for the POD.

BMR results for the visual discrimination Latency > 6 seconds test are shown in Table 18, below; the accompanying graph is shown in Figure 4. Of the four BMD models that provided an acceptable fit to the data (Linear, Power, Polynomial Degree 2 and 3), all yielded identical BMCL results, including identical AIC values. The BMD model that provided the best visual fit to the data was the Polynomial Degree 2 model (shown in bold in the table). All data were modeled using a modeled (non-constant) variance.

The 8-hour study exposure duration was adjusted for a 1-hour exposure using a modified Haber's Law equation, $C^n \times T = k$, where $n = 3$: $(709\ mg/m^3)^3 \times 8\ hr = 2.85 \times 10^9$; and $(2.85 \times 10^9 / 1\ hr)^{1/3} = 1417\ mg/m^3$ (OEHHA, 2008). This yields an adjusted $BMCL_{1SD}$ of 1417 mg/m^3 (288 ppm) 1,2,4-TMB.

Table 18. BMR Modeling Results for Latency> 6 Seconds, McKee et al. (2010) 1,2,4-Trimethylbenzene Study

BMD Model (NCV)	BMC_{1SD} (mg/m^3)	$BMCL_{1SD}$ (mg/m^3)	Goodness-of-fit *p*-value	AIC	Scaled Residual for Dose Group near BMD
Polynomial Degree 2	**970.57**	**708.96**	**0.105**	**118.59**	**0.12**
Polynomial Degree 3	970.57	708.96	0.105	118.59	0.12
Power	970.57	708.96	0.105	118.59	0.12
Linear	970.57	708.96	0.105	118.59	0.12

Abbreviations: AIC = Akaike Information Criterion; BMC_{1SD} = benchmark concentration, 1 SD change from control mean; $BMCL_{1SD}$ = lower 95% confidence limit on the benchmark concentration, 1 SD change from control mean; BMD = benchmark dose; BMR = benchmark response; NCV = non-constant variance; ppm = parts per million; TMB = trimethylbenzene. BMRs represent continuous data model runs (mean ± SD), and one SD change from control mean. Bolded values in the table indicate the data used to derive the Acute REL.

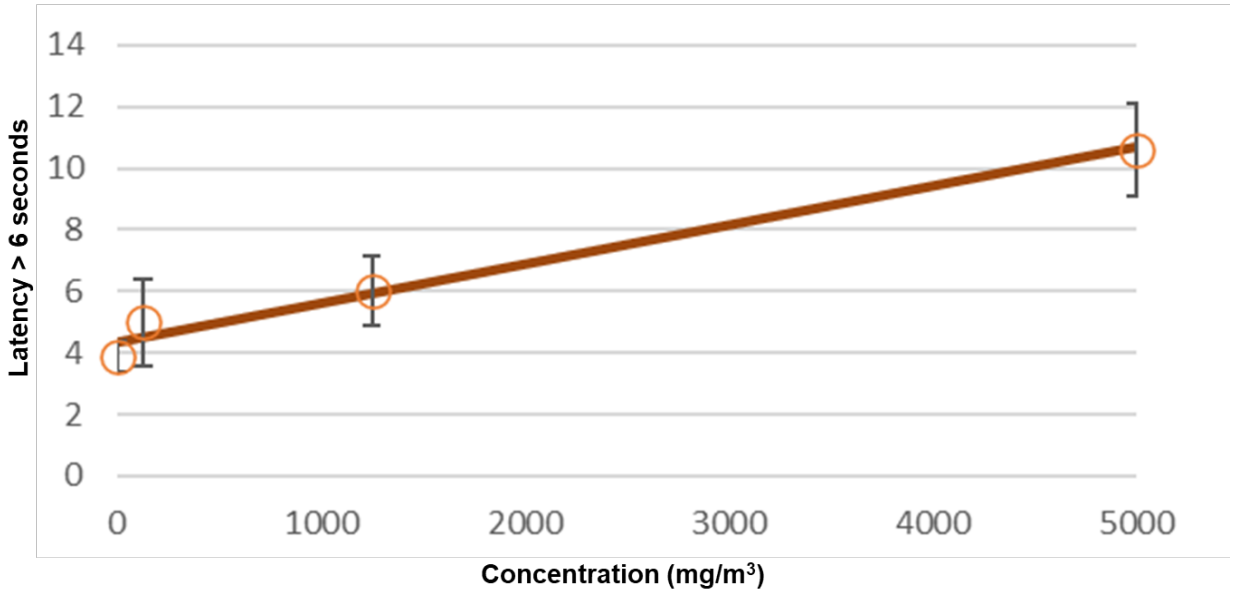


Figure 4. Polynomial Degree 2 Model (BMR_{1SD}) fit to the McKee et al. (2010) 1,2,4-Trimethylbenzene study for neurotoxicity in male rats (concentration in mg/m^3). Open circles represent data points.

For calculation of the Human Equivalent Concentration (HEC), the following equation is used:

HEC = Average Exposure Concentration × Regional Gas Dose Ratio (RDGR)

For gases with systemic effects, the RGDR is assumed to be the ratio of the animal blood:air partition coefficient $(H_{b/g})_A$ to the human blood:air partition coefficient $(H_{b/g})_H$ [RGDR = (Hb/g)A/(Hb/g)H] (OEHHA, 2008). For the 1,2,4-TMB isomer, the blood:air partition coefficients are similar, 57.7 and 59.1, respectively, for rats and humans (US EPA, 2016). This yields an RGDR of 0.98 (rounded to 1), indicating that effective rat and human TMB exposures at a given TMB concentration will be similar.

The interspecies UF = 6 (2 × √10): an interspecies toxicokinetic $UF_{A\text{-}k}$ of 2 is applied to account for residual toxicokinetic differences when using the HEC adjustment. A default interspecies toxicodynamic $UF_{A\text{-}d}$ of √10 is applied, since no data on toxicodynamic interspecies differences were available.

The intraspecies UF = 100 (10 × 10): this was comprised of a default intraspecies toxicokinetic $UF_{H\text{-}k}$ of 10, and an intraspecies toxicodynamic $UF_{H\text{-}d}$ of 10. The intraspecies toxicodynamic $UF_{H\text{-}d}$ was increased to 10 from the default factor of 3 because TMBs are neurotoxicants, and children are potentially more sensitive than

adults to neurotoxicants. The cumulative UF of 600 results in an acute REL of 2400 μg/m^3 (490 ppb) 1,2,4-TMB.

Limitations of the McKee et al. (2010) study include the following: only adult male rats were tested, data reporting for some tests were incomplete (data results were not provided for the second 8-hr exposures for visual discrimination performance tests), the duration of exposure was imprecise (*i.e.*, animals were exposed "up to 8 hours a day"), and behavioral experiments were not conducted during the exposure period. The authors state, "based on previous pharmacokinetic work with TMB, these hydrocarbons have half times in the CNS of approximately an hour". Functional observations were completed 10 to 25 minutes after termination of exposure, whereas motor activity assessment and visual discrimination performance testing was completed within 1 hour after termination of exposure. This suggests that TMB concentrations in the CNS may have decreased by the time motor activity assessment and visual discrimination performance testing was completed, which could have resulted in reduced test sensitivity.

In support of the acute REL, significantly reduced latencies in neurobehavioral tests have been observed in several other animal studies following exposure to TMBs. A significantly reduced latency in the passive avoidance test in rats was reported in several studies (males only were tested) following repeated exposure to 1,2,4-TMB or 1,3,5-TMB (Gralewicz et al., 1997; Gralewicz and Wiaderna, 2001; Wiaderna et al., 2002). Other neurotoxicity studies in male rodents (mice and rats) also observed adverse effects following acute exposure to TMBs: sensorimotor impairment (a deficit in rotarod performance and a decrease in pain sensitivity) was observed within 24 hours following exposure to 1,2,4-TMB at concentrations of 100 to 250 ppm (492 to 1230 mg/m^3) (Korsak et al., 1995, Korsak and Rydzynski, 1996).

8.2 Trimethylbenzenes Chronic Reference Exposure Level

The chronic REL is a concentration at or below which adverse noncancer health effects would not be anticipated over the person's lifetime. RELs incorporate safety factors to protect sensitive human subpopulations (see Section 6 of the Noncancer REL TSD (OEHHA, 2008).

Study	Korsak and Rydzynski, 1996
Study population	Male Wistar rats (10/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 123, 492, and 1230 mg/m^3 (0, 25, 100, or 250 ppm) 1,2,3-TMB
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Critical effects	Neurotoxic effects (pain sensitivity behavior and rotarod performance)
LOAEL	123 mg/m^3 (25 ppm)
NOAEL	Not observed
Benchmark Concentration ($BMCL_{1SD}$) (using continuous model)	47 mg/m^3 (10 ppm)
Human equivalent concentration	47 mg/m^3 (10 ppm) (calculated RGDR = 0.98, rounded to 1)
Time-adjusted exposure	8 mg/m^3 (2 ppm) = (47 mg/m^3 × 6/24 × 5/7
LOAEL uncertainty factor (UF_L)	1 (with use of $BMCL_{1SD}$)
Subchronic uncertainty factor (UFs)	√10 (13 weeks = subchronic in rodent studies)
Interspecies uncertainty factors	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factors	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	2000
Reference Exposure Level	4 µg/m^3 (1 ppb)

Aside from acute inhalation chamber studies, there are no exposure studies in humans exposed uniquely to TMBs. In humans, an occupational study reported systemic effects in a significant number of the male workers (*n* = 27) exposed for several years to a paint thinner comprised of > 80% TMBs: CNS (nervousness, anxiety and/or vertigo), hematological, and respiratory effects (Battig et al.,1956). Other occupational studies involving inhalation exposure to TMB-containing VOC mixtures show a number of

adverse neurotoxic effects: neuropsychological changes (El Hamid Hassan et al., 2013; Chen et al., 1999), deficits in short-term memory and reduced motor speed/coordination (Lee et al., 2005), and visual dysfunction (Gong et al., 2002; Pratt et al., 2000). It is not known whether these effects are primarily attributable to TMBs.

There are a number of subchronic studies in rodents, all conducted by the Nofer Institute of Occupational Medicine in Poland, that have reported similar findings in animals (*i.e.,* CNS, respiratory, and hematological effects), and including clinical chemistry and organ weight effects, following exposure to TMBs (Gralewicz et al., 1997; Korsak and Rydzynsk, 1996; Korsak et al., 1997; Korsak et al., 2000a,b; Wiaderna et al., 1998, 2002). These studies are summarized in Table 13. The Korsak and Rydzynski (1996), Korsak et al. (1997), and Korsak et al. (2000a,b) studies all similarly exposed male Wistar rats to concentrations of 0, 25, 100 or 250 ppm (123, 492, 1230 mg/m^3) pure TMBs for 90 days duration. The NOAEL/LOAELs for all these studies are shown in Table 19, below.

Table 19. NOAEL/LOAEL Values for Subchronic Trimethylbenzene Rat Inhalation Studies

Study	TMB Isomer	Endpoint	NOAEL ppm (mg/m^3)	LOAEL ppm (mg/m^3)
Korsak and Rydzynski, 1996	1,2,3-TMB	Neurotoxicity	<25 (123)	25 (123)
	1,2,4-TMB	Neurotoxicity	25 (123)	100 (492)
Korsak et al., 1997	1,2,4-TMB	Respiratory	<25 (123)	25 (123)
Korsak et al., 2000a	1,2,3-TMB	Respiratory/Clinical Chemistry	25 (123)	100 (492)
Korsak et al., 2000b	1,2,4-TMB	Respiratory/Clinical Chemistry	25 (123) (females)	25 (123) (males)

Abbreviations: LOAEL = lowest observed adverse effects level; NOAEL = no observed adverse effects level; ppm = parts per million; TMB = trimethylbenzene.
Animals in all studies were Wistar rats, exposed 6 hr/day, 5 days/week to 0, 25, 100 or 250 ppm TMBs for 90 days.

There are several studies that can be used to develop a POD for the chronic REL. Korsak and Rydzynski (1996), and Korsak et al. (2000 a; b). Korsak and Rydzynski (1996) exposed male Wistar rats 6 hours/day, 5 days/week for 90 days to targeted concentrations of 0, 25, 100 or 250 ppm (0, 123, 492, 1230 mg/m^3) 1,2,4-TMB or 1,2,3-TMB in a dynamic inhalation chamber. The study authors did not explicitly state that the reported measures of variance in their data tables were SDs. However, an independent analysis conducted by US EPA confirmed that the reported measures of variance are SDs (US EPA, 2016). A decrease in pain sensitivity, measured as an increase in the latency of the paw-lick response to a hot-plate challenge, was concentration dependent and statistically significant at 100 and 250 ppm (492 and 1230 mg/m^3) 1,2,4-TMB and 25 and 250 ppm (123 and 1230 mg/m^3) 1,2,3-TMB. Further analysis by US EPA found that the response at the 100 ppm (492 mg/m^3) 1,2,3-TMB concentration was also statistically significant (p <0.01); thus, the response was significant at all applied concentrations for the 1,2,3-TMB isomer. Latency of 60 seconds was considered as 100% inhibition of pain sensitivity. Hot-plate behavior was tested immediately after termination of exposure. Neurotoxicity was also assessed using the rotarod performance test, which measures neuromuscular function. Rotarod performance was tested before exposure, weekly during the experiment, and two weeks after termination of exposure. Exposure to 1,2,4-TMB and 1,2,3-TMB caused statistically significant effects at concentrations of 100 and 250 ppm (492 and 1230 mg/m^3) 1,2,3-TMB, and at 250 ppm (1230 mg/m^3) 1,2,4-TMB. No recovery in rotarod performance was observed two weeks post-exposure. For both endpoints, 1,2,3-TMB was more neurotoxic than 1,2,4-TMB. Thus, the TMB LOAEL for pain sensitivity (1,2,3-TMB) is 25 ppm (123 mg/m^3), the lowest dose tested, and the NOAEL/LOAEL for rotarod performance is 25 and 100 ppm (123 and 492 mg/m^3), respectively (see Table 21, below).

In the Korsak et al. (2000a,b) TMB studies, treatment-related effects included hematological, clinical chemistry, and respiratory changes. A statistically significant decrease in red blood cell counts and an increase in white blood cell counts was seen in male rats at the highest concentration in both studies; the trend analysis showed that changes were concentration dependent (Korsak et al., 2000a,b). Two weeks post-exposure, RBC counts remained low. Other hematological changes included a decrease in reticulocyte counts in females from all treated groups (changes were significant at the highest concentration), and decreased clotting time in males and females of all treated groups (changes were significant at $\geq$ 25 ppm or 123 mg/m^3) (Korsak et al., 2000b).

For clinical chemistry parameters like sorbitol dehydrogenase activity (a marker of liver damage), the lowest exposure concentration of 25 ppm (123 mg/m^3) was a LOAEL in males and a NOAEL in females (Korsak et al., 2000b). Significant increases in relative liver weight were seen in male rats exposed to the high concentration of 1,2,3-TMB,

1230 mg/m^3 (Korsak et al., 2000a). These effects point towards evidence of hepatotoxicity.

Histopathological changes were also seen in the lower respiratory system at all three exposure concentrations (Korsak et al., 2000 a; b). These changes were statistically significant at the mid- and high-concentrations for the 1,2,4-TMB isomer (Korsak et al., 2000b). Changes included lymphoepithelium formation (loss of bronchial epithelium's cuboidal character, forming lymphoepithelium) in male rats at the lowest and mid concentrations, 25 and 100 ppm (123 and 492 mg/m^3) 1,2,3-TMB, respectively. A significant increase in the intensity of lung perivascular and interstitial infiltration in male rats was seen at the highest concentration (Korsak et al., 2000a); trend analysis showed the changes were related to concentration.

Endpoints from several of these studies can be modeled using BMD methodology. Benchmark dose analysis was performed using BMDS version 3.2 (US EPA, 2020). The BMR used was equal to one standard deviation change in the model-estimated control mean for neurotoxic effects ($BMDL_{1SD}$). BMR results from the Korsak and Rydzynski (1996) and Korsak et al. (2000a,b) studies are shown in Table 20, below. The Korsak et al. (1997) rat study, which identified a LOAEL of 25 ppm (123 mg/m^3) for sensory irritation, did not report the incidences of pulmonary lesions, but rather the RD_{50} and the 95% CI of the RD_{50}. It was therefore not possible to analyze the pulmonary lesion data using BMD software.

The Korsak and Rydzynski (1996) study in rats was chosen for the development of the 8-hour REL because it is a 90-day study with a critical effect for TMBs (neurotoxicity) and yields the lowest POD, a $BMCL_{1SD}$ of 46.7 mg/m^3 (10 ppm) for 1,2,3-TMB (See Table 20, below).

Table 20. BMR Modeling Results for Different Endpoints from Korsak et al. Subchronic Trimethylbenzene Studies

Study (TMB Isomer)	Endpoint	BMD Model	CL	BMC_{1SD} (ppm)	BMC_{1SD} (mg/m^3)	$BMCL_{1SD}$ (ppm)	$BMCL_{1SD}$ (mg/m^3)
Korsak and Rydzynski, 1996 (1,2,3-TMB)	Neurotoxicity (pain sensitivity)	Exponential #4	0.95	18	86.2	10	46.7
Korsak and Rydzynski, 1996 (1,2,4-TMB)	Neurotoxicity (pain sensitivity)	Exponential #4	0.95	33	161	17	84
Korsak et al., 2000b (1,2,4-TMB)	Hematological Effects (RBC count)	Polynomial	0.95	93	456	46	227
Korsak et al., 2000a (1,2,3-TMB)	Hepatotoxicity (sorbitol dehydrogenase)	Polynomial	0.95	117	575	49	241

Abbreviations: BMC_{1SD} = benchmark concentration, 1 SD change from control mean; $BMCL_{1SD}$ = lower 95% confidence limit on the benchmark concentration, 1 SD change from control mean; BMD = benchmark dose; BMR = benchmark response; CL = confidence limit; mg/m^3 = milligram per cubic meter; ppm = parts per million; RBCs = red blood cells; TMB = trimethylbenzene. BMRs represent continuous data model runs (mean $\pm$ SD), and one SD change from control mean

Table 21, below, shows the exposure effects data used for the dose-response modeling. According to US EPA (2016), “neurobehavioral changes reported in the available TMB studies are regarded as adverse and, in particular, decreased pain sensitivity, measured as an increased latency to paw-lick in hot plate tests, represents an alteration in neurobehavioral function”.

Table 21. Pain Sensitivity (Latency of the Paw-Lick Response) Results from the Korsak and Rydzynski (1996) Trimethylbenzene Neurotoxicity Study in Rats

TMB Isomer	Endpoint	Exposure Concentration[a]			
		Control	25 ppm (123 mg/m^3)	100 ppm (492 mg/m^3)	250 ppm (1230 mg/m^3)
1,2,4-TMB	No. of Animals	9	10	9	10
	Paw-lick (sec)	15.4 ± 5.8	18.2 ± 5.7	27.6 ± 3.2**	30.1 ± 7.9**
1,2,3-TMB	No. of Animals	30	20	10	10
	Paw-lick (sec)	9.7 ± 2.1	11.8 ± 3.8*	16.3 ± 6.3##	17.3 ± 3.4**

[a] Paw-lick latency values are expressed as mean ± 1 SD.
*$p \leq 0.05$, ** $p \leq 0.01$.
Not reported as statistically significant in Table 1 from Korsak and Rydzynski (1996); however, the results of an *ad hoc* t-test (performed by US EPA) indicated significance at $p < 0.01$.
Abbreviations: mg/m^3 = milligram per cubic meter; ppm = part per million; TMB = trimethylbenzene.

For all BMD analyses of the Korsak and Rydzynski (1996) TMB data, a one standard deviation (SD) change in the control mean was used as the benchmark response (BMR) for all endpoints. For each endpoint, BMDS continuous models were fitted to the data using the maximum likelihood method. In cases where the homogeneity of the variances was rejected *(i.e.,* X^2 *p*-value <0.10), the non-constant variance was modeled. BMD modeling output is shown in Table 22, below.

Exponential (model # 4) for the 1,2,3-TMB isomer yields the lowest $BMDL_{1SD}$, 10 ppm (46.7 mg/m^3). BMD model selection is shown in boldface in Table 22, below; the accompanying graph is shown in Figure 5. Data were modeled using a non-constant variance model. BMD analysis of the 1,2,4-TMB isomer dataset yields a somewhat higher $BMDL_{1SD}$ of 17 ppm (83.6 mg/m^3). Data were modeled using a constant variance model. This BMD modeling output is also shown in Table 22.

Table 22. Lowest BMDS Model Results for Neurotoxicity (Pain Sensitivity - Latency of the Paw-Lick Response) in Male Rats following 90-day Trimethylbenzene Inhalation Exposure (Korsak and Rydzynski, 1996)

TMB Isomer	BMD Model	Variance Model	BMC_{1SD} ppm (mg/m^3)	$BMCL_{1SD}$ ppm (mg/m^3)	Goodness-of-fit *p*-value	AIC	Scaled Residual for Dose Group near BMD
1,2,4-TMB	Exponential #4	Constant	32.76 (161)	17.06 (83.9)	0.38	247.98	-0.62
1,2,3-TMB	**Exponential #4**	**Modeled**	**17.54 (86.2)**	**9.5 (46.7)**	**0.30**	**370.07**	**-0.88**

Abbreviations: AIC = Akaike Information Criterion; BMC_{1SD} = benchmark concentration, 1 SD change from control mean; $BMCL_{1SD}$ = lower 95% confidence limit on the benchmark concentration, 1 SD change from control mean; mg/m^3 = milligram per cubic meter; ppm = parts per million; TMB = trimethylbenzene.
Note: Bolded text indicates variance model and values used to derive the REL. Results are from benchmark dose analysis using Benchmark Dose Software (BMDS) version 3.2 (US EPA, 2020).

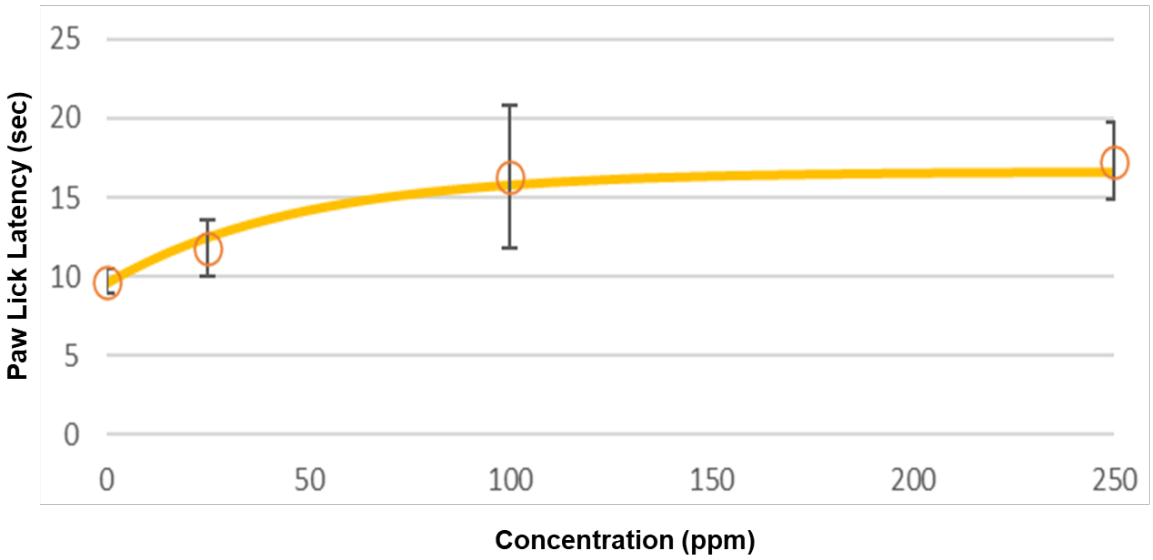


Figure 5. Exponential 4 Model (BMR_{1SD}) fit to the 90-day 1,2,3-Trimethylbenzene Korsak and Rydzynski (1996) study for neurotoxicity in male rats, non-constant variance (concentration in ppm). Open circles represent data points.

For calculation of the Human Equivalent Concentration (HEC), the following equation is used: HEC = Average Exposure Concentration × RDGR. For gases with systemic effects, such as TMBs, the RGDR is assumed to be the ratio of the animal blood:air partition coefficient $(H_{b/g})_A$ to the human blood:air partition coefficient $(H_{b/g})_H$ (OEHHA, 2008). For both the 1,2,4-TMB and 1,2,3-TMB isomers, the rat and human blood:air partition coefficients are similar, and yield an RGDR of 0.98, rounded to 1. (Refer to Table 6 for the blood:air partition coefficients).

The POD of 47 mg/m^3 (10 ppm) is then adjusted for a continuous 24-hour exposure (6/24 hours × 5/7 days per week). This yields an adjusted $BMCL_{1SD}$ of 8 mg/m^3 (2 ppm) 1,2,3-TMB.

A total UF of 2000 was then applied to the $BMDL_{1SD}$ (adj) of 8 mg/m^3 (2 ppm). A sub chronic UF of √10 was applied because the Korsak and Rydzynski (1996) study is a 13-week study and therefore considered to be a subchronic study. For interspecies uncertainty, a total overall UF of 6 was used (2 × √10); a factor of 2 for residual toxicokinetic differences (a HEC approach was used) and √10 for the toxicodynamic component of the interspecies UF ($UF_{A\text{-}d}$). For intraspecies uncertainty, a total UF of 100 (10 × 10) was used; 10 for the toxicokinetic component of the UF ($UF_{H\text{-}k}$) and 10 for the toxicodynamic component of the UF ($UF_{H\text{-}d}$) (instead of the default √10) because TMBs are neurotoxicants and are likely to impact infants/children disproportionately. This yields a chronic 1,2,4-TMB inhalation REL of 4 µg/m^3 (1 ppb).

US EPA, in its recent development of an inhalation Reference Exposure Concentration (RfC) for TMBs, also used the Korsak and Rydzynski (1996) 13-week rat neurotoxicity study as their POD (US EPA, 2016). US EPA used a physiologically-based pharmacokinetic (PBPK) model for estimating internal blood dose metrics (area under the curve, AUC) to derive HECs. US EPA based its PBPK model on a modification of the human PBPK inhalation model developed by Hissink et al. (2007). This included revising/optimizing several of the original model parameters (such as the Michaelis-Menten constants). The US EPA model also updated values for blood flow and organ volumes consistent with published sources. US EPA concluded that, "the optimized model produces acceptable simulations of venous blood 1,2,4-TMB for chronic exposure to $\leq$ 100ppm (492 mg/m^3) for rats or $\leq$ 30 ppm (147.6 mg/m^3) for humans 1,2,4,-TMB by inhalation" (but not for higher concentrations). US EPA did not parameterize the model for pregnant animals, did not include a fetal compartment, nor did they attempt to adapt the 1,2,4-TMB model to the other two isomers. Thus, the model could not be used to account for non-continuous exposures in studies that investigated effects following subchronic exposures to 1,2,3-TMB or 1,3,5-TMB, or gestational exposures to 1,2,4-TMB. Instead, US EPA used default HEC conversion factors for these two chemicals, and duration-adjusted PODs for maternal/developmental effects.

US EPA made very limited use of the modified PBPK model in its RfC analysis (*i.e.,* only used for HEC adjustment) for 1,2,4-TMB. In order to avoid the uncertainty around enzyme induction in rats above 100 ppm 1,2,4-TMB, US EPA relied on external exposure concentrations as the dose metric in BMDS model runs (as did OEHHA). US EPA then used the PBPK model to convert the POD (in ppm) to AUC in venous blood in rats, followed by conversion of the AUC to a continuous exposure in humans. The US EPA chronic TMB RfC is 0.061 mg/m^3. OEHHA opted to use the default HEC calculations and chose not to use the PBPK approach given the limitations and uncertainties associated with the US EPA's modified model and paucity of data on the appropriate dose metrics based on mode of action.

8.3 Trimethylbenzenes 8-hour Reference Exposure Level

Study	Korsak and Rydzynski, 1996
Study population	Male Wistar rats (10/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 123, 492, and 1230 mg/m^3 (0, 25, 100, or 250 ppm) to 1,2,3-TMB
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Critical effects	Neurotoxic effects (pain sensitivity behavior and rotarod performance)
LOAEL	123 mg/m^3 (25 ppm)
NOAEL	Not observed
BMCL1SD	47mg/m^3 (10 ppm)
Human equivalent concentration	47 mg/m^3 (10 ppm) (RGDR = 0.98, rounded to1)
Time-adjusted exposure	17 mg/m^3 (3 ppm) = 47 mg/m^3 × 6/24 × 5/7 × 20/10)
LOAEL uncertainty factor (UFL)	1 (with use of BMCL1SD)
Subchronic uncertainty factor (UFs)	√10 (13 weeks = subchronic in rodent studies)
Interspecies uncertainty factors	
Toxicokinetic (UFA-k)	2
Toxicodynamic (UFA-d)	√10
Intraspecies uncertainty factors	
Toxicokinetic (UFH-k)	10
Toxicodynamic (UFH-d)	10
Cumulative uncertainty factor	2000
Reference Exposure Level	8 µg/m^3 (2 ppb)

The 8-hour REL is based on the same study as the chronic REL (Korsak and Rydzynski, 1996) and uses the same benchmark dose analysis with a POD of 10 ppm (47 mg/m^3) 1,2,3-TMB. In this instance the time adjusted exposure reflects conversion from a continuous to an intermittent exposure: the POD of 10 ppm (47 mg/m^3) is adjusted for a continuous 24-hour exposure (6/24 hours × 5/7 days per week), and then multiplied by 2 (20 m^3/10 m^3) to represent an active worker breathing half the 24-hour inspiration volume of air during an 8-hour workday. This yields an adjusted $BMCL_{1SD}$ of 3 ppm (17 mg/m^3) 1,2,3-TMB. The same uncertainty factors apply to give a cumulative UF of 2000 and an 8-hour REL of 8 µg/m^3 (2 ppb).

8.4 RELs for the Mixed TMB Isomers

The acute TMB REL was developed using 1,2,4-TMB isomer data and the chronic and 8-hour RELs using 1,2,3-TMB isomer data because the critical studies in each instance generated lower REL values using those isomers than did data for the other TMB isomers. BMDS analyses showed the 1,2,4-TMB and 1,2,3-TMB isomers to be comparable in their toxicities for the critical endpoint evaluated, neurotoxicity. The three TMB isomers share multiple similarities regarding their chemical, toxicokinetic, and toxicological properties that support adopting one isomer's value for the others. Thus, it is expected that the REL values developed using the 1,2,4- and 1,2,3-TMB isomers would be adequately health protective for the other TMB isomers. US EPA has adopted a similar approach in the development of their RfCs for the TMB isomers (US EPA, 2016).

9. Evidence for Differential Sensitivity of Children

Infants and children may be more susceptible to the toxic effects of TMB exposure due to developmental/physiological differences between children and adults, and also because of the type of adverse effects (neurotoxic, respiratory) associated with exposure to TMBs. That is, developing organisms may show greater sensitivity to neurological and respiratory toxicants than adults. Children have higher rates of respiration, and greater lung surface area than adults. Infants and children may be more susceptible to the effects of neurotoxicants and respiratory system toxicants because their nervous systems are still developing. OEHHA considers substances that cause either neurotoxicity or respiratory toxicity to disproportionately impact infants and children (OEHHA, 2001).

The activities of the enzyme systems that metabolize TMBs (*i.e.,* the CYT P450s) and render them less toxic (*i.e.,* glucuronic acid, glycine, and sulfates) are reduced in children up to 1 year of age (Ginsberg et al., 2004). Renal clearance in young infants, up to 2 months of age, is also decreased in early life (Ginsberg et al., 2004). Potentially, at similar exposure levels, blood concentrations of the parent TMBs in infants and young children may be higher than adults and may persist longer.

Additionally, individuals with pre-existing respiratory conditions (*e.g.*, asthma, allergies) may be more sensitive to the respiratory effects resulting from exposure to TMBs. One study in France reported a significant association between 1,2,4-TMB concentrations in indoor air and asthma (Billionnet et al., 2011) (see Section 6.1). Prevalence rate statistics indicate that a significantly higher percentage of children have asthma than adults, indicating that chemicals that induce or exacerbate asthma will have a

disproportional impact on children (OEHHA, 2001). The acute, 8-hour and chronic RELs include UFs to account for these potential differences.

10. References

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