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Toxicological Review of Trimethylbenzenes

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

Supplemental Information

August *2013*

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

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Figure C-13. Plot of mean response by dose for decreased red blood cells in male Wistar rats, with fitted curve for Hill model with constant variance. C-34

ABBREVIATIONS

1,2,3-TMB	1,2,3-trimethylbenzene
1,2,4-TMB	1,2,4-trimethylbenzene
1,3,5-TMB	1,3,5-trimethylbenzene
AAQC	Ambient air quality criterion
ABR	amount of 1,2,4-TMB in the brain
ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, distribution, metabolism and excretion
AEGL	Acute exposure guideline limit
AIC	Akaike Information Criterion
BAL	bronchoalveolar lavage
BMD	benchmark dose
BMDL	lower confidence limit on the benchmark dose
BMDS	benchmark dose software
BMR	benchmark response
BW	body weight
CAS	Chemical Abstracts Service
CI	confidence interval
CMIX	average of arterial and venous blood concentrations
CNS	central nervous system
CV	concentration in venous blood
CVS	concentration in venous blood exiting slowly perfused tissues
CXEQ	concentration in exhaled breath
DMBA	dimethylbenzoic acid
DMHA	dimethylhippuric acid
EC_{50}	half maximal effective concentration
EPA	U.S. Environmental Protection Agency
GD	gestational day
HEC	human equivalent concentration
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
K_m	Michaelis-Menten constant
LOAEL	lowest-observed-adverse-effect level
NCEA	National Center for Environmental Assessment
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No-observed-adverse-effect level
OMOE	Ontario Ministry of the Environment
p	probability value
PBPK	physiologically based pharmacokinetic (model)
POD	point of departure
POI	Point of impingement
QPC	alveolar ventilation rate
QRTOTC	sum of fractional flows to rapidly perfused tissues, liver, and brain
QSTOTC	sum of fractional flows to slowly perfused tissues
RBC	red blood cell
RD_{50}	50% respiratory rate decrease
REL	Recommended exposure limit
RfC	reference concentration
RfD	reference dose
ROS	reactive oxygen species
SD	standard deviation
SE	standard error
TLV	threshold limit value
TMB	trimethylbenzene
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UV	ultraviolet
VLC	volume of fat
V_{max}	½ maximal enzyme rate
VOC	volatile organic compound
W	watt
WBC	white blood cell
WS	white spirit
χ^2	chi-squared

APPENDIX A. HEALTH ASSESSMENTS AND REGULATORY LIMITS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

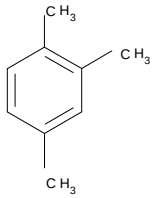
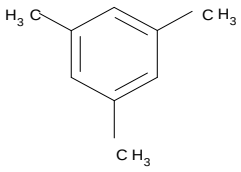
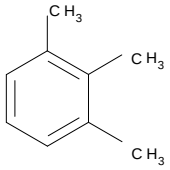
Table A-1. Other national and international health agency assessments for TMBs

Agency	Toxicity value
National Institue for Occupational Safety and Health (NIOSH, 1992, 1988)	Recommended Exposure Limit (REL) for TMBs – 25 ppm (123 mg/m^3) time weighted average for up to a 10 hour work day and a 40 hour work week, based on the risk of skin irritation, central nervous system depression, and respiratory failure (Battig et al., 1956)
American Conference of Governmental Industrial Hygienists (ACGIH, 2002)	Threshold Limit Value (TLV) for VOC mixture containing 1,2,4-TMB and 1,3,5-TMB – 25 ppm (123 mg/m^3) time weighted average for a normal 8-hour work day and a 40-hour work week, based on the risk of irritation and central nervous system effects (Battig et al., 1956)
National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (U.S. EPA, 2007)	Acute Exposure Guideline Level (AEGL)-1 (nondisabling) – 180 ppm (890 mg/m^3) to 45 ppm (220 mg/m^3) (10 min to 8 hrs, respectively) (Korsak and Rydzyński, 1996) AEGL-2 (disabling) – 460 ppm (2,300 mg/m^3) to 150 ppm (740 mg/m^3) (10 min to 8 hrs, respectively) (Gage, 1970)
Ontario Ministry of the Environment (MOE, 2006)	For TMBs: 24 hr Ambient Air Quality Criterion (AAQC) – 0.3 mg/m^3 based on CNS effects; half-hour Point of Impingement (POI) – 0.9 mg/m^3 based on CNS effects (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997b; Korsak and Rydzyński, 1996)

APPENDIX B. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-REPONSE ANALYSIS

B.1. PHYSICAL AND CHEMICAL PROPERTIES

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

Property	1,2,4 TMB	1,3,5 TMB	1,2,3 TMB
CAS Registry Number	95-63-6	108-67-8	526-73-8
Synonym(s)	1,2,4-Trimethylbenzene, pseudocumene, asymmetrical trimethylbenzene	1,3,5-Trimethylbenzene, mesitylene, symmetrical trimethylbenzene	1,2,3-Trimethylbenzene, hemimellitene, hemellitol, pseudocumol
Molecular formula	C_9H_{12}	C_9H_{12}	C_9H_{12}
Molecular weight	120.19	120.19	120.19
Chemical structure			
Melting point, °C	-43.8	-44.8	-25.4
Boiling point, °C @ 760 mm Hg	168.9	164.7	176.1
Vapor pressure, mm Hg @ 25°C	2.10	2.48	1.69
Density, g/mL at 20 °C	0.8758	0.8637	0.8944
Flashpoint, °C	44	50	44
Water solubility, mg/L at 25 °C	57	48.2	75.2
Other solubilities	ethanol, benzene, ethyl ether, acetone, petroleum ether	alcohol, ether, benzene, acetone, oxygenated and aromatic solvents	ethanol, acetone, benzene, petroleum ether
Henry's law constant, atm m^3/mol	6.16×10^{-3}	8.77×10^{-3}	4.36×10^{-3}
Log K_{OW}	3.78	3.42	3.66
Log K_{OC}	2.73	2.70-3.13	2.80–3.04
Bioconcentration factor	439	234	133–259
Conversion factors	1 ppm = 4.92 mg/m^3 1 mg/m^3 = 0.2 ppm	1 ppm = 4.92 mg/m^3 1 mg/m^3 = 0.2 ppm	1 ppm = 4.92 mg/m^3 1 mg/m^3 = 0.2 ppm
Source: (HSDB, 2011a, b, c; U.S. EPA, 1987)			

B.2. TOXICOKINETICS

There has been a significant amount of research conducted on the toxicokinetics of 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB in experimental animals and humans. In vivo studies have been conducted to evaluate the adsorption, distribution, metabolism and excretion (ADME) of all isomers following exposure via multiple routes of exposure in rats (Swiercz et al., 2006; Tsujimoto et al., 2005; Swiercz et al., 2003; Swiercz et al., 2002; Tsujino et al., 2002; Tsujimoto et al., 2000; Eide and Zahlsen, 1996; Zahlsen et al., 1990; Huo et al., 1989; Dahl et al., 1988; Mikulski and Wiglusz, 1975) and human volunteers (Janasik et al., 2008; Jones et al., 2006; Järnberg et al., 1997a; Järnberg et al., 1997b; Kostrzewski et al., 1997; Järnberg et al., 1996; Kostrewski and Wiaderna-Brycht, 1995; Fukaya et al., 1994; Ichiba et al., 1992). The following sections provide a summary of the toxicokinetic properties for all three isomers. For complete details regarding the toxicokinetics of TMB isomers in humans and animals, see Tables B-46-B-64 in Appendices B.6-B.8.

B.2.1. Absorption

Both humans and rats readily absorb 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB into the bloodstream following exposure via inhalation. Humans (n = 9-10, Caucasian males) exposed to 25 ppm (123 mg/m^3) 1,2,4-TMB or 1,3,5-TMB for 2 hours exhibited similar maximum capillary blood concentrations (6.5 ± 0.88 and 6.2 ± 1.6 µM, respectively [digitized data]), whereas absorption for 1,2,3-TMB was observed to be higher (7.3 ± 1.0 µM [digitized data]) (Järnberg et al., 1998, 1997a; Järnberg et al., 1996). Kostrewski et al. (1997) observed equivalent maximal capillary blood concentrations in humans (n = 5) exposed to 30.5 ppm (150 mg/m^3) 1,2,4-TMB or 1,3,5-TMB for 8 hours (8.15 ± 1.4 and 6.3 ± 1.0 µM, respectively). In the same study, human volunteers exposed to 100 mg/m^3 (20.3 ppm) 1,2,3-TMB had capillary blood concentrations of 4.3 ± 1.1 µM. In humans (n = 4, 2 male, 2 female) exposed to 25 ppm (123 mg/m^3) 1,3,5-TMB for 4 hours, venous blood concentrations were markedly lower (0.85 µM [no SD reported]), but this may be related to measurement of 1,3,5-TMB in the venous blood (Jones et al., 2006). 1,3,5-TMB has a higher blood:fat partition coefficient (230) than 1,2,4-TMB (173) or 1,2,3-TMB (164) (Järnberg and Johanson, 1999) and therefore much of the 1,3,5-TMB absorbed into capillary blood may preferentially distribute to adipose tissue before entering into the venous blood supply. Measurements of respiratory uptake of 1,2,4-TMB, 1,2,3-TMB, or 1,3,5-TMB are similar in humans (n = 10, Caucasian males) (60 ± 3%, 48 ± 3%, and 55 ± 2%, respectively).

In rats, rapid absorption into the bloodstream was observed in many studies following single exposures to 1,2,4-TMB, with maximal blood concentrations of 537 ± 100, 221 (no SD reported), and 64.6 ± 13.6 µM observed after exposures to 1,000 ppm (4,920 mg/m^3) for 12 hours, 450 ppm (2,214 mg/m ^{3}) for 12 hours, and 250 ppm (1,230 mg/m^3) for 6 hours (Swiercz et al., 2003; Eide and Zahlsen, 1996; Zahlsen et al., 1990). Zahlsen et al. (1990) observed a decrease in blood concentrations of 1,2,4-TMB following repeated exposures, which they attribute to induction of metabolizing enzymes; a similar decrease in 1,2,4-TMB blood concentrations following repeated exposures was not observed in Swiercz et al. (2003). Using a 4-comparment toxicokinetic model, Yoshida et al. (2010) estimated that a rat exposed to 50 µg/m^3 1,2,4-TMB for 2 hours would absorb 6.6 µg/kg body weight (no SD reported). Using this same model, the authors estimated that humans exposed to 24 µg/m^3 1,2,4-TMB for 2 hours would absorb 0.45 µg/kg body weight (no SD reported). 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB have also been observed to be absorbed and distributed via blood circulation following oral and dermal exposures in rats (Tsujino et al., 2002; Huo et al., 1989). Lastly, calculated blood:air partition coefficients for 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB (43.0 [40.8-45.2], 66.5 [63.7-69.3], and 59.1 [56.9-61.3], respectively) were similar in humans (n = 10, 5 male, 5 female), indicating that the two isomers would partition similarly into the blood (Järnberg and Johanson, 1995). Additionally, the blood:air partition coefficients between humans and rats were very similar for all three isomers: 1,2,4-TMB (43.0 vs. 55.7), 1,2,3-TMB (66.5 vs. 62.6), and 1,3,5-TMB (59.1 vs. 57.7) (Meulenberg and Vijverberg, 2000). This further indicates patterns of absorption would be similar across species.

B.2.2. Distribution

No information exists regarding the distribution of any isomer in adult humans. However, experimentally calculated tissue-specific partition coefficients were similar for all three isomers across a number of organ systems (fat, brain, liver, muscle, and kidney) (Meulenberg and Vijverberg, 2000). This strongly indicates that 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB can be expected to partition similarly into these various organ systems. Trimethylbenzenes (unspecified isomer) have also been detected in cord blood, and therefore can be expected to partition into the fetal compartment (Cooper et al., 2001; Dowty et al., 1976). In rats, 1,2,4-TMB was observed to distribute widely to all examined organ systems following oral exposure, with the highest concentrations found in the stomach (509 ± 313 μg/g) and adipose tissue (200 ± 64 μg/g) (Huo et al., 1989). Following inhalation exposures, 1,2,4-TMB and 1,3,5-TMB were observed to distribute to all tissues examined, with tissue-specific concentrations dependent on the external exposure concentration (Swiercz et al., 2006; Swiercz et al., 2003; Eide and Zahlsen, 1996). 1,2,4-TMB distributed to the adipose tissue to a much higher degree than to the brain, liver, or kidneys (Eide and Zahlsen, 1996). Venous blood concentrations of 1,2,4-TMB and 1,3,5-TMB and liver concentrations of 1,2,4-TMB were observed to be significantly lower in repeatedly exposed animals versus animals exposed only once to higher concentrations (Swiercz et al., 2006; Swiercz et al., 2003; Swiercz et al., 2002). Kidney concentrations of 1,3,5-TMB were observed to be lower in repeatedly exposed animals versus animals exposed once, but only at the lowest exposure concentration. The authors suggest that lower tissue concentrations of TMB isomers observed in repeatedly-exposed animals is mostly likely due to induction of metabolizing enzymes at higher exposure concentrations. This hypothesis is supported by the observation of P-450 enzyme induction in the livers, kidneys, and lungs of rats exposed to 1,200 mg/kg/day 1,3,5-TMB for 3 days (Pyykko, 1980).

1,2,4-TMB was also observed to distribute to individual brain structures, with the brainstem and hippocampus having the highest concentrations following exposure (Swiercz et al., 2003). Zahlsen et al. (1990) also observed decreasing blood, brain, and adipose tissue concentrations following repeated exposures versus single day exposures in rats exposed to 1,000 ppm (4,920 mg/m^3). In the only study to investigate distribution following dermal exposure, 1,2,4-TMB preferentially distributed to the kidneys (Tsujino et al., 2002). Concentrations in the blood, brain, liver, and adipose tissue were similar to one another, but 1,2,4-TMB concentrations only increased in a dose-dependent manner in adipose tissue, and continued to accumulate in that tissue following the termination of exposure. Similar results were reported for 1,2,3-TMB and 1,3,5-TMB, but specific data were not presented. Detailed information regarding the distribution of 1,2,3-TMB in rats following inhalation or oral exposures is lacking. However, similar tissue-specific partition coefficients for 1,2,3-TMB compared to 1,2,4-TMB and 1,3,5-TMB were similar across a number of organ systems (Meulenberg and Vijverberg, 2000), indicating similar patterns of distribution can reasonably be anticipated.

B.2.3. Metabolism

The metabolic profiles for each isomer were qualitatively similar between humans and rats, although in some cases, quantitative differences were reported. In humans (n = 10, Caucasian males), all three isomers are observed to be metabolized to benzoic and hippuric acids. Approximately 22% of inhaled 1,2,4-TMB was collected as hippuric acid metabolites in urine 24 hours after 2 hour exposures to 25 ppm (123 mg/m^3) 1,2,4-TMB (Järnberg et al., 1997b). 3,4-dimethylhippuric acid (DMHA) comprised 82% of the dimethylhippuric acids collected after exposure to 1,2,4-TMB, indicating that steric factors are important in the oxidation and/or glycine conjugation of 1,2,4-TMB in humans. Approximately 11% of inhaled 1,2,3-TMB was collected as hippuric acid metabolites (Järnberg et al., 1997b). As with 1,2,4-TMB, steric influences seem to play an important role in the preferential selection of which metabolites are formed: 2,3-DMHA comprised 82% of all hippuric acid metabolites collected. Urinary hippuric acid metabolites for 1,3,5-TMB following the same exposure protocol accounted for only 3% of inhaled dose. The lower levels of hippuric acids recovered in urine following exposure to 1,3,5-TMB may be a result of differing pK_a values. The DMHA metabolite of 1,3,5-TMB has the highest pK_a value of any DMHA metabolite, indicating it ionizes to a lesser degree in urine. This may lead to increased reabsorption in the kidney tubules, consequently lowering the total amount of DMHA metabolite excreted within 24 hours (Järnberg et al., 1997b). Greater amounts of urinary benzoic and hippuric acid metabolites (73%) were observed in humans (n = 5) following exposure to higher amounts of 1,3,5-TMB (up to 30.5 ppm) for 8 hours (Kostrzewski et al., 1997; Kostrewski and Wiaderna-Brycht, 1995). Following occupational exposure to 1,2,4-TMB or 1,3,5-TMB, urinary benzoic acid and hippuric acid metabolites in workers (n = 6-12) were highly correlated with TMB isomer air concentrations (Jones et al., 2006; Fukaya et al., 1994; Ichiba et al., 1992).

Following oral exposures in animals, the quantitative metabolic profiles of the three isomers appears to differ. Mikulski and Wiglusz (1975) observed that 73% of the administered dose of 1,3,5-TMB was recovered as glycine (i.e., hippuric acid, 59.1 ± 5.2%), glucuronide (4.9 ± 1.0), or sulfate (9.2 ± 0.8%) conjugates in the urine of rats within 48 hours after exposure. However, the total amount of metabolites recovered following exposure to 1,2,3-TMB and 1,2,4-TMB was much less (33.0% and ~37%, respectively). The major terminal metabolites for 1,2,4-TMB and 1,3,5-TMB are dimethylhippuric acids (23.9 ± 2.3% and 59 .1 ± 5.2 % total dose, respectively). Dimethylhippuric acid metabolites represent a smaller fraction (10.1 ± 1.2 %) of the metabolites produced following 1,2,3-TMB exposure. When an estimate of the total amount of metabolite was calculated, differences between isomers remained but were in closer agreement: 93.7% (1,3,5-TMB), 62.6% (1,2,4-TMB), 56.6% (1,2,3-TMB) (no SD reported). It is important to note that Mikulski and Wiglusz (1975) did not measure other TMB metabolites, such as mercapturic acid conjugates, trimethylphenols, or dimethylbenzoic acids. Huo et al. (1989) reported that total amount of metabolites (phenols, benzyl alcohols, benzoic acids, and hippuric acids) recovered with 24 hours following exposure to 1,2,4-TMB was 86.4 ± 23% of administered dose (~100 mg/kg).

Similar profiles in metabolism were observed in rabbits: DMBAs and DMHAs were observed following oral exposure of rabbits to either 1,2,4-TMB or 1,3,5-TMB (Laham and Potvin, 1989; Cerf et al., 1980). Specifically for 1,3,5-TMB, 68.5% of the administered oral dose was recovered as the DMHA metabolite, with only 9% recovered as the DMBA metabolite. Additionally, a minor metabolite not observed in rats, 5-methylisophthalic acid was observed following exposure of rabbits (Laham and Potvin, 1989). Additional terminal metabolites for the three isomers include: mercapturic acids (~14–19% total dose), phenols (~12% total dose), and glucuronides and sulphuric acid conjugates (4–9% total dose) for 1,2,4-TMB; mercapturic acids (~5% total dose), phenols (<1–8% total dose), and glucuronides and sulphuric acid conjugates (8–15% total dose) for 1,2,3-TMB; and phenols (~4–8% total dose) and glucuronides and sulphuric acid conjugates (~5–9% total dose) for 1,3,5-TMB (Tsujimoto et al., 2005; Tsujimoto et al., 2000, 1999; Huo et al., 1989; Wiglusz, 1979; Mikulski and Wiglusz, 1975).

Phenolic metabolites were also observed in rabbits following oral exposures to 1,2,4-TMB or 1,3,5-TMB, although the amounts recovered were quite small (0.05–0.4 % of total dose) (Bakke and Scheline, 1970). As observed in humans, the influence of steric factors appeared to play a dominant role in determining the relative proportion of metabolites arising from oxidation of benzylic carbons: the less sterically hindered 3,4-DMHA comprised 79.5% of the collected hippuric acid metabolites (Huo et al., 1989). Steric factors appear to be minimal regarding oxidation of the aromatic ring itself: the most hindered phenol metabolites of 1,2,4-TMB and 1,2,3-TMB were either formed in equal or greater proportions compared to less sterically hinder metabolites (Huo et al., 1989)(Tsujimoto et al., 2005). The proposed metabolic schemes for 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB are shown in Figures B-1, B-2, and B-3.

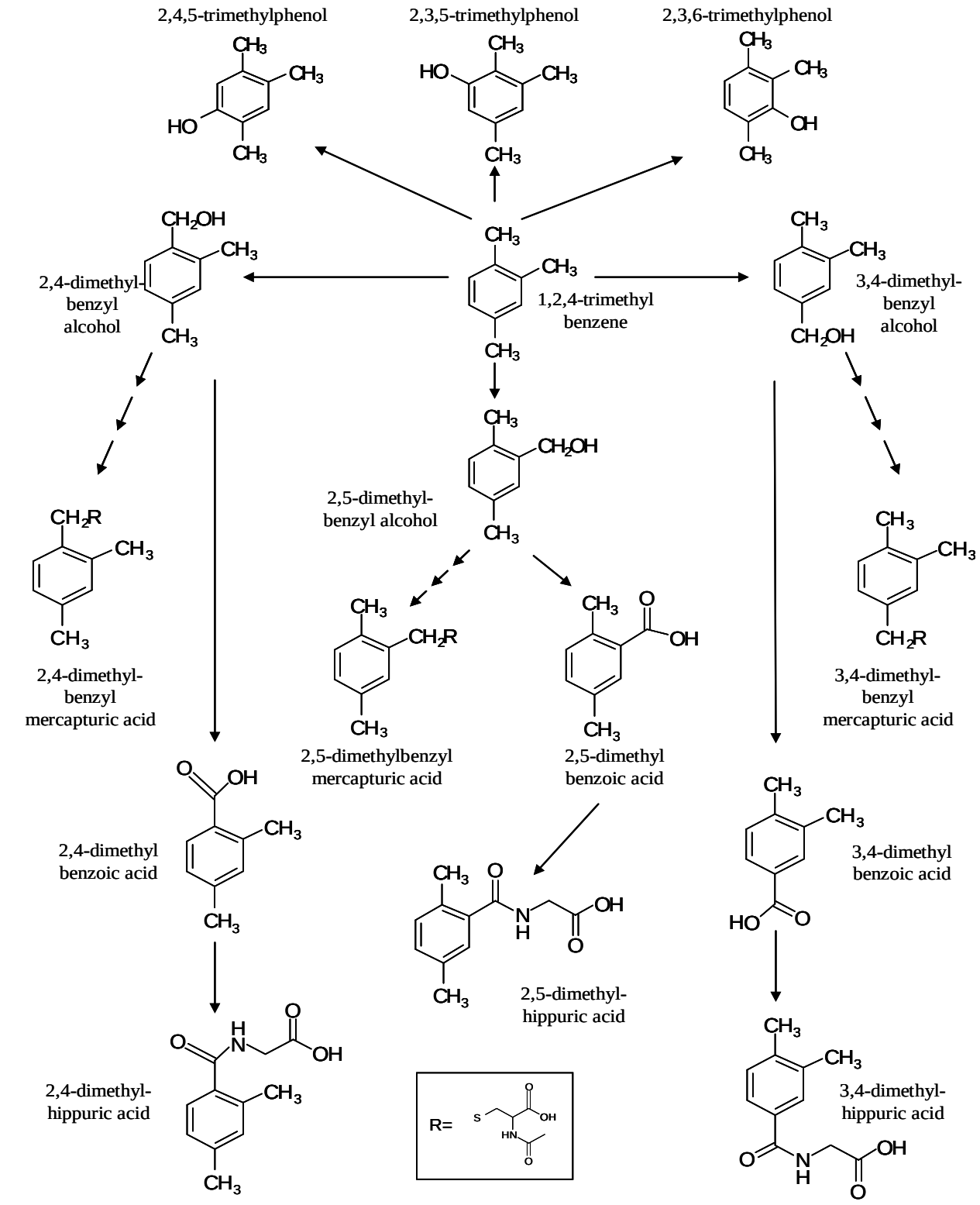


Figure B-1. Metabolic scheme for 1,2,4-TMB.

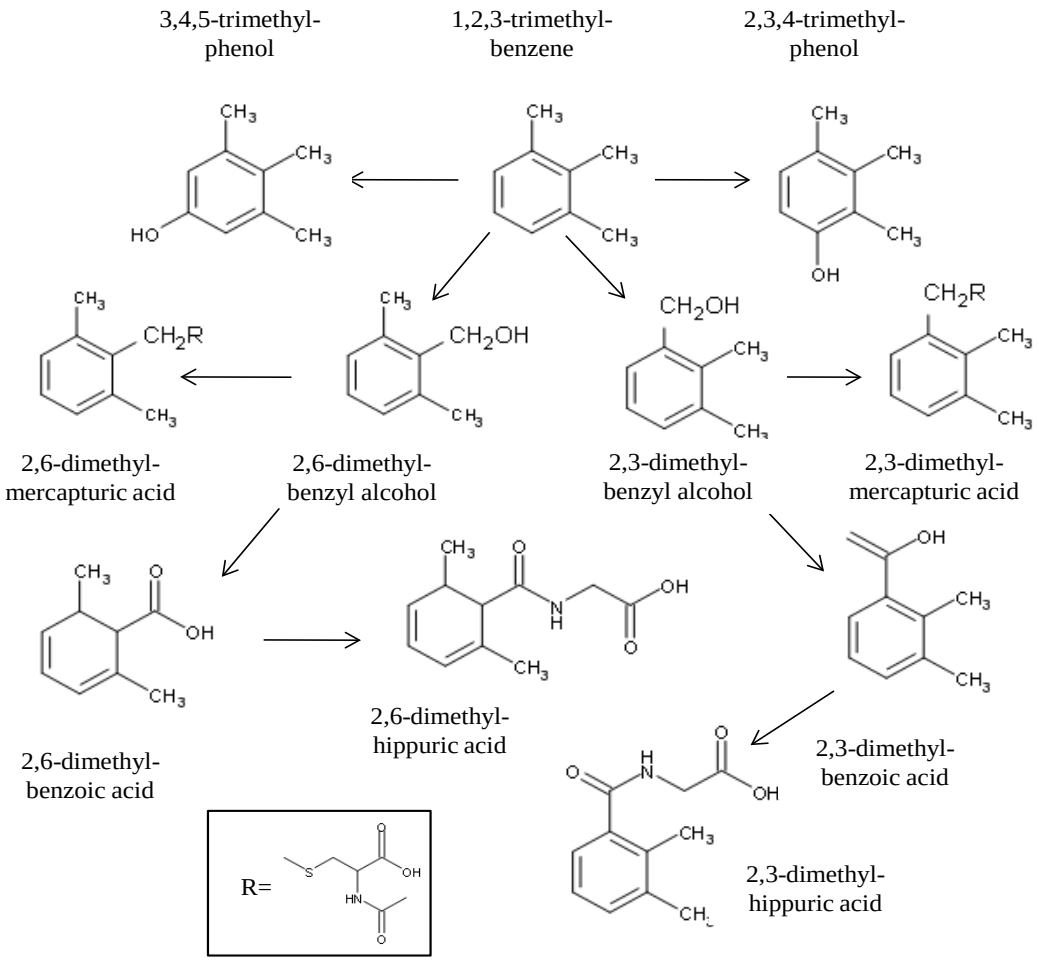


Figure B-2. Metabolic scheme for 1,2,3-TMB.

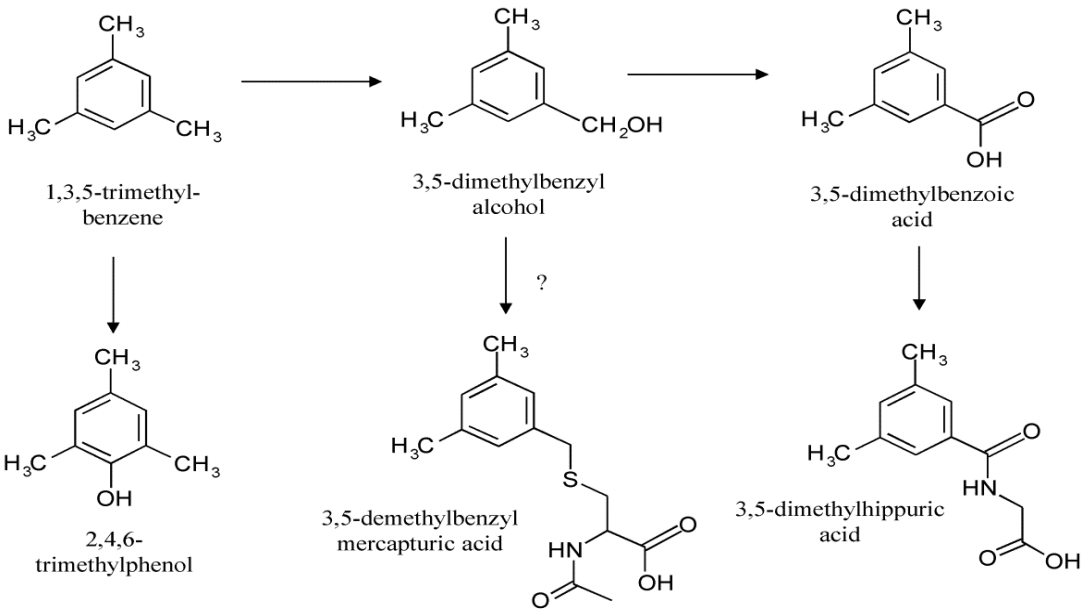


Figure B-3. Metabolic scheme for 1,3,5-TMB.

B.2.4. Excretion

In humans (n = 10, Caucasian males) at low doses (25 ppm [123 mg/m^3]), half-lives of elimination from the blood of all TMB isomers were split into four distinct phases, with the half-lives of the first three phases being similar across isomers: 1,2,4-TMB (1.3 ± 0.8 min, 21 ± 5 min, 3.6 ± 1.1 hr), 1,2,3-TMB (1.5 ± 0.9 min, 24 ± 9 min, 4.7 ± 1.6 hr), and 1,3,5-TMB (1.7 ± 0.8 min, 27 ± 5 min, 4.9 ± 1.4 hr) (Järnberg et al., 1996). 1,3,5-TMB had a higher total blood clearance value compared 1,2,4-TMB or 1,2,3-TMB (0.97 ± 0.06 L/hr/kg vs. 0.68 ± 0.13 or 0.63 ± 0.13 L/hr/kg, respectively). The half-life of elimination for 1,3,5-TMB in the last and longest phase is much greater than those for 1,2,4-TMB or 1,2,3-TMB (120 ± 41 hr vs. 87 ± 27 and 78 ± 22 hr, respectively). Urinary excretion of unchanged parent compound was extremely low (<0.002%) in humans (n = 6-10, male) for all three isomers (Janasik et al., 2008; Järnberg et al., 1997b). The half-life of elimination of hippuric acid metabolites from the urine was also greater for 1,3,5-TMB, compared to 1,2,4-TMB or 1,2,3-TMB (16 hr vs. 3.8–5.8 and 4.8–8.1 hr, respectively) (Järnberg et al., 1997b).

Differences in the values of terminal half-lives may be related to interindividual variation in a small sample population (n = 8–10) and difficulty measuring slow elimination phases. All three isomers were eliminated via exhalation: 20–37% of the absorbed dose of 1,2,4-TMB, 1,2,3-TMB, or 1,3,5-TMB was eliminated via exhalation during exposure to 123 mg/m^3 (25 ppm) for 2 hours (Järnberg et al., 1996) and elimination of 1,3,5-TMB via breath was bisphasic with an initial half-life of 60 minutes, and a terminal half-life of 600 minutes (Jones et al., 2006). Following exposure of rats to 25 ppm (123 mg/m^3) 1,2,4-TMB or 1,3,5-TMB for 6 hours, the terminal half-life of elimination of 1,3,5-TMB from the blood (2.7 hours) was shorter than that for 1,2,4-TMB (3.6 hours) (Swiercz et al., 2006; Swiercz et al., 2002). As dose increased, the half-lives for elimination from blood following single exposures to 1,2,4-TMB (17.3 hours) became much longer than those for 1,3,5-TMB (4 hours). This same pattern was observed for 4-week repeated exposures as well.

B.3. PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS

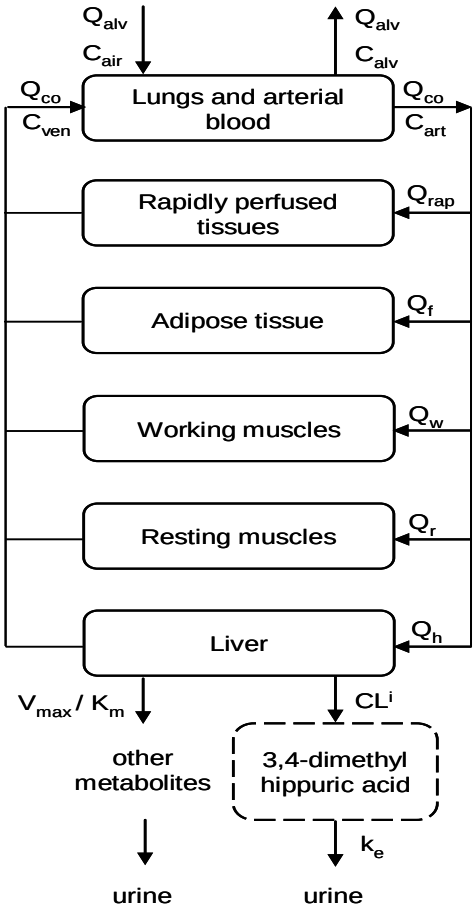
B.3.1. Summary of Available PBPK models for 1,2,4-TMB

B.3.1.1. Järnberg and Johanson (1999)

Järnberg and Johanson (1999) describe a PBPK model for inhalation of 1,2,4-TMB in humans. The model is composed of six compartments (lungs, adipose, working muscles, resting muscles, liver, and rapidly perfused tissues) for the parent compound and one (volume of distribution) for the metabolite, 3,4-DMHA (see Figure B-4). The lung compartment includes lung tissue and arterial blood. Excretion of parent compound is assumed to occur solely by ventilation. As 1,2,4-TMB has a pronounced affinity to adipose tissue, a separate compartment for fat is incorporated into the model. Remaining non-metabolizing compartments are rapidly perfused tissues, comprising the brain, kidneys, muscles, and skin.

Because previous experimental data was gathered during exercise (Järnberg et al., 1997a; Järnberg et al., 1996), the muscle compartment was divided into two equally large compartments, resting and working muscles. Two elimination pathways (a saturable Michaelis-Menten pathway for all metabolites other than 2,4-DMHA [pathway I] and a first order pathway [pathway II] for formation of 3,4-DMHA) from the hepatic compartment were included. Metabolism was assumed to occur only in the liver compartment. Tissue:blood partition coefficients of 1,2,4-TMB were calculated from experimentally determined blood:air, water:air, and olive oil:air partition coefficients (Järnberg and Johanson, 1995) (Table B-2).

The model was used to investigate how various factors (work load, exposure level, fluctuating exposure) influence potential biomarkers of exposure (end-of-shift and prior-to-shift concentrations of parent compound in blood and 3,4-DMHA in urine). Biomarker levels estimated at end-of-shift remained fairly constant during the week, whereas biomarker levels prior-to-shift gradually increase throughout the week. This indicates end-of-shift values represent the same day's exposures, whereas prior-to-shift values reflect cumulative exposure during the entire work week. Increased work load increased uptake of 1,2,4-TMB. For example, a work load of 150 W over an exposure period of 8 hours increased the level of 1,2,4-TMB in the blood more than 2-fold, compared to levels of 1,2,4-TMB in the blood after an 8 hour exposure at rest. Simulated 8-hour exposures at air levels 0 to 100 ppm (0 to 492 mg/m^3) shows that overall metabolism is saturable, and that the metabolic pathway yielding 3,4-dimethylbenzene becomes more important as exposure concentrations increase.



Legend: C: concentration of 1,2,4-TMB; C_{air}: concentration in ambient air; C_{art}: concentration in arterial blood; C_{ven}: concentration in venous blood; Q_{alv}: alveolar ventilation; Q_{CO}: cardiac output; Q_i: blood flow to compartment i (where i = rap = rapidly perfused tissues; f = adipose tissue; w = working muscles, r = resting muscles, h = liver); V_{max}: maximum rate of metabolism, pathway I; Km: Michaelis-Menten constant for metabolic pathway I; CL^i: intrinsic hepatic clearance of metabolic pathway II; k_e: excretion rate constant of 3,4-DMHA. Adapted from Järnberg and Johanson (1999).

Figure B-4. Physiological based toxicokinetic model for 1,2,4-TMB in humans.

Table B-2. Measured and calculated partition coefficients for TMB isomers at 37°C

Substance	Measured values[a]			Calculated values
	$P_{Saline:Air}$ n = 42	$P_{Oil:Air}$ n = 25	Human $P_{Blood:Air}$ n = 39	Human $P_{Blood:Air}$[b]
1,3,5-TMB	1.23 (1.11–1.35)	9,880 (9,620–10,140)	43.0 (40.8–45.2)	60.3
1,2,4-TMB	1.61 (1.47–1.75)	10,200 (9,900–10,400)	59.1 (56.9–61.3)	62.2
1,2,3-TMB	2.73 (2.54–2.92)	10,900 (10,500–11,300)	66.5 (63.7–69.3)	67.5

[a]Mean values and 95%CI.

[b]Calculated as $(0.79 \times P_{Saline:Air}) + (0.006 \times P_{Oil:Air})$; where 0.79 is the relative content of saline in blood and 0.006 is the relative content of fat in blood (Fiserova-Bergerova, 1983).

Adapted from Järnberg and Johanson (1995).

Previously performed experimental human exposures to 1,2,4-TMB were used to estimate the metabolic parameters and alveolar ventilation (Järnberg et al., 1997a; Järnberg et al., 1996). Individual simulated arterial blood concentrations and exhalation rates of 1,2,4-TMB, as well as the urinary excretion rate of 3,4-DMHA, were simultaneously adjusted to the experimentally obtained values by varying the alveolar ventilation at rest. One individual's compound-specific and physiological parameters were then used for subsequent model predictions (Table B-3).

Table B-3. PBPK model parameters for 1,2,4-TMB toxicokinetics in humans using the Järnberg and Johanson (1999) model structure

Parameters	Rest	Both[a]	50 W
Body height (m)		1.78	
Body weight (kg)		75.5	
V_{max} (µmol/min)		3.49	
K_m (µM)		4.35	
CL^i (L/min)		0.149	
Elimination rate constant (min^{-1})		0.0079	
Alveolar ventilation (L/min)	9.05		20.2
Compartment volumes (L)			
Lungs and arterial blood		1.37	
Liver		1.51	
Fat		25.0	
Brain and kidneys		1.49	
Working muscles		16.6	
Resting muscles		16.6	
Blood flows (L/min)			
Cardiac output	5.17		9.16
Liver	1.67		
Fat	0.55		
Brain and kidneys	1.86		1.78
Working muscles	0.55		4.3
Resting muscles	0.55		0.55
Partition coefficients			
Blood:air		59	
Fat:blood		125	
Liver:blood		5	
Rapidly perfused tissues:blood		5	
Muscle:blood		5	

[a]Parameters used for both working and resting conditions.

Adapted from Järnberg and Johanson (1999).

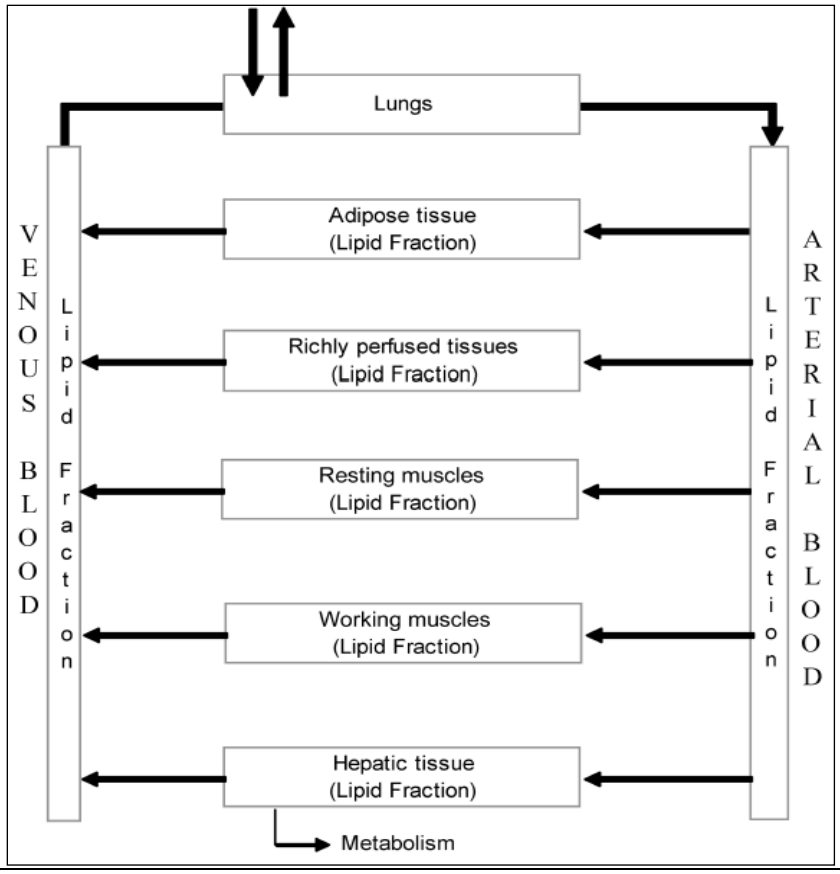
B.3.1.2. Emond and Krishnan (2006)

The Emond and Krishnan (2006) model was not developed specifically for 1,2,4-TMB, but rather to test a modeling concept. The PBPK model developed was to test the hypothesis that a model could be developed for highly lipophilic volatile organic chemicals (HLVOCs) using the neutral lipid-equivalent (NLE) content of tissues and blood as the basis. This NLE-based modeling approach was tested by simulating uptake and distribution kinetics in humans for several chemicals including α-pinene, d-limonene, and 1,2,4-TMB. The focus of this model review is to use of the model for the prediction of 1,2,4-TMB kinetics and distribution.

This model consisted of five compartments (see Figure B-5) with systemic circulation, where the tissue volumes corresponded to the volumes of the neutral lipids (i.e., their neutral lipid–equivalents), rather than actual tissue volume as more commonly found. NLE is the sum of the neutral (nonpolar) lipids and 30% of the tissue phospholipid (fraction of phospholipids with solubility similar to neutral lipids) content. The model describes inhalation of 1,2,4-TMB using a lumped lung/arterial blood compartment. Clearance of 1,2,4-TMB is described in the model with exhalation, but more significantly through first order hepatic metabolism. First-order metabolism is appropriate in the low dose region (< 100 ppm [< 492 mg/m^3]), where metabolism is not expected to be saturated.

In the study description, the mixed lung/arterial blood compartment is not a standard structure for the lung/blood/air interface. The concentration in lung tissue is assumed equal to alveolar blood, and the exhaled air concentration is equal to the lung/blood concentration divided by the blood air partition coefficient. This approach is appropriate, and appears to be accurately represented mathematically by the authors.

Physiological parameters appear to be within ranges normally reported. The calculation of the NLE fraction is clearly explained and values used in the calculations are clear and transparent. Other model parameters (e.g., alveolar ventilation, cardiac output, blood flows, and volumes of compartments) were taken from Järnberg and Johanson (1999) and converted to the approximate NLE. Hepatic clearance rates were taken from literature on in vivo human clearance calculations and then expressed in terms of NLE. The NLE-based model was able to adequately predict human blood concentrations of 1,2,4-TMB following inhalation of 2 or 25 ppm (9.8 or 123 mg/m^3) for 2 hours without alteration to model parameters obtained from literature.



Note: Arrows represent blood flows, gas exchange, and metabolism as indicated. Source: Emond and Krishnan (2006).

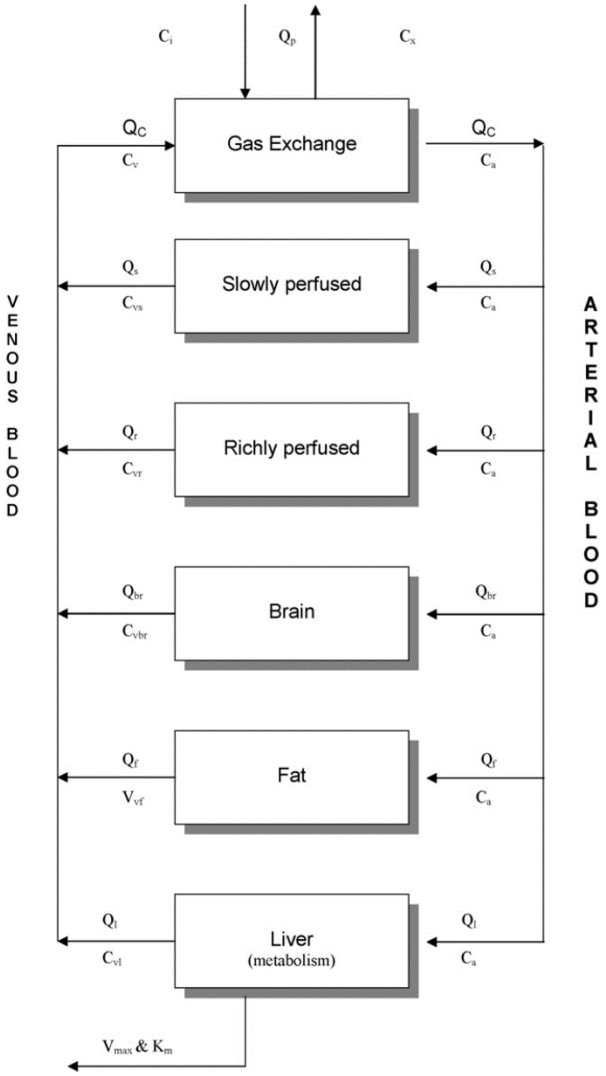
Figure B-5. Schematic of human model structure for 1,2,4-TMB using the NLE-based model approach.

The PBPK model developed by Emond and Krishnan (2006) is used to test the hypothesis that a model could be developed for HLVOCs using the NLE content of tissues and blood as the basis. To test this NLE-based approach, the uptake and distribution kinetics in humans for several chemicals including 1,2,4-TMB were simulated. The model appeared to accurately reflect experimental data; however, a rodent model is needed for this assessment for animal–to–human extrapolation and no known rodent NLE model for 1,2,4-TMB is available.

B.3.1.3. Hissink et al. (2007)

This model was developed to characterize internal exposure following white spirit (WS) inhalation. Since WS is a complex mixture of hydrocarbons, including straight and branched paraffins, two marker compounds were used including 1,2,4-TMB and *n*-decane. The rat models were developed to predict the levels of 1,2,4-TMB and *n*-decane in blood and brain, then the rat model was scaled allometrically to obtain estimates for human blood following inhalation. Toxicokinetic data on blood and brain concentrations in rats of two marker compounds, 1,2,4-TMB and n-decane, together with in vitro partition coefficients were used to develop the model. The models were used to estimate an air concentration that would produce human brain concentrations similar to those in rats at the no-observed-effect-level (NOEL) for central nervous system (CNS) effects.

This is a conventional five compartment PBPK model for 1,2,4-TMB similar to previously published models for inhaled solvents. The five compartments were: liver, fat, slowly perfused tissues, rapidly perfused tissues, and brain (see Figure B-6).



Note: Boxes represent tissue compartments, while solid arrows represent blood flows, gas exchange, and metabolism as indicated. Source: Hissink et al. (2007).

Figure B-6. Schematic of rat and human PBPK model structure.

All compartments are described as well mixed/perfusion limited. A lung compartment is used to describe gas exchange. The liver was the primary metabolizing organ where 1,2,4-TMB metabolism was described as saturable using Michaelis-Menten kinetics. Since the brain is the target organ for CNS effects due to exposure to hydrocarbon solvents, it was included as a separate compartment. For the rat, the authors reported that K_m and V_{max} values were obtained by fitting predicted elimination time courses to observed blood concentration profiles at three different exposure levels (obtained from the rat exposure portion of the study). For the human model, rat V_{max} data was scaled to human body weight ($BW^{0.74}$) and K_m values were used unchanged.

The model appears to effectively predict blood concentrations in rats and humans and in the brains of rats following inhalation of WS. Changes to the rat model parameters to fit the human data were as expected. The model is simple and includes tissues of interest for potential dose metrics.

In rats, the model-predicted blood and brain concentrations of 1,2,4-TMB were in concordance with the experimentally derived concentrations. In humans, experimental blood concentrations of 1,2,4-TMB were well predicted by the model, but the predicted rate of decrease in air concentration between 4–12 hours was lower compared to measured values. The authors did not provide information on how model predictions compared to data from animals or humans exposed to pure 1,2,4-TMB. Based on good model fits of experimental data, the model was valid for the purpose of interspecies extrapolation of blood and brain concentrations of 1,2,4-TMB as a component of WS.

B.3.2. 1,2,4-TMB PBPK Model Selection

All available 1,2,4-TMB PBPK models were evaluated for potential use in this assessment. Of the three deterministic PBPK models available for 1,2,4-TMB (Hissink et al., 2007; Emond and Krishnan, 2006; Järnberg and Johanson, 1999), the Hissink et al. (2007) model was chosen to utilize in this assessment because it was the only published 1,2,4-TMB model that included parameterization for both rats and humans, the model code was available, and the model adequately predicted experimental data in the dose range of concern. The Hissink et al. (2007) model was thoroughly evaluated, including a detailed computer code analysis (details follow in Section B.3.3).

B.3.3. Details of Hissink et al. (2007) Model Analysis

B.3.3.1. Review and Verification of the Hissink et al. (2007) 1,2,4-TMB PBPK Model

Verification of accuracy of the model code

In general, the model code and the description of the model in Hissink et al. (2007) were in agreement. The one significant discrepancy was that the model code contained an element that changed the metabolism rate (V_{max}) during exposure in a manner that was not documented in the paper. This additional piece of model code, when used in 8 hour rat simulations with a body weight of 0.2095 kg, resulted in V_{max} holding at 1.17 from the beginning of exposure to t = 1 hr, then increasing linearly to 1.87 by the end of the exposure and to 2.67 by the end of the post exposure monitoring period (t = 16 hrs, 8 hrs after the end of exposure). The published rat simulations, however, did not appear to be entirely consistent with the inclusion of these V_{max} adjustments, raising questions as to whether the code that was verified was the code that was actually used in the final analyses done for the published simulations. The impact of this deviation from the published V_{max} value is described below in regards to the verification of the Hissink et al. (2007) model.

Other minor issues were identified by examining the code and comparing it to the model documentation in Hissink et al. (2007). The code contained some elements that were not necessary (e.g., i.v. dosing, repeated exposure, interruptions in daily exposure), but since these do not hinder proper functioning of the model, these elements were not removed or modified. The mass balance equation omitted one term, the amount of 1,2,4-TMB in the brain (ABR); this term has been added. The coding for the blood flow was not set up so as to ensure flow/mass balance. That is, values of sum of fractional flows to rapidly perfused tissues, liver, and brain (QRTOTC) and sum of fractional flows to slowly perfused tissues (QSTOTC) were selected such that their sum equals one, but if one value were to be changed, the model code would not automatically compensate by changing the other. Therefore, the code was modified so that QSTOTC = 1 – QRTOTC, to facilitate future sensitivity analyses.

Human exhaled breath concentrations were compared to CXEQ (= CV/PB based on the model code and consistent with the description of the experiment), which would be equivalent to the end-exhaled alveolar air after breath holding, but the method used to calculate CXEQ was not noted in Hissink et al. (2007). This is important because there can be different definitions of exhaled breath depending on the measurement technique. For example, mixed exhaled breath is typically calculated as 70% alveolar air and 30% "inhaled" concentration, due to dead space.

Comparisons between the computer .m files and published descriptions (Hissink et al., 2007) indicated minor discrepancies and uncertainties in exposure concentrations and body weight. Exposure concentrations in the simulations were set at the nominal exposure levels, rather than analytically determined levels. The maximum deviation between the nominal level and analytically determined levels occurred in the rat high exposure group, with a nominal exposure of 4,800 mg/m^3 WS (7.8% [38.4 mg/m^3] 1,2,4-TMB) and mean analytical concentrations ranging from 4,440 to 4,769 mg/m^3—as much as 9.2% lower. Rat body weights at time of exposure were reported as 242 to 296 g (Hissink et al., 2007), but the .m files use values of 210.01, 204.88, and 209.88 g in the low-, mid-, and high-exposure groups, respectively. Human volunteer body weights reportedly ranged from 69 to 82 kg, and the text states that the fitted V_{max} and K_m were obtained for a 70 kg male (Hissink et al., 2007), but a body weight of 74.9 kg was used in the .m file. No changes to these parameters were made in the model code, based on the assumption that additional data were available to the model authors.

Measured human blood concentrations were compared to the average of arterial and venous blood concentrations (CMIX), while the protocol states that blood was taken from the cubital vein, so a more appropriate measure may have been venous blood exiting the slowly perfused tissues compartment (CVS). This choice of dose metric is unlikely to have contributed significantly to any errors in parameterizing the model (i.e., estimating best-fit metabolism parameters) because the difference between the two values is generally small. Revised model code and modeling results are provided on EPA's Health Effects Research Online (HERO) database (U.S. EPA, 2011a).

Verification of model parameter plausibility

Anatomical and physiological parameters

The anatomical physiological parameters used by Hissink et al. (2007) were taken from U.S. EPA (1988), but more current convention is to use the parameters in Brown et al. (1997). Comparisons of the rat anatomical and physiological parameters in these sources are found in Table B-4. Many disagreements in values were identified, particularly with respect to the blood flows. In interpreting the blood flow percentages, it should be noted that the percentages enumerated by Brown et al. (1997) do not sum to 100%, which is of course a physiological requirement. Perfusion rates of various depots of fat may differ, so the single value or fractional blood flow to fat given by Brown et al. (1997) of 7%, may be deemed sufficiently uncertain that the Hissink et al. (2007) value of 9% is considered acceptable. Brown et al. (1997) report substantially higher blood flow percentages to slowly perfused tissues (skin: 5.8% and muscle: 27.8%, for a total of 33.6%) than the value of 15% used by Hissink et al. (2007). The difference cannot be due to a smaller set of tissues being "lumped" into this compartment, because Hissink et al. (2007) assign a larger volume fraction of tissue to this compartment. Hissink et al. (2007) also assign a higher percentage of blood flow to the liver than indicated by Brown et al. (1997). Because no sensitivity analyses were conducted by the authors, it is unclear what impact these discrepancies may have had on the predicted 1,2,4-TMB kinetics and visual optimization of metabolism parameters.

Comparisons of the human anatomical and physiological parameters in Hissink et al. (2007) and Brown et al. (1997) are found in Table B-5. In general, the agreement was better for humans than it was for rats. Brown et al. (1997) propose a higher default body fat percentage than was used by Hissink et al. (2007), but Hissink et al. (2007) used values derived from measurements of the volunteers participating in the study. Because these volunteers had relatively low percentages of body fat, it is appropriate that the volume of slowly perfused tissue (including muscle) should be increased to compensate.

Table B-4. Comparison of rat anatomical and physiological parameters in Hissink et al. (2007) to those of Brown et al. (1997)

Parameter	Hissink et al. (2007)[a]	Range from Brown et al. (1997)	Values in agreement?
Alveolar ventilation rate (L/hr/kg$^{0.7}$)	20	12–54[b]	Yes
Total cardiac output (L/hr/kg$^{0.7}$)	20	9.6–15	No
Blood flow (% cardiac output)			
Liver (total)	25	13.1–22.1	No
Fat	9	7	Acceptable[c]
Brain	1.2	1.5–2.6	No
Rapidly perfused (total)	49.8	15.3–27.4	No
Adrenals		0.2–0.3	
Heart		4.5–5.1	
Kidneys		9.5–19	
Lung		1.1–3	
Slowly perfused (total)	15	33.6	No
Muscle		27.8	
Skin		5.8	
Total	100	70.5–92.7	
Tissue volume (% body weight)			
Liver	4	2.14–5.16	Yes
Fat	7	3.3–20.4	Yes
Brain	0.72	0.38–0.83	Yes
Rapidly perfused	4.28	3.702–6.11	Yes
Adrenals		0.01–0.31	
Stomach		0.4–0.6	
Small intestine		0.99–1.93	
Large intestine		0.8–0.89	
Heart		0.27–0.4	
Kidneys		0.49–0.91	
Lungs		0.37–0.61	
Pancreas		0.24–0.39	
Spleen		0.13–0.34	
Thyroid		0.002–0.009	
Slowly perfused	75	51.16–69.1	Acceptable[c]
Muscle		35.36–45.5	
Skin		15.8–23.6	
Total	91	60.682–101.6	

[a]Values from U.S. EPA (1988).
[b]Assuming a standard 250 g rat.
[c]Hissink et al. (2007) value outside of literature range, but acceptable (see discussion in text).
Data source: Hissink et al. (2007) and Brown et al. (1997).

Table B-5. Comparison of human anatomical and physiological parameters in Hissink et al. (2007) to those of Williams and Leggett (1989) as reported by Brown et al. (1997)

Parameter	Hissink et al. (2007)[a]	Range from Brown et al. (1997)	Values in agreement?
Alveolar ventilation rate (L/hr/kg$^{0.7}$)	20	15	Acceptable
Total cardiac output (L/hr/kg$^{0.7}$)	20	16	Acceptable
Blood flow (% cardiac output)			
Liver (total)	26	11–34.2	Yes
Fat	5	3.7–11.8	Yes
Brain	14	8.6–20.4	Yes
Rapidly/Richly perfused (total)	30	19.9-35.9	Yes
Adrenals		0.3	
Heart		3–8	
Kidneys		12.2–22.9	
Lung		2.5	
Thyroid		1.9–2.2	
Slowly perfused (total)	25	9–50.8	Yes
Muscle		5.7–42.2	
Skin		3.3–8.6	
Total	100	52.2–153.1	
Tissue Volume (% body weight)			
Liver	2.6	2.57	Yes
Fat	14.6	21.42	Acceptable (measured)[a]
Brain	2	2	Yes
Rapidly/Richly perfused	3	3.77	Acceptable
Adrenals		0.02	
Stomach		0.21	
Small intestine		0.91	
Large intestine		0.53	
Heart		0.47	
Kidneys		0.44	
Lungs		0.76	
Pancreas		0.14	
Spleen		0.26	
Thyroid		0.03	
Slowly perfused	66.4	43.71	Acceptable
Muscle		40	
Skin		3.71	
Total	88.6	73.47	

[a]The Hissink et al. (2007) value differs from Brown et al. (1997), but is acceptable (see discussion in text).

Data source: Hissink et al. (2007); and Williams and Leggett (1989) [as reported by Brown et al. (1997)].

Chemical-specific parameters

The chemical-specific model parameters, the partition coefficients, and the metabolic parameters are summarized in Table B-6.

Table B-6. Comparison of chemical-specific parameters in Hissink et al. (2007) to literature data

Parameter	Hissink et al. (2007)		Literature		Values in agreement?
	Value	Technique	Value	Technique	
Partition coefficients					
Saline:Air	3	In vitro	1.47–1.75[a]	In vitro	Acceptable
Olive oil:Air	13,200	In vitro	9,900–10,400[a]	In vitro	Acceptable
Blood:Air - human	85	In vitro	59.6–61.3[a]	In vitro	Acceptable
Blood:Air - rat	148	In vitro	--		
Rapidly perfused:Blood	2.53	Calculated	--		
Slowly perfused:Blood	1.21	Calculated	--		
Fat:Blood	62.7	Calculated	63[b]	In vivo	Yes
Brain:Blood	2.53	Calculated	2[b]	In vivo	Acceptable
Liver:Blood	2.53	Calculated	--		
Metabolism					
$V_{max}C$ – rat (mg/hr/kg$^{0.7}$)	3.5	Visual optimization	--		
$V_{max}C$ – human (mg/hr/kg$^{0.7}$)	3.5	Assumed equal to rat	1.2–21[c]	Optimization	Yes
K_m – rat (mg/L)	0.25	Visual optimization	--		
K_m – human (mg/L)	0.25	Assumed equal to rat	0.42–4.0[c]	Optimization	No
$V_{max}C/K_m$ – human (L/hr/kg$^{0.7}$)	14	Assumed equal to rat	2.6–15[c]	Optimization	Yes

[a]Järnberg and Johanson (1995).

[b]Zahlsen et al. (1990).

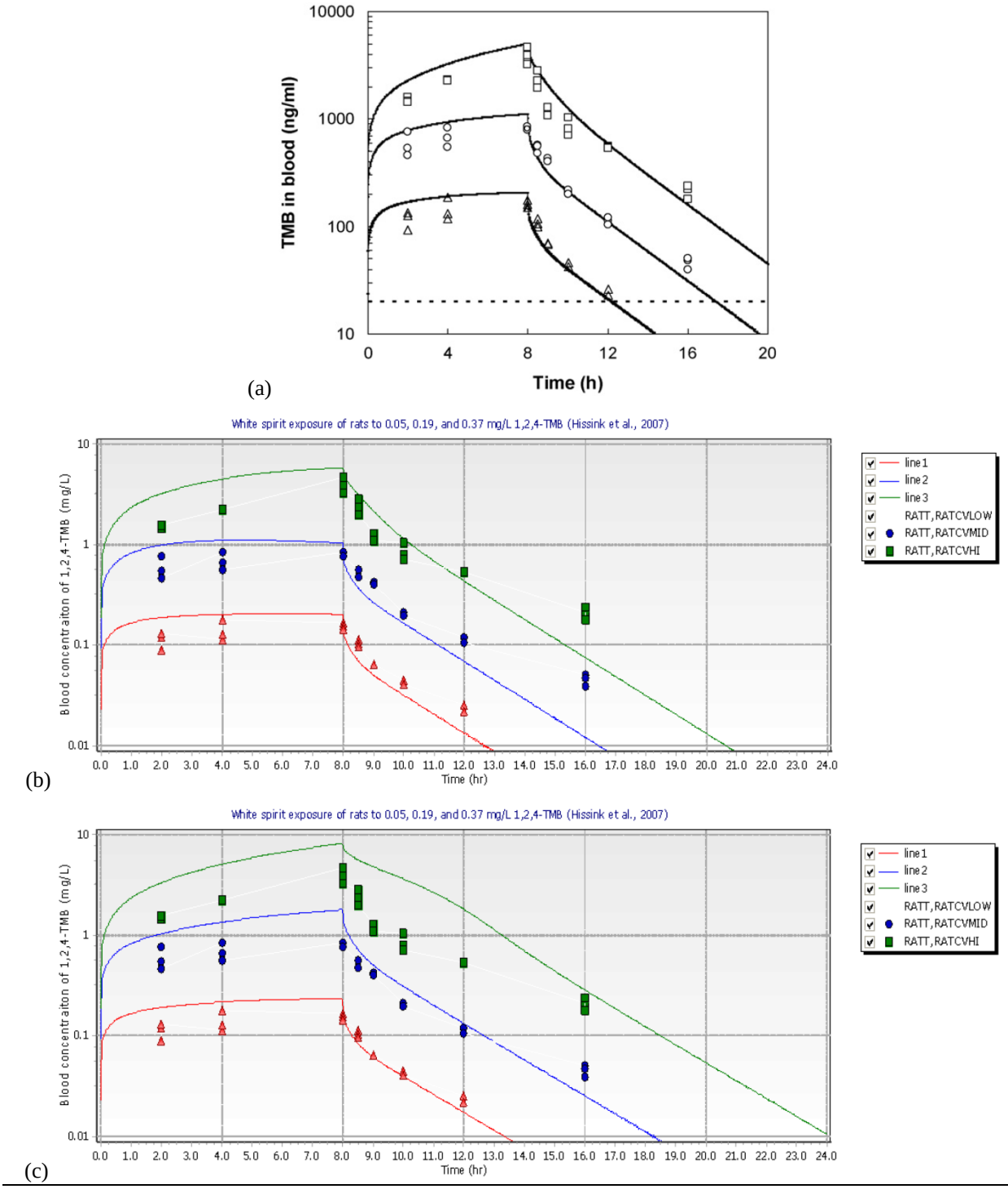
[c]Järnberg and Johanson (1999).

Source: Hissink et al. (2007)

Where data were available, the agreement is generally acceptable. While the rat-derived K_m is less than the lower 95% confidence interval value for the human K_m, the human $V_{max}C/K_m$ ratio is in acceptable agreement. When considering sufficiently low exposure concentrations, the performance of the Hissink et al. (2007) human model metabolism parameters would be consistent with the Järnberg and Johanson (1999) value.

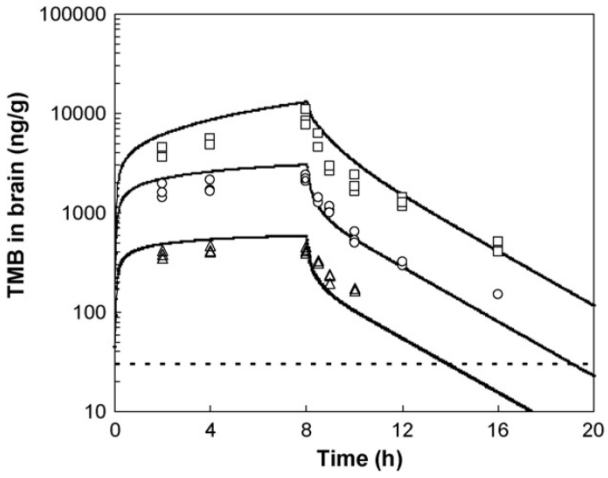
Verification that the model can reproduce all figures and tables in the publication by Hissink et al. (2007)

The experimental data in Hissink et al. (2007) were estimated by use of Plot Digitizer (version 2.4.1) to convert the symbols on the relevant figures into numerical estimates. The model code provided (adapted for acslX), with a variable value for V_{max}, does not appear to perfectly reproduce the rat simulations in Hissink et al. (2007) (Figures B-7a and b and B-8a and b) (please note that the Hissink et al. (2007) figures have been "stretched" to produce approximately the same x-axis scale found in the acslX figures). It appears to yield end-of exposure blood and brain concentrations that are about the same as in the Hissink et al. (2007) simulations, but the post-exposure clearance appears faster in EPA's calculations (see, for example, the 16 hr time points for the high exposures). When the simulations were run with V_{max} constant (Figures B-7c and B-8c), as documented in Hissink et al. (2007), the rat simulations yield higher blood and tissue concentrations than depicted in Hissink et al. (2007), most notably at the high exposure concentration. Similar results were obtained for the rat brain concentrations (Figure B-8). The human simulations of blood and exhaled air appear to be faithfully reproduced by the model (Figure B-9). The predicted brain concentration for humans exposed to 600 mg/m^3 WS (45 mg/m^3 1,2,4-TMB) for 4 hours was reported as 721 ng/g (0.721 mg/L) in Hissink et al. (2007), whereas the current simulation predicts a concentration of 0.818 mg/L.

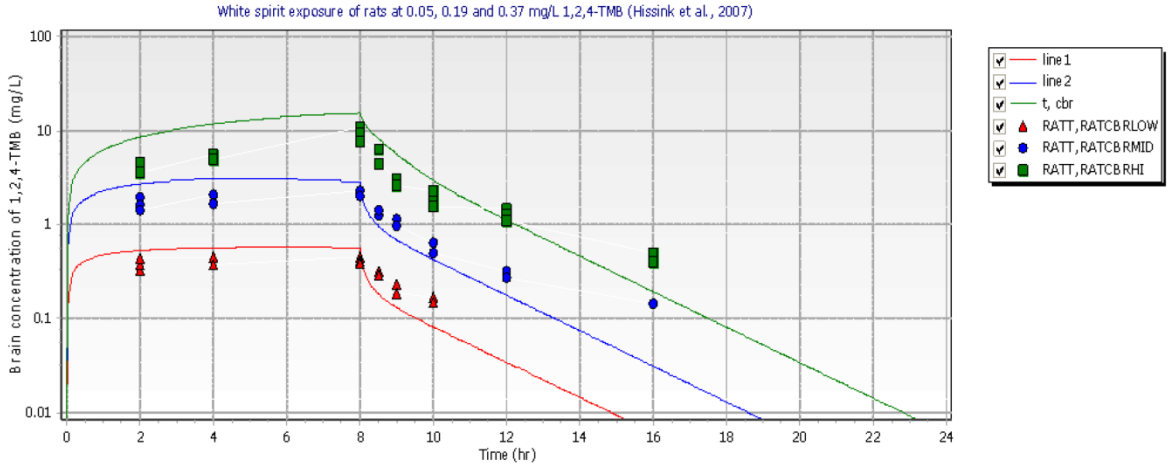


(a) Hissink et al. (2007), Figure 2, lower panel (b) variable V_{max}, (c) constant V_{max}.

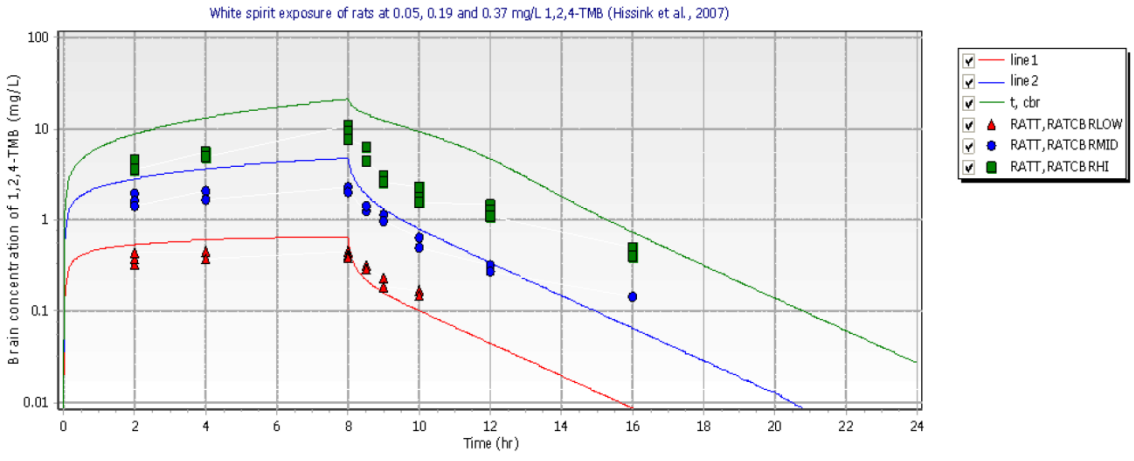
Figure B-7. Simulated and measured blood concentrations of 1,2,4,-TMB in rats exposed to 600, 2,400, or 4,800 mg/m³ WS for 8 hours.



(a)



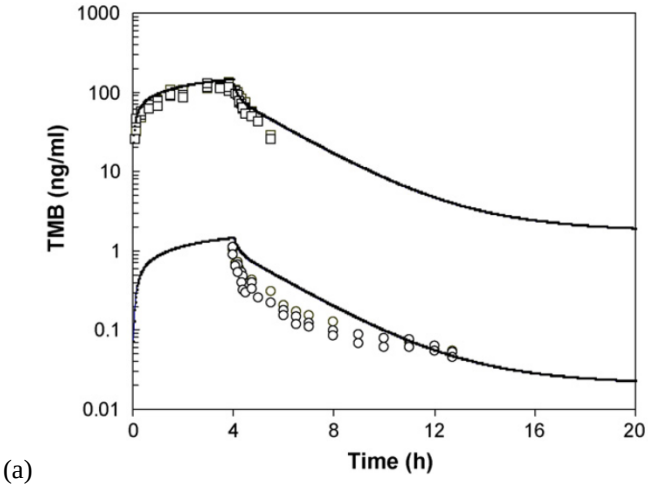
(b)



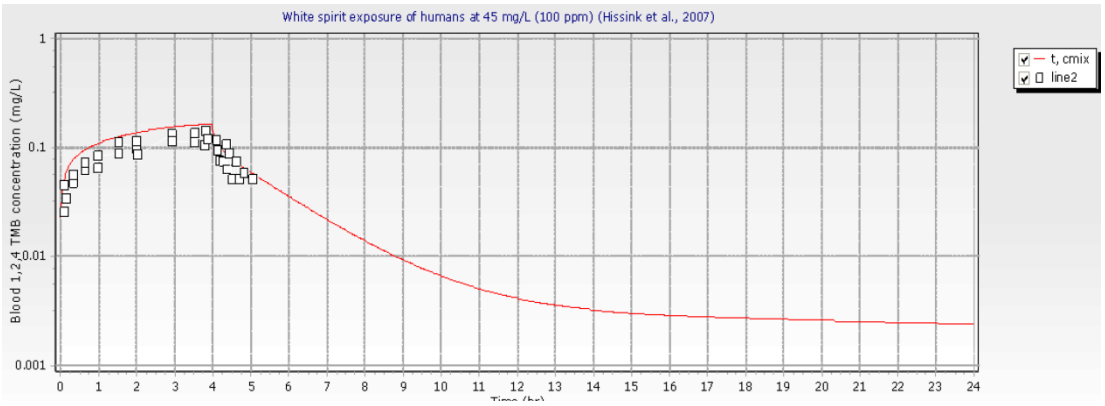
(c)

(a) Hissink et al. (2007), Figure 3, lower panel. (b) variable V_{max} (c) constant V_{max}.

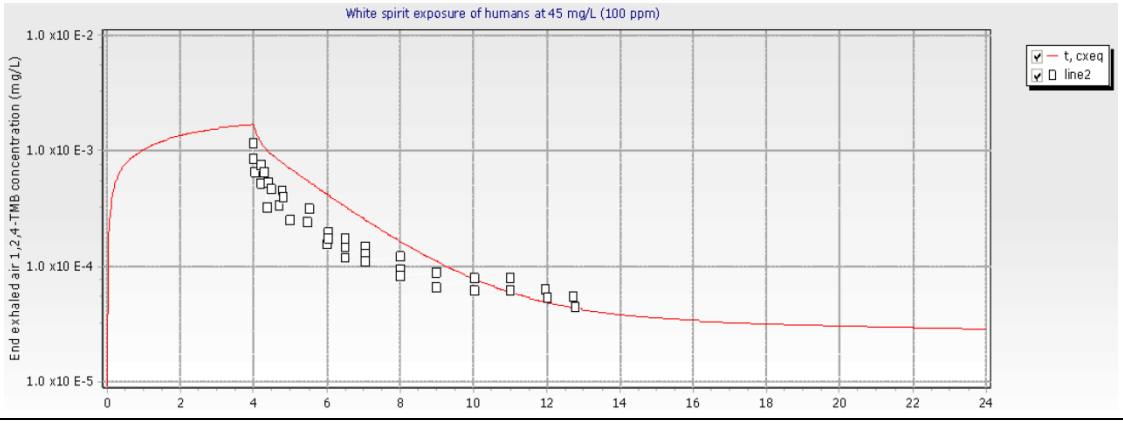
Figure B-8. Simulated and measured brain concentrations of 1,2,4-TMB in rats exposed to 600, 2,400, or 4,800 mg/m³ WS for 8 hours.



(a)



(b)



(c)

(a) Hissink et al. (2007), Figure 4; (b) model simulation during exposure; and (c) model simulation after exposure.

Figure B-9. Simulated and measured exhaled air concentrations of 1,2,4-TMB in three volunteers exposed to 600 mg/m³ WS for 4 hours.

B.3.3.2. PBPK Model Optimization and Validation

Methods and Background

For all optimizations, the Nelder-Mead algorithm was used to maximize the log-likelihood function (LLF). A constant heteroscedasticity value of 2 (i.e., relative error model) was assumed. Statistical significance of an increase in the LLF was evaluated for 95% confidence per Collins et al. (1999). All kinetic studies were conducted with adult animals or adult human volunteers. In many cases, blood and tissue concentration data in a numerical form were available from the literature (Swiercz et al., 2003; Swiercz et al., 2002; Kostrzewski et al., 1997; Eide and Zahlsen, 1996; Zahlsen et al., 1992; Dahl et al., 1988). The 1,2,4-TMB blood, brain, and exhaled breath concentration data in Hissink et al. (2007) were published in graphical format and a colleague of Dr. Hissink also provided these in numerical form to Dr. Lisa Sweeney for use in this analysis.

Average estimates of the blood concentrations of 1,2,4-TMB (average and standard deviation) in humans exposed only to 1,2,4-TMB as presented in graphs in Järnberg et al. (1998, 1997a; 1996) were used in this evaluation. Estimates of the blood and tissue 1,2,4-TMB concentrations in rats presented in graphs in Zahlsen et al. (1990) were also used in this evaluation. Prior to model optimization, physiological parameters were modified from those in Hissink et al. (2007) to better reflect a more recent literature compilation (Brown et al., 1997) than the references cited by Hissink et al. (2007) (Table B-7). Where possible, study specific body weights and measured concentrations (rather than nominal concentrations) have been used, as detailed in the .m files (U.S. EPA, 2011a). For the Zahlsen et al. (1990) 14-day study, body weights for exposures after the first exposure were estimated based on European growth curves for male Sprague-Dawley rats (linear regression of weights for weeks 6–9) (Harlan Laboratories, 2012).

Table B-7. Parameter values for the rat and human PBPK models for 1,2,4 TMB used by EPA

Parameter	RAT	HUMAN (AT REST)
Body weight (kg)	0.230–0.390[a]	70
Alveolar ventilation rate (L/hr/kg$^{0.70}$)	14	15
Total cardiac output (L/hr/kg$^{0.70}$)	14	16
Blood flow (% of total cardiac output)		
Liver	17.6	17.5
Fat	9	8.5
Brain	2.0	11.4
Rapidly perfused	37.8	37.7
Slowly perfused	33.6	24.9
Volume (% of body weight)		
Liver	4	2.6
Fat	7	21.42
Brain	0.57	2
Rapidly perfused	4.43	3
Slowly perfused	75	59.58
Partition coefficients (dimensionless)		
Blood: air	148	85
Rapidly perfused: blood	2.53	4.4
Slowly perfused: blood	1.21	2.11
Fat: blood	62.7	109
Brain: blood	2.53	4.4
Liver: blood	2.53	4.4
Liver metabolism		
$V_{max}C$ (mg/hr/kg$^{0.70}$)	4.17	
K_m (mg/L)	0.322	

[a]Study specific.

Source: (U.S. EPA, 2011a).

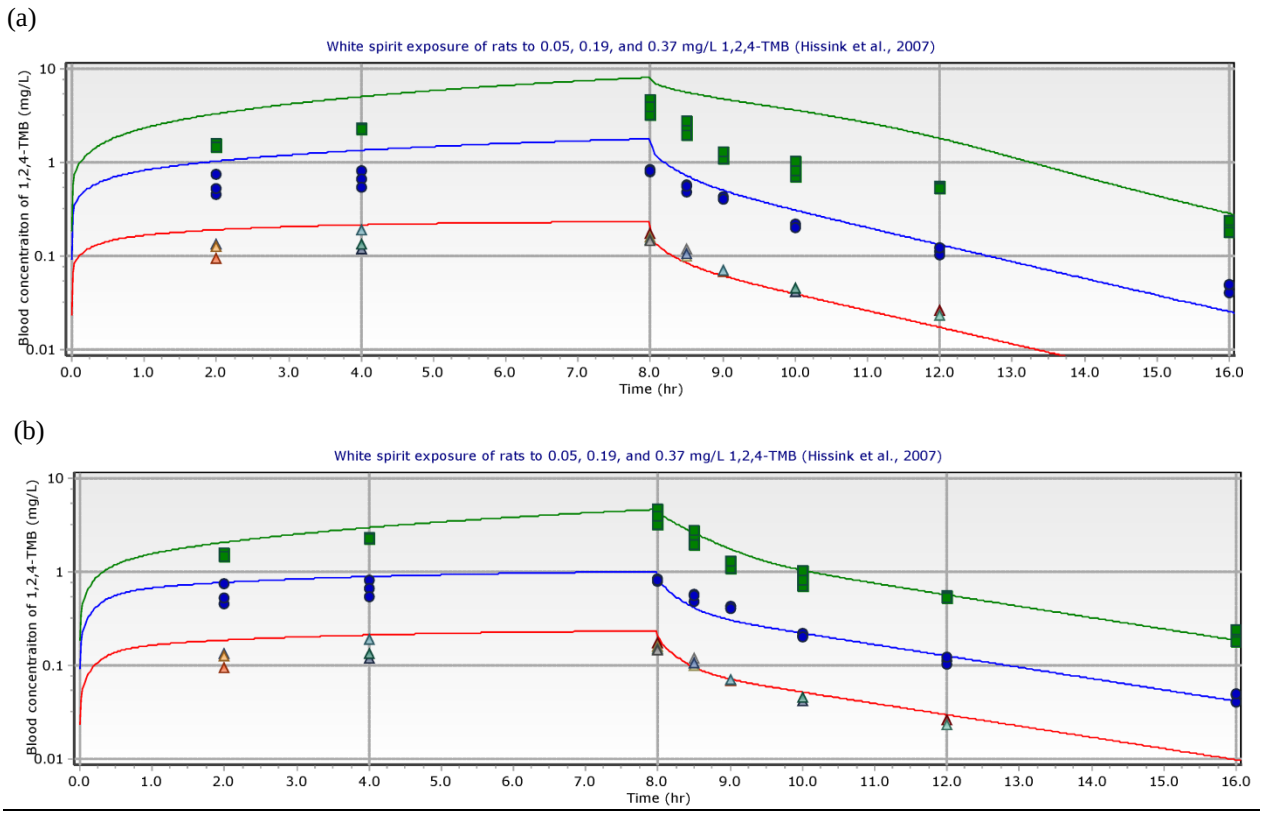
Rat Model Optimization

The rat studies considered in model optimization and model testing (validation) are summarized in Table B-8.

Table B-8. Rat 1,2,4-TMB kinetic studies used in model development and testing

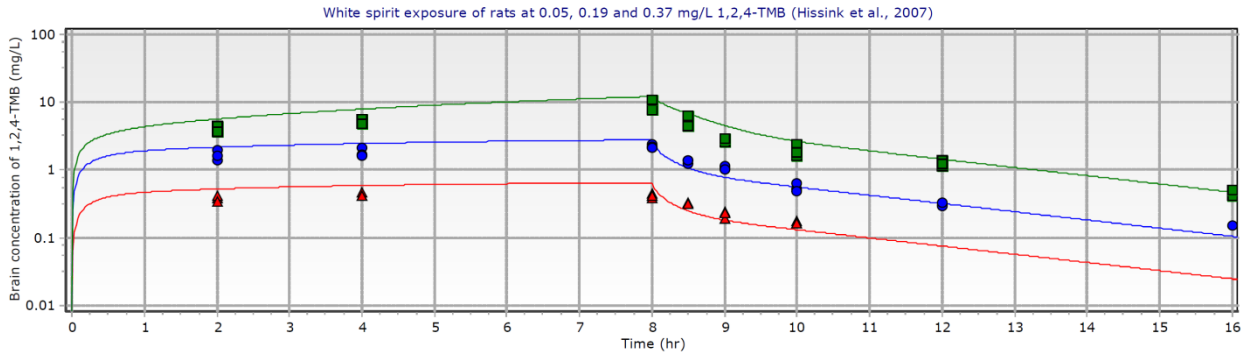
Reference	Strain	Gender	Nominal concentration	Exposure regimen	1,2,4-TMB measurement	Use in model evaluation	Form of comparison
Hissink et al. (2007)	WAG/RijCR/BR (Wistar derived)	Male	102, 410, 820 ppm WS (7.8% 1,2,4-TMB [39.1, 157.3, 314.7 mg/m^3])	8 hr	Mixed blood time course	Optimization (1,2,4-TMB in mixture)	Figure B-10
					Brain time course	Testing	Figure B-11
Swiercz et al. (2003)	Wistar	Male	25, 100, 250 (123, 492, 1,230 mg/m^3)	6 hr/day, 5 days/week 4 weeks	Venous blood time course	Optimization (1,2,4-TMB only)	Figure B-12
					Arterial blood, liver, brain	Testing	Table B-9
				6 hr	Arterial blood, liver, brain	Testing	Table B-9
Swiercz et al. (2002)	Wistar	Male	25, 100, 250 (123, 492, 1,230 mg/m^3)	6 hr	Venous blood time course	Testing	Figure B-13
Zahlsen et al. (1990)	Sprague-Dawley	Male	1,000 (4,920 mg/m^3)	12 hr/day 14 days	Blood, brain, perirenal fat on days 1, 3, 7, 10, and 14	Testing	Table B-12
Zahlsen et al. (1992)	Sprague-Dawley	Male	100 492 mg/m^3)	12 hr/day 3 days	Blood, brain, liver, kidney, and perirenal fat at end of exposures and after 12 hr recovery	Testing	Table B-10
Eide and Zahlsen (1996)	Sprague-Dawley	Male	75, 150, 300, 450 369, 738, 1,476, 2,214 mg/m^3)	12 hr	Blood, brain, liver, kidney, and perirenal fat	Testing	Table B-11
Dahl et al. (1988)	F344/N	Male	100 (492 mg/m^3)	80 min	Inhalation uptake	Testing	Text

Values for $V_{max}C$ and K_m were numerically optimized based on the fit of the model predictions to the measured blood concentrations of 1,2,4-TMB of Hissink et al. (2007) for rats exposed once to one of three concentrations of 1,2,4-TMB as a component of WS. The optimized value of $V_{max}C$ was only modestly different from the value determined by Hissink et al. (2007) (initial: 3.5 vs. optimized: 3.08 mg/hr/kg$^{0.7}$) from visual optimization (with slightly different physiological parameters), but the K_m value differed by 5-fold (initial: 0.25 vs. optimized: 0.050 mg/L). The increase in the LLF from 42.6 to 58.2, with two adjustable parameters, indicates that the improvement in fit (Figure B-10) is statistically significant. The percentage of variation explained increased from 82.3 to 90.4%, and the fit by visual inspection appears to be very good during exposure (modestly overpredicting) and excellent in the post-exposure period. Using the optimized kinetic parameters, the rat brain concentrations of 1,2,4-TMB were also well-predicted (Figure B-11).



Note: Rats exposed to 1,2,4-TMB in white spirit (WS) (Hissink et al., 2007) (a) before and (b) after numerical optimization. See Legend, Figures B-7 and B-8.

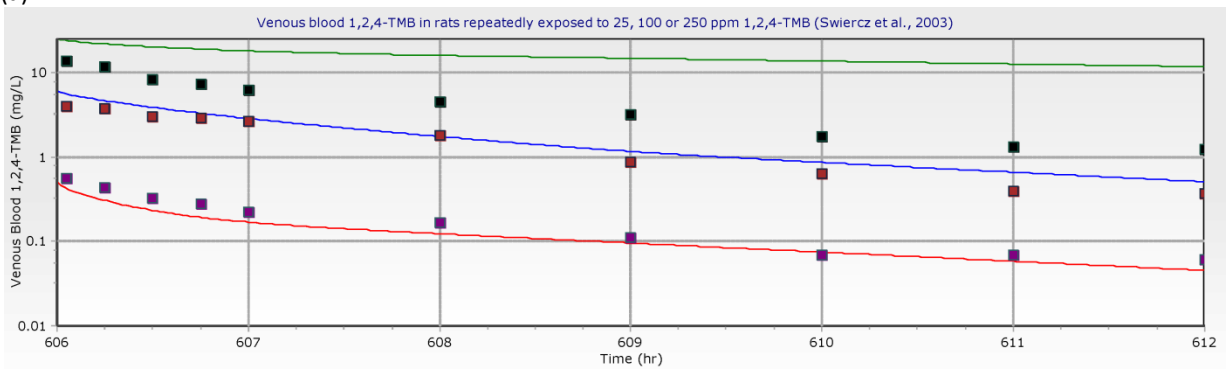
Figure B-10. Comparisons of model predictions to measured blood concentrations in rats exposed to 1,2,4-TMB in WS.

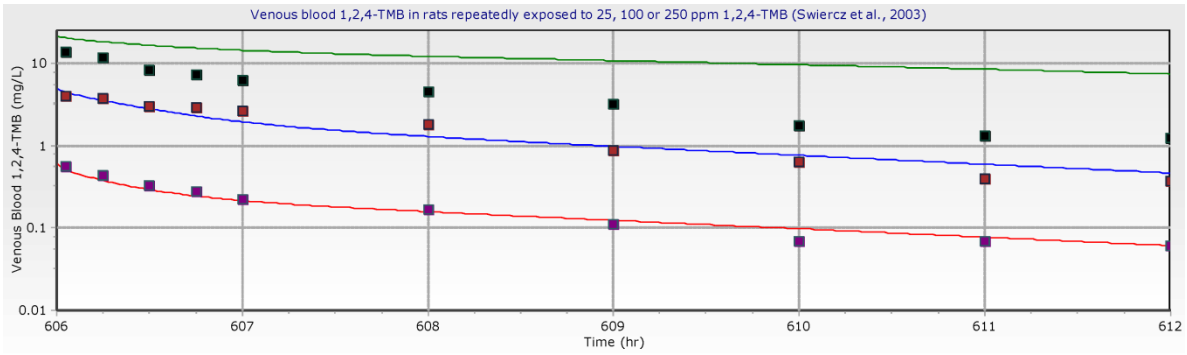


Note: Rats exposed to 1,2,4-TMB in white spirit (WS) (Hissink et al., 2007), using model parameters optimized for fit to Hissink et al. (2007) rat blood data. See Legend in Figures B-7 and B-8.

Figure B-11. Comparisons of model predictions to measured brain concentrations in rats exposed to 1,2,4-TMB in WS.

(a)





(b)

Swiercz et al. (2003) in rats repeatedly exposed to 1,2,4-TMB: (a) before and (b) after numerical optimization. See Legend in Figures B-7 and B-8.

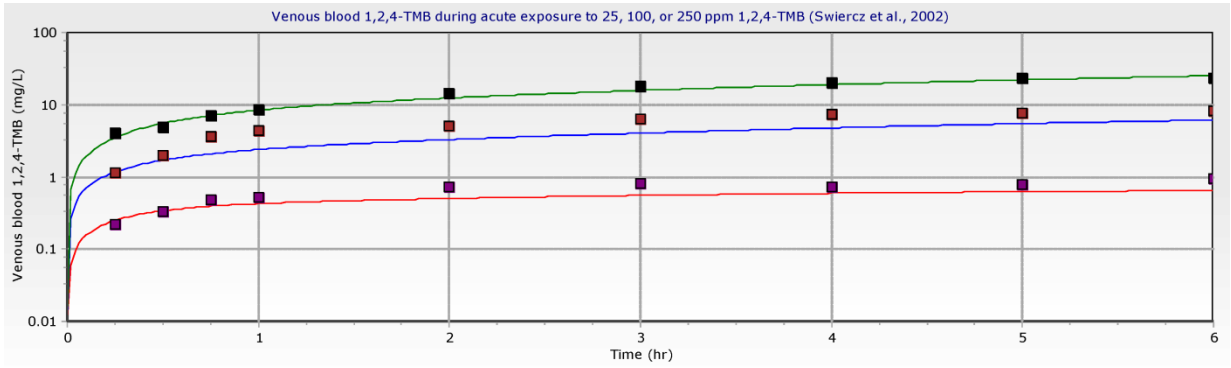
Figure B-12. Comparisons of model predictions to measured venous blood concentrations by Swiercz et al. (2003) in rats repeatedly exposed to 1,2,4-TMB.

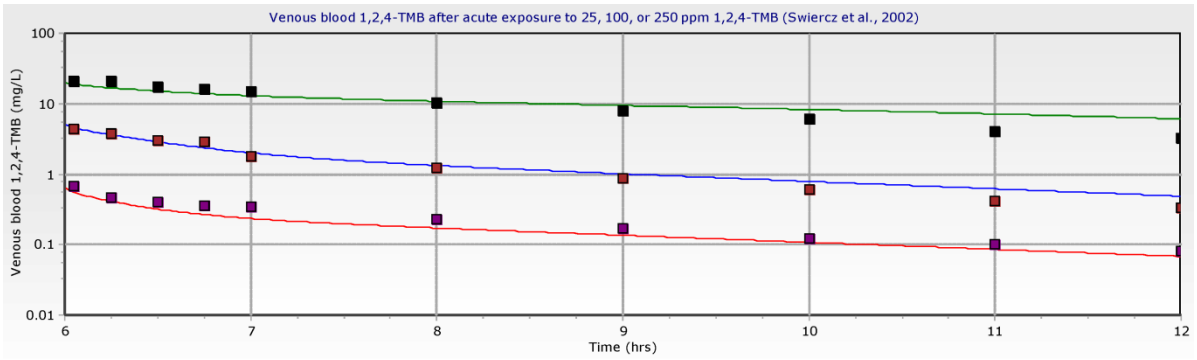
The $V_{max}C$ and K_m values derived from optimization to the Hissink et al. (2007) rat data were used as the starting values for optimizing fit to the venous blood data of Swiercz et al. (2003), in which exposure was to 1,2,4-TMB (only) repeatedly for 4 weeks. Venous blood samples were collected from the tail vein. The best fit parameters of $V_{max}C$ = 4.17 mg/hr/kg$^{0.7}$ and K_m= 0.322 mg/L produced an increase in the LLF from -28.1 to -15.6, a statistically significant improvement, which increased the variation explained from 47.9 to 68.1% (Figure B-12). The deviation between the model and experimental data is primarily exhibeted on the high concentration data set. When this set is not considered, the percent variation explained the remaining two sets is 94.5%. Optimization to the low and middle concentrations alone (omitting the high concentration) does not substantially change the parameters or increase the LLF (simulations not shown). Optimization using the high concentration alone yields $V_{max}C$ and K_m estimates of 7.91 mg/hr/kg$^{0.7}$ and 0.11 mg/L, respectively, with 96.7 percent of variation explained (simulations not shown).

Rat Model Validation

The parameters derived from the Swiercz et al. (2003) venous blood optimizations were used to simulate other studies in which rats and humans (see below) were exposed to 1,2,4-TMB alone (without co-exposures). The fit to the Swiercz et al. (2002) venous blood data was very good (Figure B-13). In fact, the fit to the acute, high-exposure blood concentrations was superior to the fit to the repeated, high-exposure data (Figure B-12b). This may reflect adaptation (induction of metabolism) resulting from repeated, high concentration exposures.

(a)





(b)

Swiercz et al. (2002) in acutely exposed rats: (a) during and (b) after exposure. See Legend in Figures B-7 and B-8.

Figure B-13. Comparisons of model predictions to measured rat venous blood concentrations by Swiercz et al. (2002) in acutely exposed rats.

The model predictions of arterial blood and tissues in the repeated-exposure Swiercz et al. (2003) study were not very accurate, considering that the venous blood data from the same study were used for optimization (Table B-9). The discrepancies between seemingly contemporaneous venous and arterial blood measurements were noted by the authors of the original study and may be due to collection delays (i.e., tail vein for venous blood, decapitation for arterial samples). The geometric mean error ratio (greater of model/experiment or experiment/model) for these data was 2.8.

Table B-9. Model simulated and experimental measured concentrations of 1,2,4 TMB in male Wistar rats exposed to 1,2,4-TMB, Swiercz et al. (2003)

	Exposure concentration	Model (mg/L)	Experiment (mg/L)[a]	Model: Experiment ratio
Repeated exposure (Model t = 606 hr)				
Arterial blood	25 ppm (123 mg/m^3)	0.61	0.33	1.8
	100 ppm (492 mg/m^3)	5.0	1.54	3.2
	250 ppm (1,230 mg/m^3)	22.8	7.52	3.0
Brain	25 ppm (123 mg/m^3)	1.91	0.45	4.2
	100 ppm (492 mg/m^3)	14.6	2.82	5.2
	250 ppm (1,230 mg/m^3)	59.0	18.6	3.2
Liver	25 ppm (123 mg/m3)	0.41	0.45	0.91
	100 ppm (492 mg/m^3)	10.5	3.00	3.5
	250 ppm (1,230 mg/m^3)	54.6	22.5	2.4
Acute exposure (Model t = 6 hr)				
Arterial blood	25 ppm (123 mg/m^3)	0.53	0.31	1.7
	100 ppm (492 mg/m^3)	7.10	1.24	5.7
	250 ppm (1,230 mg/m^3)	18.6	7.76	2.4
Brain	25 ppm (123 mg/m^3)	2.19	0.49	4.5
	100 ppm (492 mg/m^3)	20.6	2.92	7.0
	250 ppm (1,230 mg/m^3)	62.1	18.3	3.4
Liver	25 ppm (123 mg/m^3)	0.49	0.44	1.1
	100 ppm (492 mg/m^3)	16.3	7.13	2.3
	250 ppm (1,230 mg/m^3)	57.7	28.2	2.0

[a]Data source: Swiercz et al. (2003).

Zahlsen and co-workers (Eide and Zahlsen, 1996; Zahlsen et al., 1992; Zahlsen et al., 1990) conducted studies in which male Sprague-Dawley rats were exposed to 1,2,4-TMB by inhalation for 12 hr/day. For the studies conducted at concentrations similar to those in the Swiercz studies (Tables B-11 and B-10), the model error was similar to that of the arterial blood and tissue measurements in the Swiercz studies (geometric mean error of 3.3 for Zahlsen et al. (1990), and 2.9 for Eide and Zahlsen (1996).

Table B-10. Model simulated and experimental measured concentrations of 1,2,4-TMB in male Sprague-Dawley rats exposed to 100 ppm (492 mg/m^3) 1,2,4-TMB (12 hr/day, for 3 days) at the end of exposure or 12 hours after the last exposure

	Day	Model (mg/L)	Experiment (mg/L)[a]	Model: Experiment ratio
Venous blood	1	8.52	1.71	5.0
	2	8.71	1.51	5.8
	3	8.72	2.06	4.2
	Recovery[b]	1.08	0.024	7.6
Brain	1	22.6	4.58	4.9
	2	23.1	4.19	5.5
	3	23.1	4.39	5.3
	Recovery[b]	0.46	Nondetect	Not calculated
Liver	1	18.2	4.93	3.7
	2	18.7	3.67	5.1
	3	18.7	4.25	4.4
	Recovery[b]	0.077	0.072	1.1
Kidney (compared to rapidly perfused)	1	22.6	13.7	1.7
	2	23.1	17.1	1.4
	3	23.1	12.5	1.9
	Recovery[b]	0.46	0.24	1.9
Fat	1	491	210	2.3
	2	503	165	3.1
	3	504	129	3.9
	Recovery[b]	29.1	14.4	2.0

[a]Data from Zahlsen et al. (1992).

[b]Recovery period is designated as 12 hr after the last exposure.

There was essentially no difference in the measured venous blood concentration of 1,2,4-TMB in the Zahlsen et al. (1992) study at 100 ppm (492 mg/m^3) and at 75 ppm (369 mg/m^3) in the Eide and Zahlsen (1996) study ((1.70 and 1.69 mg/L, respectively), so there is evidently some inter-study variability or subtle differences in how the studies were conducted, perhaps in the rapidity of sample collection. The Zahlsen et al. (1990) study, which used a higher nominal concentration of 1,000 ppm (4,920 mg/m^3), exhibited greater deviation between predicted and measured blood and tissue 1,2,4-TMB concentrations (Table B-12), which generally increased with a greater number of exposure days and then plateaued (geometric mean errors of 2.7, 8.4, 12.6, 13.9, and 12.1 on exposure days 1, 3, 7, 10, and 14, respectively).

Table B-11. Model simulated and experimental measured concentrations of 1,2,4-TMB in male Sprague-Dawley rats exposed to 1,2,4-TMB at the end of 12 hour exposure

	Exposure concentration	Model (mg/L)	Experiment (mg/L)[a]	Model: Experiment ratio
Venous blood	75 ppm (369 mg/m^3)	4.21	1.69	2.5
	150 ppm (738 mg/m^3)	17.8	6.9	2.6
	300 ppm (1,476 mg/m^3)	48.3	13.9	3.5
	450 ppm (2,252 mg/m^3)	78.6	26.6	3.0
Brain	75 ppm (369 mg/m^3)	11.5	2.83	4.1
	150 ppm (738 mg/m^3)	46.6	11.7	4.0
	300 ppm (1,476 mg/m^3)	125	26.5	4.7
	450 ppm (2,252 mg/m^3)	203	48.0	4.2
Liver	75 ppm (369 mg/m^3)	7.39	6.41	1.2
	150 ppm (738 mg/m^3)	42.2	14.8	2.9
	300 ppm (1,476 mg/m^3)	120	30.8	3.9
	450 ppm (2,252 mg/m^3)	198	56.2	3.5
Kidney (compared to Rapidly perfused)	75 ppm (369 mg/m^3)	11.5	6.41	1.8
	150 ppm (738 mg/m^3)	46.6	20.2	2.3
	300 ppm (1,476 mg/m^3)	125	33.9	3.7
	450 ppm (2,252 mg/m^3)	203	59.1	3.4
Fat	75 ppm (369 mg/m^3)	255	61.9	4.1
	150 ppm (738 mg/m^3)	987	457	2.2
	300 ppm (1,476 mg/m^3)	2,636	1,552	1.7
	450 ppm (2,252 mg/m^3)	4,276	2,312	1.8

[a]Data from Eide and Zahlsen (1996).

Dahl et al. (1988) exposed male F344 rats to 1,2,4-TMB at 100 ppm (492 mg/m^3) for 80 minutes and monitored the total uptake. Under the conditions of the experiment, it was determined that average rat took up 3.28 (trial 1) or 3.89 (trial 2) mg 1,2,4-TMB. In a model simulation, the predicted uptake was 3.61 mg. Geometric mean model error for the two trials was 1.2.

Table B-12. Model simulated and experimental measured concentrations of 1,2,4-TMB in male Sprague-Dawley rats exposed to 1,000 ppm (4,920 mg/m^3) 1,2,4-TMB (12 hr/day, for 14 days) at the end of exposure

	Day	Model (mg/L)	Experiment (mg/L)[a]	Model: Experiment ratio
Venous blood	1	181	63.5	2.8
	3	293	43.1	6.8
	7	372	33.4	11.1
	10	395	34.0	11.6
	14	399	35.2	11.3
Brain	1	465	120	3.9
	3	747	64.9	11.5
	7	946	63.5	14.9
	10	1,005	62.1	16.2
	14	1,014	71.5	14.2
Fat	1	9,919	5,860	1.7
	3	17,328	2,282	7.6
	7	22,323	1,835	12.2
	10	23,763	1,677	14.2
	14	23,961	2,169	11.0

[a]Data from Zahlsen et al. (1990).

Human Model Validation

Kinetic parameters derived from optimal fit for rat venous blood data (described above) were tested for the applicability to human kinetics by comparison to studies in which humans were exposed to 1,2,4-TMB alone or 1,2,4-TMB in co-exposures with WS (Table B-13). The key data set for validation in humans was deemed to be Kostrzewski et al. (1997) because these volunteers were exposed to 1,2,4-TMB alone (no co-exposure, as in Hissink et al. (2007)) under sedentary conditions (i.e., level of effort was not elevated, as in Järnberg et al. (1998, 1997a; 1996)).

Using the $V_{max}C$ and K_m derived from the Swiercz et al. (2003) rat repeated exposure data, the simulated blood concentration underestimated those measured during exposure of human volunteers by Kostrzewski et al. (1997), then overpredicted blood concentrations up to 7 hours post-exposure, and underpredicted subsequent measured blood concentrations (Figure B-14). Of 21 blood measurements, only two differed from the simulated value by more than a factor of 2 (maximum: 2.6), with a geometric mean deviation of 1.5-fold between the simulated and measured values. The percent variation explained was 69.74%. When K_m was held constant and $V_{max}C$ was optimized (final value: 3.39 mg/hr/kg$^{0.7}$), the improvement in fit was minimal (72.14% of variation explained), and not statistically significant, so the rat-derived values were considered acceptable (see the subsection regarding Rat Model Optimization, in Section B.3.3.2).

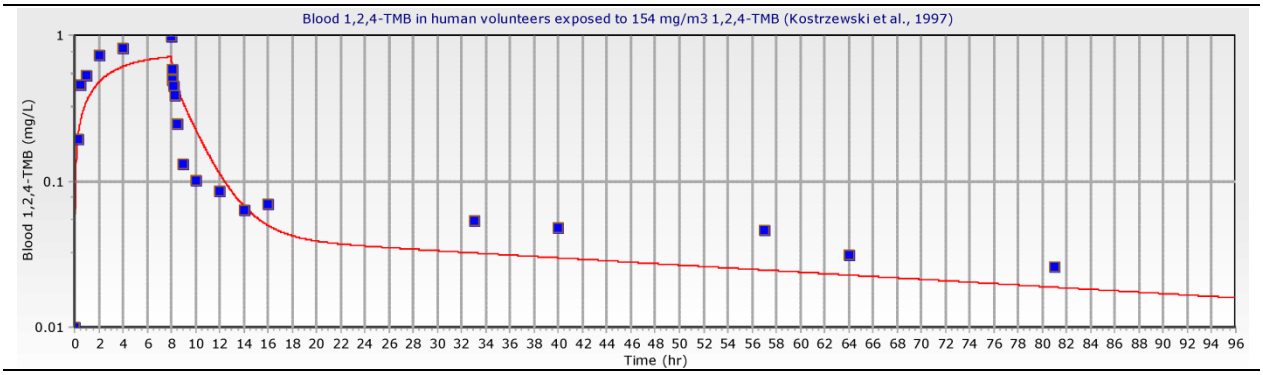
Table B-13. Human kinetic studies of 1,2,4-TMB used in model validation

Reference	Ethnicity	Gender	Nominal concentration	Exposure regimen	1,2,4-TMB measurements	Use in model evaluation	Form of comparison
Kostrzewski et al. (1997)[a]	Not stated; conducted in Poland	Sex not stated. Assumed male.	30 ppm (147.6 mg/m^3)	8 hr	Venous blood time course	Testing	Figure B-14
Jarnberg et al. (1999; 1998, 1997a; 1996)[b]	Caucasian; conducted in Sweden	Male	2 and 25 (~10 and 123 mg/m^3)	2 hr at 50 W (bicycle)	Venous blood and exhaled air time course	Testing (blood data only)	Figure B-15
Hissink et al. (2007)[c]	Not stated; spoke Dutch as "native language"	Male	100 ppm WS with 7.8% 1,2,4-TMB (~38.3 mg/m^3 1,2,4-TMB)	6 hr	Venous blood and end exhaled air time course	Testing	Figure B-16

[a]Five volunteers, ages 24–37, with no known occupational exposure to 1,2,4-TMB. Height of 1.70 to 1.86 m and BW of 70–97 kg. The average of the high and low values for age, height, and weight plus assumed gender (male) were used to calculate central tendency estimate of 22.44% for volume of body fat (VFC), per Deurenberg et al. (1991) . QPC estimated from the midpoint of the range for total ventilation (0.56 to 1 m^3/hr), average of high and low body weights, BW$^{0.74}$ scaling, and an assumption that alveolar ventilation was 2/3 of total ventilation.

[b]Ten volunteers, average age 35, range 26-48, with no known occupational exposure to solvents; volunteers were instructed to avoid contact with organic solvent and to refrain from taking drugs or drinking alcoholic beverages for 2 days before exposure. Average BW 76.5 kg. Alveolar ventilation rate (QPC) estimated from the mean value for total ventilation rate during exposure, average body weights, BW$^{0.74}$ scaling, and an assumption that alveolar ventilation was 2/3 of total ventilation. Digitized blood data (group averages) extracted from figures.

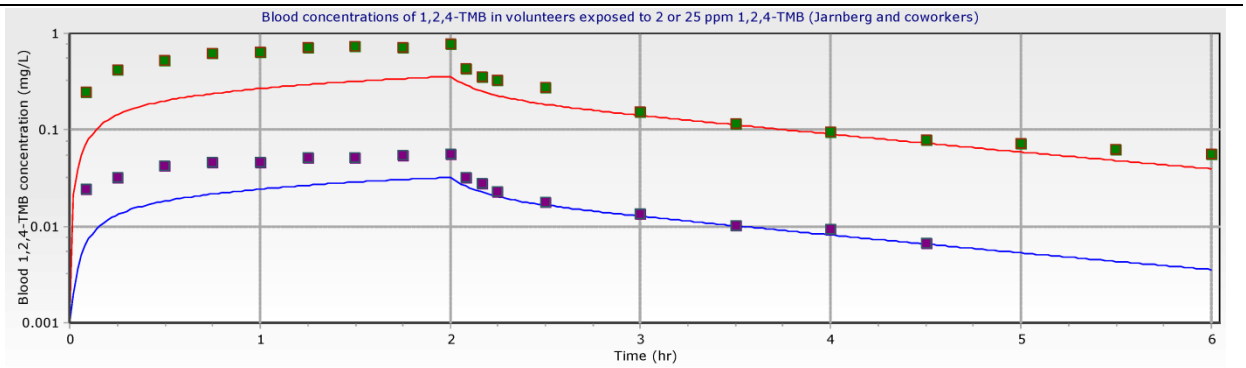
[c]Three volunteers, ages 23–26, BW 69–82 kg, mean body fat of 14.6% (skin caliper measurement); alcohol consumption 10–15 drinks/week (all subjects), one smoker (4 cigarettes per day).



Note: Kostrzewki et al. (1997) in human volunteers exposed to 154 mg 1,2,4-TMB/m^3 for 8 hours.

Figure B-14. Comparisons of model predictions to measured human venous blood concentrations in Kostrzewki et al. (1997) in human volunteers exposed to 154 mg 1,2,4-TMB/m^3 for 8 hours.

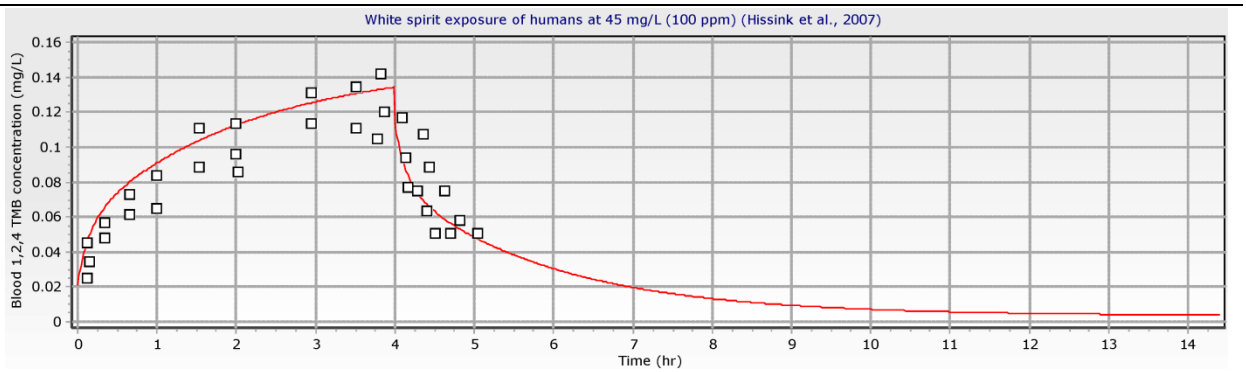
For comparisons between the Järnberg and Johason (1999) and Järnberg et al. (1998, 1997a; 1996) data and the model, simulations were conducted with QPC (calculated as described in footnote to Table B-13) at the elevated (working) level throughout the simulation, but with no other adjustments made for exercise conditions. The model consistently underpredicted the measured venous blood concentrations of 1,2,4-TMB (Figure B-15). At 25 ppm (123 mg/m^3), blood concentrations were underpredicted by a factor of 2.1 to 3.5 during exposure and by a factor of 1.04 to 1.5-fold in the post-exposure period, for a geometric mean discrepancy of 1.7 for this concentration. At 2 ppm (~10 mg/m^3), blood concentrations were underpredicted by factors of 1.7 to 2.7 during exposure and 1.01 to 1.2 in the post-exposure period, for a geometric mean discrepancy of 1.6 for this concentration.



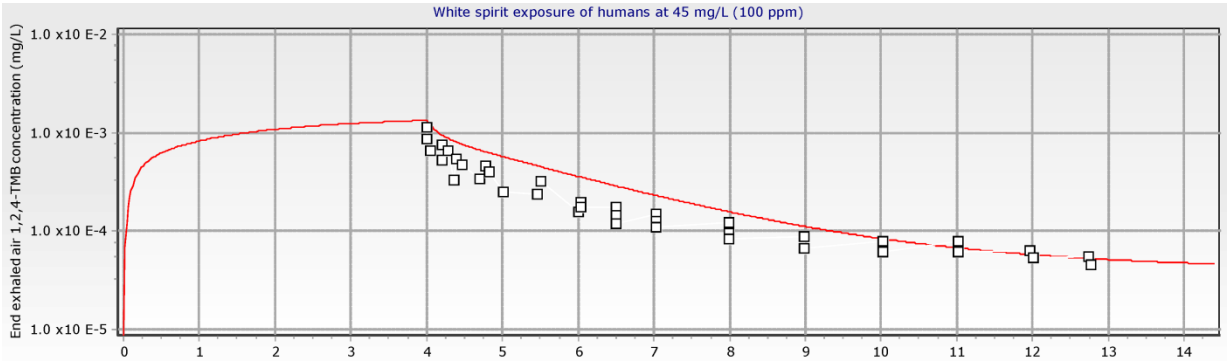
Note: Järnberg et al. (1998, 1997a; 1996) in volunteers exposed to 2 or 25 ppm (~10 or 123 mg/m^3) 1,2,4-TMB for 2 hours while riding a bicycle (50 W).

Figure B-15. Comparisons of model predictions to measured human venous blood concentrations of Järnberg et al. (1998, 1997a; 1996) in volunteers exposed to 2 or 25 ppm (~10 or 123 mg/m^3) 1,2,4-TMB for 2 hours while riding a bicycle (50 W).

Comparisons of model predictions and experimental data were also made for the human study described in Hissink et al. (2007) in which volunteers inhaled 100 ppm WS with 7.8% 1,2,4-TMB (38.4 mg/m^3 1,2,4-TMB) for 4 hours (Figure B-16). The agreement between simulated and measured concentrations of 1,2,4-TMB in blood during exposure was excellent. The agreement between the modeled and measured 1,2,4-TMB in end-exhaled air during the post-exposure period was very good.



(a)



(b)

Note: (a) human venous blood and (b) end of exposure exhaled air 1,2,4-TMB in human volunteers exposed to 100 ppm WS with 7.8% 1,2,4-TMB (38.4 mg/m^3 1,2,4-TMB) (Hissink et al., 2007).

Figure B-16. Comparisons of model predictions to measured (a) human venous blood and (b) end of exposure exhaled air 1,2,4-TMB in human volunteers exposed to 100 ppm WS with 7.8% 1,2,4-TMB (38.4 mg/m^3 1,2,4-TMB).

Summary of Optimization and Validation

Numerical optimization of the fit to the rat data in Hissink et al. (2007) produced a similar $V_{max}C$, but smaller K_m than the values determined by Hissink et al. (2007) using visual optimization. Changes made to values of physiological parameters may have contributed to the differences in optimized values. Because the rats in the Hissink et al. (2007) study were co-exposed to other components of WS, the potential for these other components to alter the kinetics of 1,2,4-TMB was noted as a possible concern for predicting the kinetics of 1,2,4-TMB in test animals with no co-exposures. Another concern was the potential for kinetic changes with repeated exposure. As the Swiercz et al. (2003) rat kinetic study involved repeated exposure to 1,2,4-TMB without potentially confounding co-exposures, and provides post-exposure venous blood time course data, it appears to be the most suitable for describing kinetics relevant to chronic RfC and RfD development. The $V_{max}C$ and K_m values from the numerical optimization to the Hissink et al. (2007) rat data were used as starting values for optimization of the fit to the Swiercz et al. (2003) venous blood data. The improvement in fit for the low and middle concentrations (25 and 100 ppm [123 and 492 mg/m^3]) was apparent from careful visual inspection and was statistically significant, and these values were used in subsequent validation simulations.

In general, the model simulations of venous blood concentrations in exposed Wistar rats, uptake by F344 rats, and venous blood and exhaled breath of human volunteers were acceptable. The measured Wistar rat arterial blood and tissue concentrations were consistently overpredicted by the model, suggesting collection delays in the studies. The model also consistently overpredicted the measured Sprague-Dawley rat tissue and blood concentrations, including the "recovery" (12 hr post-exposure) samples, which should not be subject to collection delays. Many of the "validation" comparisons were made at exposure concentrations (250 ppm [1,230 mg/m^3]or greater) for which the optimized model did not provide accurate venous blood concentrations. It cannot be determined with the available data whether the 2–3-fold differences between the model and Sprague-Dawley rat blood concentrations at lower concentrations (75 and 150 ppm [369 and 738 mg/m^3]) are due to methodological differences (e.g., in sample collections and analysis) or true strain differences. Overall, we conclude that the optimized model produces acceptable simulations of venous blood 1,2,4-TMB for chronic exposure to ≤ 100 ppm (492 mg/m^3) for rats or ≤ 30 ppm (147.6 mg/m^3) for humans 1,2,4-TMB by inhalation. If rat exposures of interest exceed 100 ppm (492 mg/m^3), consideration should be given to reassessing model validation at high concentrations using $V_{max}C$ and K_m parameters optimized for repeated, high concentration exposures [e.g., 250 ppm (1,230 mg/m^3) from Swiercz et al.(2003)].

B.3.3.3. Sensitivity Analysis of Rat Model Predictions

The primary objective of the sensitivity analysis was to evaluate the ability of the available data to unambiguously determine the values of both $V_{max}C$ and K_m (i.e., parameter identifiability). Toward this end, sensitivity analyses were conducted using acslX. Because the selected key data set was the venous blood concentrations in the Swiercz et al. (2003) study, simulations were conducted to see how small changes in parameters changed the estimated venous blood concentrations under the conditions of this study, simulating the first 12 hours (6 hrs exposure, 6 hrs post-exposure), conditions that are essentially identical to those in Swiercz et al. (2002). The evaluations were limited to the lowest (25 ppm [123 mg/m^3]) and highest (250 ppm [1,230 mg/m^3]) exposure concentrations. It should be noted that after the optimization (Figure B-13b), the agreement between the model and the experimental data at the lower exposure concentration was superior to the agreement at the high concentration, so the low concentration sensitivity analysis results are somewhat more meaningful than the high concentration results. The results are calculated as normalized sensitivity coefficients (NSC) (i.e., percent change in output/percent change in input, calculated using the central difference method).

The interpretation of the sensitivity analysis outputs focused on the times during which blood concentrations were measured, so the sensitivity analyses for the first 15 minutes of exposure were not considered relevant. Parameters are grouped (Table B-14) as relatively insensitive (maximum|NSC| < 0.2 for 0.25 hr < t < 12 hr), moderately sensitive (0.2 < maximum|NSC| < 1.0), or highly sensitive (maximum|NSC| > 1.0).

$V_{max}C/K_m$ was identifiable from the data (as opposed to $V_{max}C$ and K_m each being identifiable), one would expect that the NSC for these parameters would always be opposite in sign, and equal in magnitude, which is not the case. We conclude that K_m and $V_{max}C$ are distinctly identifiable using the Swiercz et al. (2003; 2002) data.

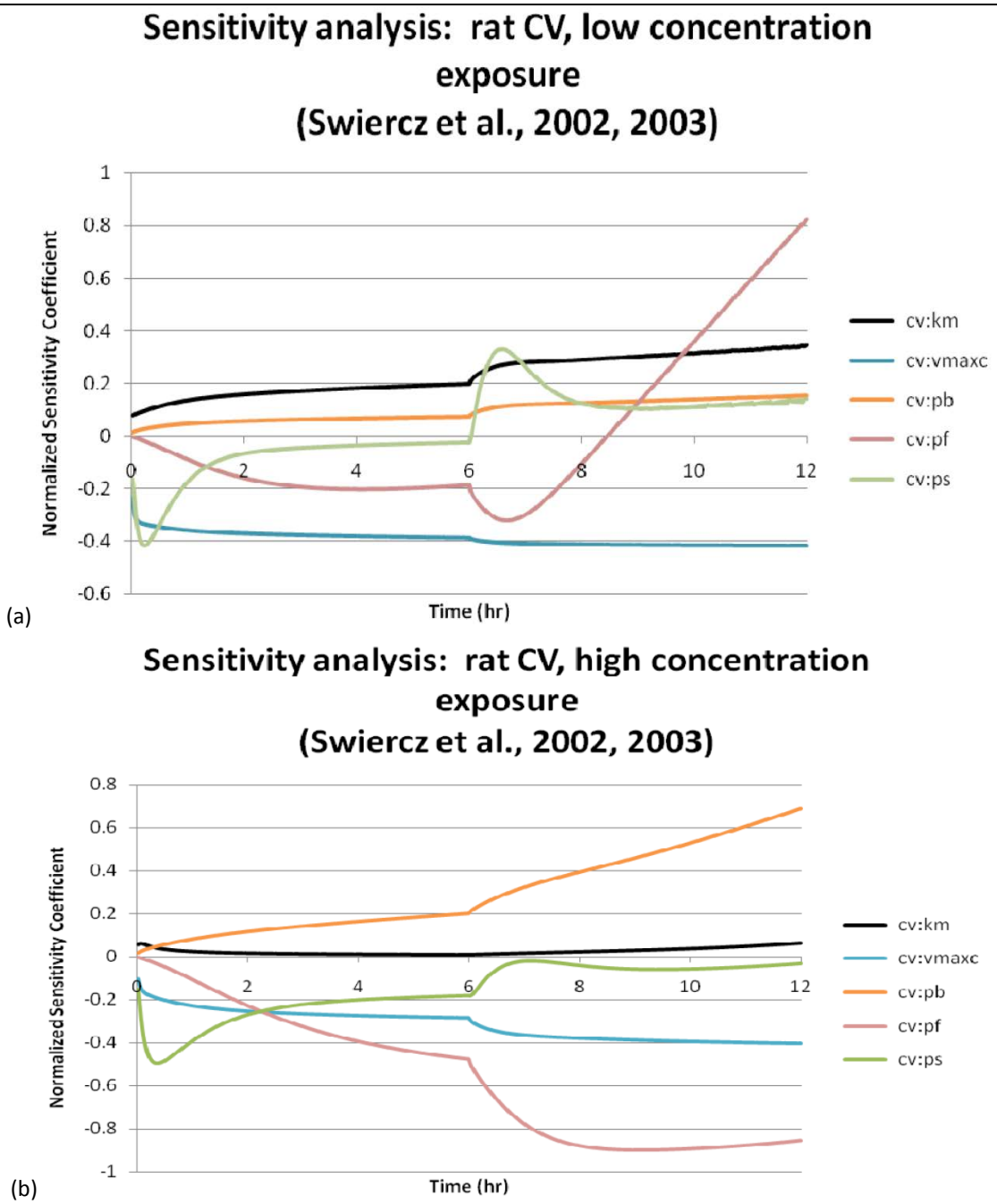
While the focus of this sensitivity analysis was to evaluate the identifiability of chemical-specific parameters from the available data, additional insights can be obtained by considering the other "sensitive" parameters. Predicted blood concentrations were sensitive to the value of QPC (ventilation rate). If high concentrations produce a sedative effect, decreases in ventilation could contribute to the model's greater over-prediction of the experimentally measured values at high concentrations [e.g., as high as 1,000 ppm (4,920 mg/m^3), in Zahlen et al. (1990)]. The accuracy of the predicted net uptake in the Dahl et al. (1988) study indicates that, at 100 ppm (492 mg/m^3), the model value of QPC is likely appropriate, since net uptake in this relatively short experiment (80 minutes) is highly sensitive to the breathing rate (simulations not shown). The fractional volumes of the fat and slowly perfused tissues compartments are also moderately important parameters (with time courses similar to those of the corresponding partition coefficients shown in Figure B-15). The volume of the fat compartment in particular is known to vary with age and strain (Brown et al., 1997), so using the same value for all studies might have an impact on the predicted kinetics.

Table B-14. Parameter sensitivity for venous blood 1,2,4-TMB concentration in rats exposed to 1,2,4-TMB via inhalation

Parameter	Insensitive (maximum |NSC| < 0.2)	Moderately sensitive (0.2 < maximum |NSC| <1.0)	Highly sensitive (maximum |NSC| > 1.0)
BW		L, H	
CONC			L, H
QPC			L, H
$V_{max}C$		L, H	
K_m	H	L	
PB	L	H	
		L, H	
PS		L, H	
PR	L, H		
PL	L, H		
PBR	L, H		
VFC		L, H	
VSTOTC		L, H	
VRTOTC	L, H		
VLC	L, H		
VBRC	L, H		
QCC		H	L
QFC		L, H	
QRTOTC		L, H	
QLC	H		L
QBRC	L, H		

L = low exposure concentration (25 ppm [123 mg/m^3]), H = high exposure concentration (250 ppm [,1230 mg/m^3]).

Body weight (BW), concentration of 1,2,4-TMB in the air (CONC), alveolar ventilation rate (QPC), Michaelis-Menten maximum rate of metabolism ($V_{max}C$), Michaelis-Menten constant: concentration where $V_{m,ax}$ is half-maximal (V_{max}), blood:air partition coefficient (PB), fat:blood partition coefficient (PF), slowly perfused:blood partition coefficient (PS), rapidly perfused:blood partition coefficient (PR), liver:blood partition coefficient (PL), brain:blood partition coefficient (PBR), volume of fat (VFC), volume of slowly perfused tissues (VSTOTC), volume of rapidly perfused tissues (VRTOTC), volume of liver (VLC), volume of brain (VBRC), cardiac output (QCC), blood flow to fat (QFC), blood flow to slowly perfused tissues (QRTOTC), blood flow to liver (QLC), blood flow to brain (QBRC)



Note: Rats exposed to (a) 25 ppm (123 mg/m^3) or (b) 250 ppm (1,230 mg/m^3) of 1,2,4-TMB via inhalation for 6 hours (Swiercz et al., 2003; Swiercz et al., 2002).

Figure B-17. Time course of normalized sensitivity coefficients of moderately sensitive chemical-specific parameters (response: venous blood concentration) in rats exposed to (a) 25 ppm (123 mg/m^3) or (b) 250 ppm (1,230 mg/m^3) of 1,2,4-TMB via inhalation for 6 hours.

B.3.3.4. Sensitivity Analysis of Human Model Predictions

A sensitivity analysis for human model predictions to all parameters was conducted for continuous inhalation exposures, and results are shown in Table B-15. The results are presented as normalized sensitivity coefficients (i.e., percent change in output/percent change in input, calculated using the central difference method; NSC). Similar to analyses performed for the rat, parameters are noted as relatively insensitive (|NSC| < 0.2), moderately sensitive (0.2 < |NSC| < 1.0), or highly sensitive (|NSC| > 1.0). To bracket the range of human equivalent concentrations (HECs), inhalation sensitivities were evaluated at 10 and 150 ppm (49.2 and 738 mg/m^3) concentration. The resulting coefficients (Table B-15) are not surprising. The two fitted metabolic parameters, $V_{max}C$ and K_m both influence model predictions. The $V_{max}C$ sensitivity is higher at 150 ppm (738 mg/m^3) (|0.8873|) than at 10 ppm (49.2 mg/m^3) (|0.238|) due to the slight metabolic saturation.

Table B-15. Parameter sensitivity for steady-state venous blood 1,2,4-TMB concentration in humans exposed to 1,2,4-TMB via inhalation

Parameter	Insensitive (maximum|NSC| < 0.2)	Moderately sensitive (0.2 < maximum|NSC| < 1.0)	Highly sensitive (maximum|NSC| > 1.0)
BW	L, H		
CONC		L	H
QPC		L, H	
$V_{max}C$		L, H	
K_m	L, H		
PB	L, H		
	L, H		
PS	L, H		
PR	L, H		
PL	L, H		
PBR	L, H		
VFC	L, H		
VSTOTC	L, H		
VRTOTC	L, H		
VLC	L, H		
VBRC		L, H	
QCC	L, H		
QFC	L, H		
QRTOTC		L, H	
QLC	L, H		

L = low exposure concentration (10 ppm [49.2mg/m^3]), H = high exposure concentration (150 ppm [738 mg/m^3]).

Body weight (BW), concentration of 1,2,4-TMB in the air (CONC), alveolar ventilation rate (QPC), Michaelis-Menten maximum rate of metabolism ($V_{max}C$), Michaelis-Menten constant: concentration where $V_{m,ax}$ is half-maximal (V_{max}), blood:air partition coefficient (PB), fat:blood partition coefficient (PF), slowly perfused:blood partition coefficient (PS), rapidly perfused:blood partition coefficient (PR), liver:blood partition coefficient (PL), brain:blood partition coefficient (PBR), volume of fat (VFC), volume of slowly perfused tissues (VSTOTC), volume of rapidly perfused tissues (VRTOTC), volume of liver (VLC), volume of brain (VBRC), cardiac output (QCC), blood flow to fat (QFC), blood flow to slowly perfused tissues (QRTOTC), blood flow to liver (QLC), blood flow to brain (QBRC)

B.3.3.5. Modification of the Hissink et al. (2007) model to include oral route of exposure

For derivation of an oral RfD, the updated 1,2,4-TMB PBPK model based on Hissink et al. (2007) was further modified by adding code for continuous oral ingestion. It was assumed that 100% of the ingested 1,2,4-TMB is absorbed by constant infusion of the oral dose into the liver compartment. There were no oral data available to calibrate the model for oral absorption and no data were available evaluate the model predictions following oral ingestion either. Thus, although the assumption that 100% of the dose would enter the liver is a common assumption, it does represent an area of uncertainty in the route-to-route extrapolation used to derive oral reference values.

The contribution of the first-pass metabolism in the liver for oral dosing was evaluated by simulating steady state venous blood levels (at the end of 50 days continuous exposure) for a standard human at rest (70 kg) for a range of concentrations and doses. For ease of visual comparison (Figure B-18), concentrations were converted to daily doses based on the amount of 1,2,4-TMB inhaled, as computed by the model. (An inhaled concentration of 0.001 mg/L [0.20 ppm (0.98 mg/m^3)] is equivalent to an inhaled dose of 0.12 mg/kg/day.) At both very low and very high daily doses by inhalation or oral dosing, steady state CV is essentially linear with respect to the daily dose, but with different CV/dose ratios and a transition zone between 1 and 100 mg/kg/day. At low daily doses, equivalent inhalation doses result in steady state blood concentrations 4-fold higher than an equivalent oral dose due to the hepatic first-pass effect. The first-pass effect becomes insignificant with respect to steady-state venous blood concentrations for daily doses in excess of ~50 mg/kg/day.

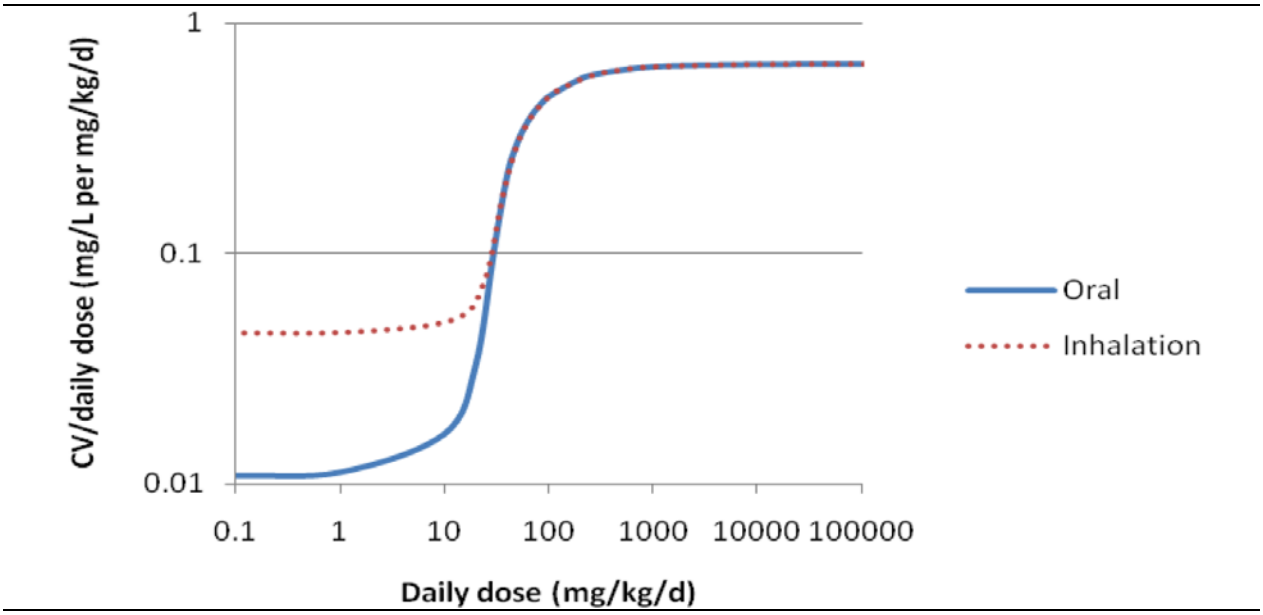


Figure B-18. Effect of route of exposure and dose rate on steady-state venous blood concentration (t = 1,200 hr) for continuous human exposure to 1,2,4-TMB.

B.3.3.6. Conclusions

Several changes were made to the model for use in this assessment: (1) Updated physiological parameters were implemented (Brown et al., 1997); (2) Hepatic metabolism was revised to omit variation over time and new $V_{max}C$ and K_m values were estimated through numerical optimization; and (3) An oral dosing component was added to the model as constant infusion into the liver compartment. The values were optimized to Hissink et al. (2007) data and resulted in a $V_{max}C$ of 4.17 mg/hr/kg$^{0.7}$ and K_m of 0.322 mg/L. In addition, the model was tested for its ability to predict published rat data resulting from exposure to 1,2,4-TMB alone (Swiercz et al., 2003; Swiercz et al., 2002; Eide and Zahlsen, 1996; Zahlsen et al., 1992; Zahlsen et al., 1990; Dahl et al., 1988). Using the optimized values, the model adequately predicted the data and lower concentrations. Human data (Hissink et al., 2007; Järnberg and Johanson, 1999; Järnberg et al., 1998, 1997a; Kostrzewski et al., 1997; Järnberg et al., 1996) were also utilized to validate model predictions.

B.3.4. Summary of Available PBPK models for 1,3,5-TMB or 1,2,3-TMB

There are currently no available PBPK models for rodents or humans for either 1,3,5-TMB or 1,2,3-TMB.

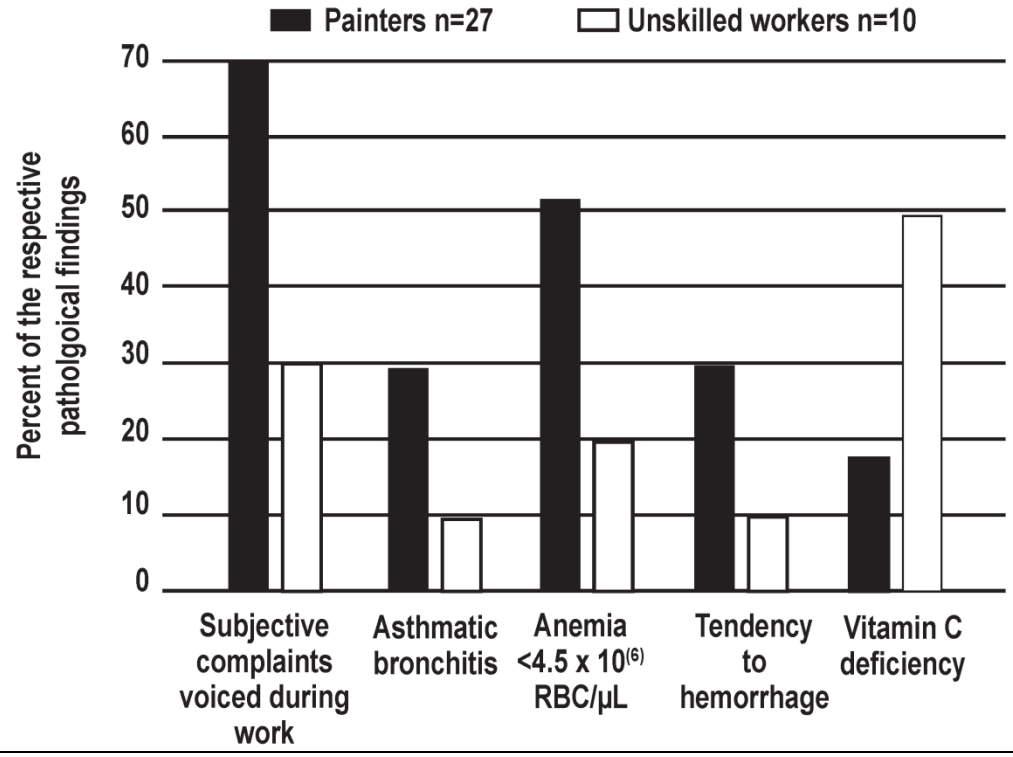
B.4. HUMAN STUDIES

Table B-16. Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB. Battig et al. (1956), as reviewed by Baettig et al. (1958)

Study (location)	**Outcome assessment**
• Transportation plant in Switzerland	• Survey was conducted to investigate the CNS, respiratory, hematological effects of long-term TMB exposure • Additional information on working history, personal history, and psychiatric health was collected
POPULATION CHARACTERISTICS	
Exposed population	**Referent or control description**
• 27 TMB-exposed workers that worked primarily in the painting shop of the transportation plant	• 10 unskilled workers from the same plant that were not exposed to TMB vapors.
Exposure assessment	**Statistical analysis**
• Exposure level: 10–60 ppm (49.2–295 mg/m^3) in working rooms • Exposure duration: approximately 10 years • Compounds to which study participants were exposed: Fleet-X DV-9, a solvent that contained 1,2,4-TMB and 1,3,5-TMB (50% and 30%, respectively) for approximately 10 years. Fleet-X DV-99 also potentially contained 1,2,3-TMB and numerous methylethylbenzenes.	• No statistical analyses were reported.

Table B-16 (Continued): Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB. Battig et al. (1956), as reviewed by Baettig et al. (1958)

RESULTS
Exposure subgroup
• Increased self reports of vertigo, headaches, and drowsiness during work. • Increased presence of chronic asthmatic bronchitis, anemia, and altered blood clotting characteristics (e.g., increased clotting time and tendency to hemorrhage). • Increased vitamin C deficiency was observed in controls, but the authors attribute this to nutritional deficiencies in this population.
Effect estimate (95% CI)
Figure 1. Clinical findings obtained from workers exposed to TMB compared to unskilled worker controls not exposed to TMB.



Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

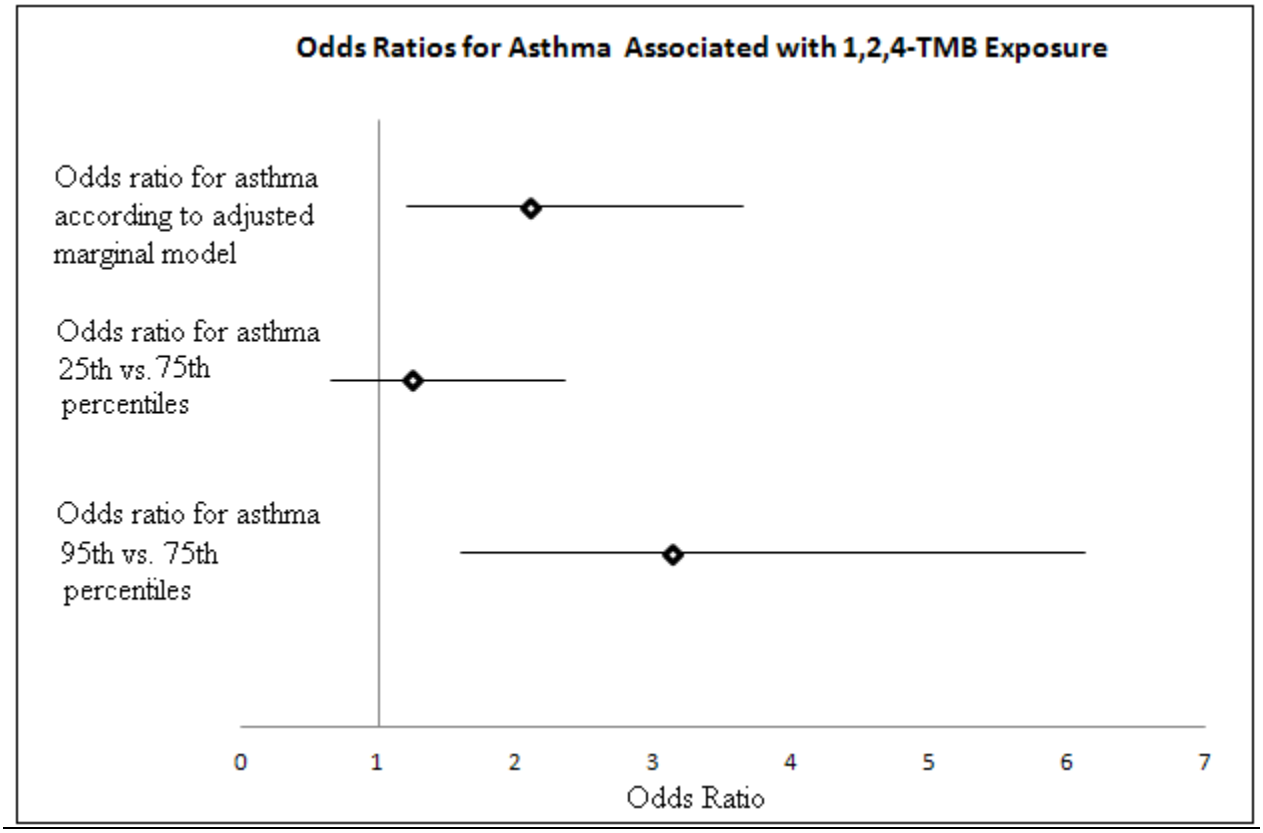
Data source: Battig et al. (1956), as reviewed by Baettig et al. (1958)

Table B-17. Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB; Billionnet et al. (2011)

Study (location)	**Outcome assessment**
• Random selection of dwellings throughout France	• Standardized, self-administered questionnaire was completed by participants to determine number and severity of respiratory effects, particularly asthma and rhinitis. • Additional information on daily habits, smoking status, and sociodemographic variables was collected. • Diagnosis of rhinitis or asthma was not confirmed by a physician.
POPULATION CHARACTERISTICS	
Exposed population	**Referent or control description**
• 1,612 individuals living in 567 dwellings, aged 15 or older. • Surveys were conducted and air samples were collected over a period of one week.	• The study cohort was also used as the control group. Dwellings with low levels of individual volatile organic compound (VOCs) were used as controls for that particular compound.
Exposure assessment	**Statistical analysis**
• Exposure level: For 1,2,4-TMB, exposure varied from undetectable to 111.7 µg/m^3, with median concentration 4.0 µg/m^3. • Exposure duration: Not reported; reported measurements represent the means of one week of monitoring.	• Pollutant correlations tested by Spearman's rank correlation coefficient. • Generalized estimating equation approach used to adjust for correlations between individuals within same dwelling. • Global VOC score was created to address exposure to multiple pollutants. • All models were adjusted for age, sex, and smoking status.

Table B-17 (Continued): Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB; Billionnet et al. (2011)

RESULTS
Exposure subgroup
• Statistically significant increase in odds ratios for asthma following 1,2,4-TMB exposure. • No statistically significant increase in odds ratio for rhinitis and 1,2,4-TMB exposure.
Effect estimate (95% CI)
Figure 1. Odds ratios for asthma and asthma/rhinitis and exposure to 1,2,4-TMB. For all models, data was adjusted for confounders.



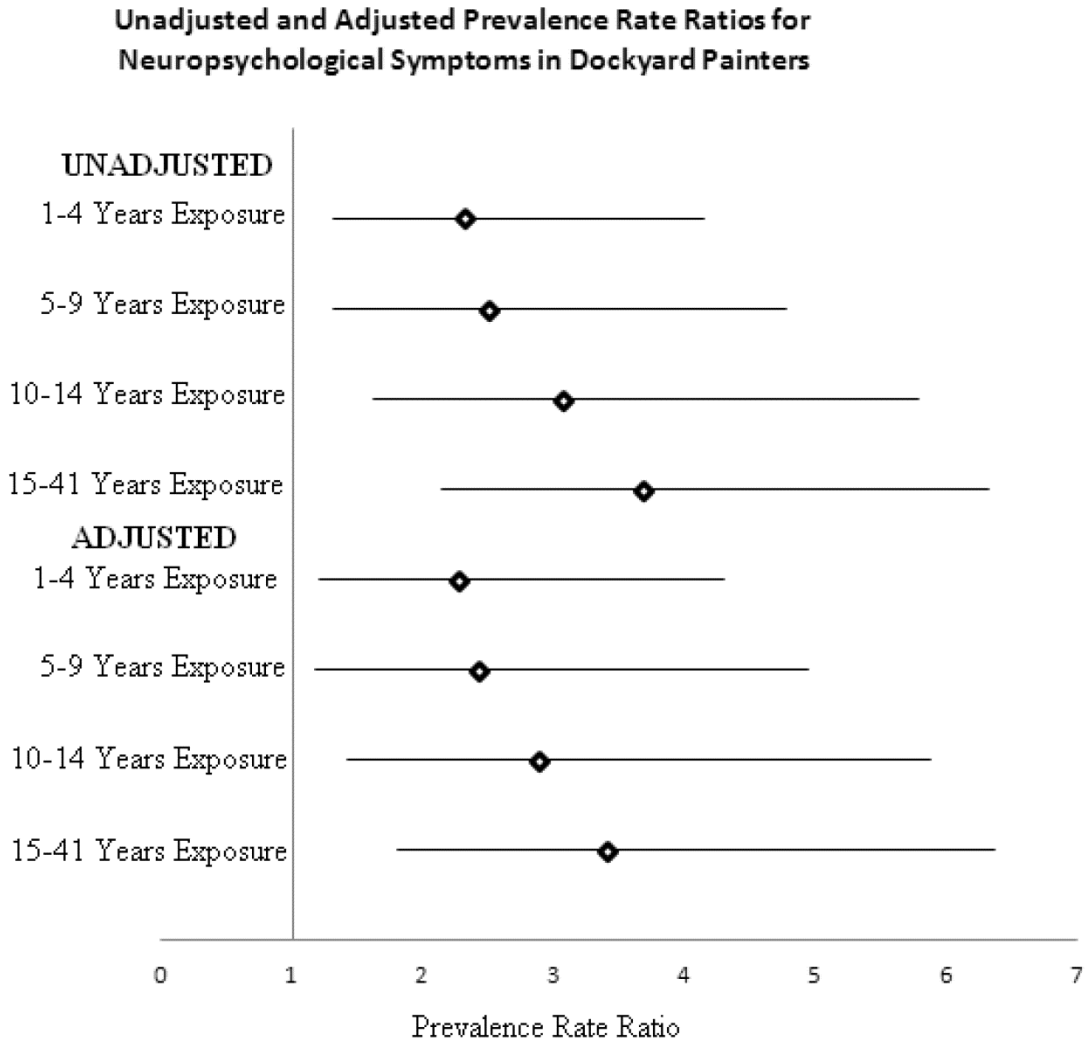
Source: Billionnet et al. (2011)

Table B-18. Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Chen et al. (1999)

Study (location)	**Outcome assessment**
• Dockyard in Scotland, United Kingdom	• Survey was conducted to determine mortality, symptoms, and risks of paint exposure. • Additional information on age, education, smoking, alcohol consumption, and personality was collected.
POPULATION CHARACTERISTICS	
Exposed cohort	**Referent or control description**
• 1292 TMB-exposed males who worked as painters in a dockyard for at least 1 yr between 1950 and 1992. • Follow up period extended from 1960 through 1994	• 953 individuals matched by age and selected from lists of patients of local primary care physicians.
Exposure assessment	**Statistical analysis**
• Exposure level: Specific concentrations not discussed • Exposure duration: at least 1 yr; range 1–41 years • Compounds to which study participants were exposed: white spirit (1,2,4-TMB), xylene, TMB (unspecified), n-butanol, trichlorethylene, naptha, and cumene.	• Intra-cohort proportional mortality ratios were calculated, as were standardized mortality ratios for comparison with all Scottish males. 95% confidence intervals calculated assuming a Poisson distribution. • χ^2 test used to assess differences in neuropsychological symptoms between painters and non-painters. • Brestow-Cox model used to adjust for covariates including educational level, smoking, alcohol consumption, and social conformity. • Log-regression model used for case-control study.

Table B-18 (Continued): Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Chen et al. (1999)

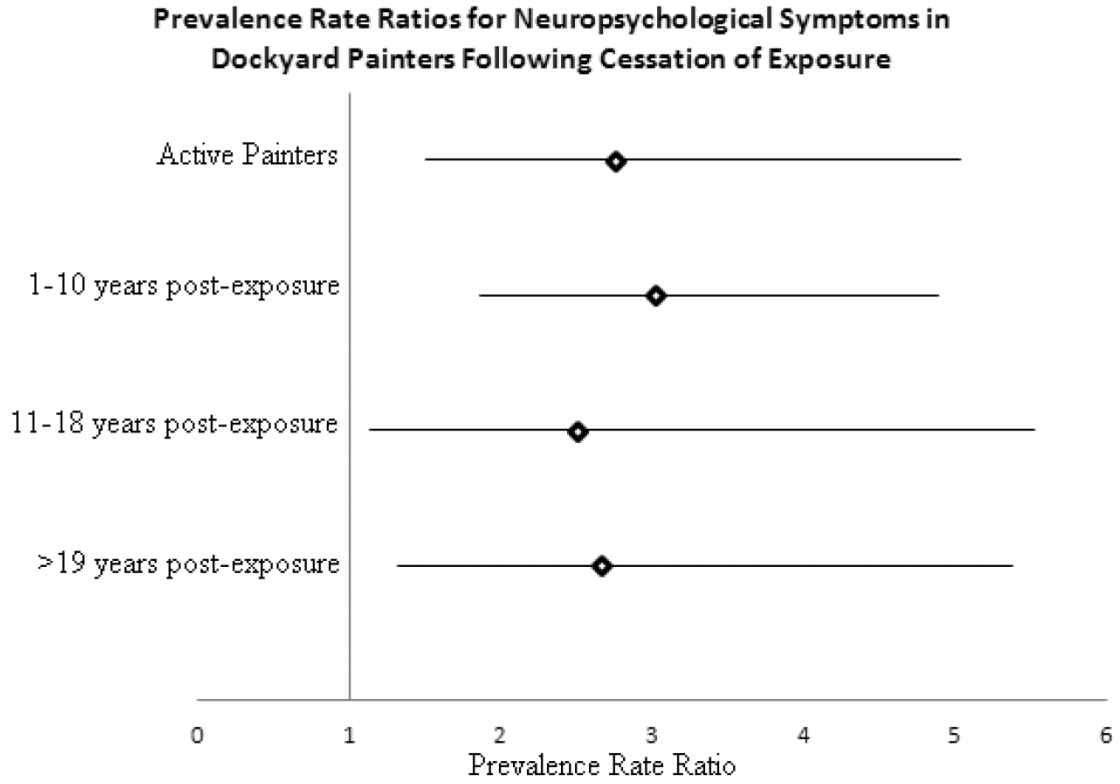
RESULTS
Exposure subgroup
• Increased prevalence rate ratios for neuropsychological symptoms amongst painters. • Rate ratios increased significantly with increasing number of years of exposure, even after adjustment for possible confounders. • Multivariate-adjusted odds ratios within nested case-control analysis showed same relationship.
Effect estimate (95% CI)
Figure 1. Unadjusted and adjusted prevalence rate ratios for neuropsychological symptoms in dockyard painters vs. controls. With increasing years of exposure, rate ratios were found to increase. Symptoms included difficulty in buttoning and unbuttoning, trembling hands, or unsteadiness in arms or legs. For trend in unadjusted rate ratios, p<0.00001.



Source: Chen et al. (1999).

Table B-18 (Continued): Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Chen et al. (1999)

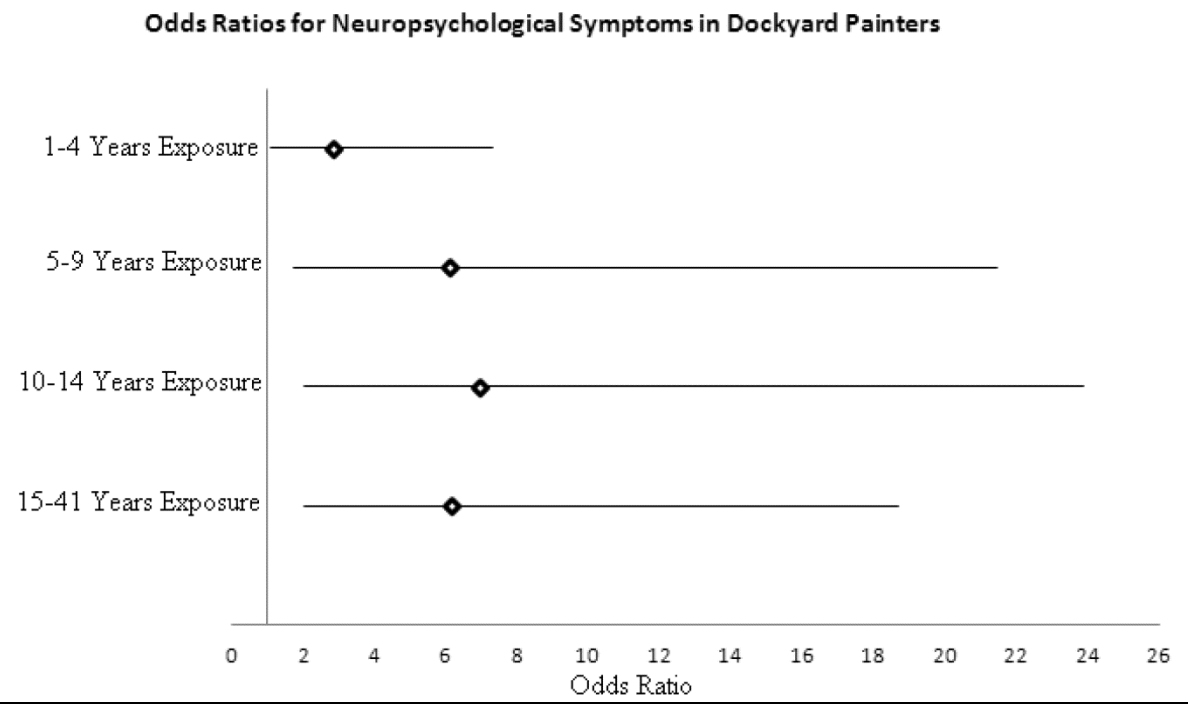
Figure 2. The effect of elapsed time since cessation of painting on all symptoms. Values reported are prevalence rate ratios for painters vs. non-painters. No significant decrease in risk with increasing post-exposure time was found.



Source: Chen et al. (1999).

Table B-18 (Continued): Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Chen et al. (1999)

Figure 3. The effect of exposure duration on odds ratio for neuropsychological symptoms. With increasing years of exposure, odds ratios were found to increase.



Source: Chen et al. (1999).

Table B-19. Characteristics and quantitative results for controlled human exposure study of exposure to 1,2,4-TMB in WS. Lammers et al. (2007)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Humans	M	12	Inhalation	57 or 570 mg/m^3	4 hrs

Additional Study details

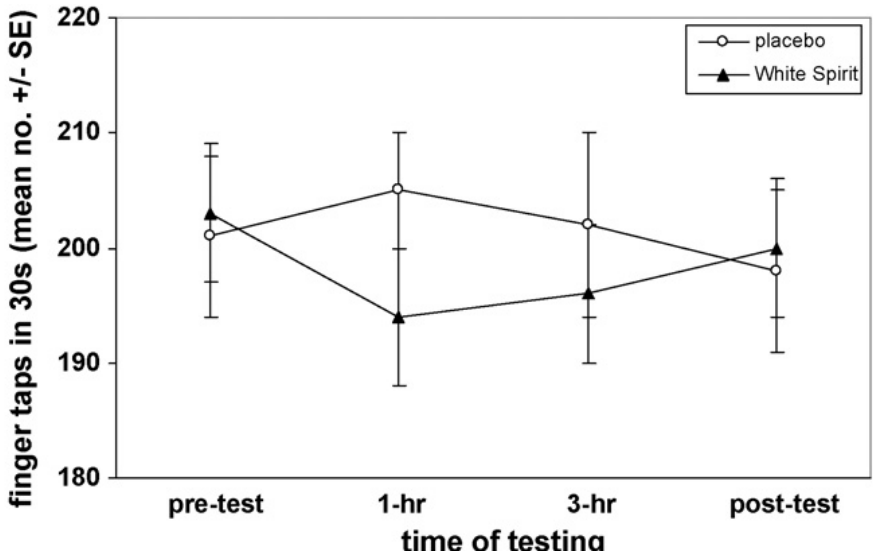
- Human volunteers were exposed to 57 or 570 mg/m^3 during two test sessions separated by 1 week, each lasting 4 hrs.
- Several tests were conducted to evaluate impact of WS on CNS. These included tests of observation, reaction time, and hand-eye coordination.
- In humans, attention deficit was observed following WS inhalation.
- The study protocol was approved by the TNO's Institutional Review Board

	Test scores (mean ± SD) at various time points in humans exposed to 57 or 570 mg/m^3 WS, for 4 hrs	
Observation	**57 mg/m^3**	**570 mg/m^3**
Mood and affect		
Fatigue (scale score)		
Pre-test	1.11 ± 0.04	1.11 ± 0.05
1 hr	1.06 ± 0.03	1.17 ± 0.09
3 hrs	1.21 ± 0.12	1.29 ± 0.13
Post-test	1.38 ± 0.15	1.51 ± 0.23
Vigor (scale score)		
Pre-test	3.35 ± 0.20	3.53 ± 0.09
1 hr	3.58 ± 0.16	3.23 ± 0.20
3 hrs	3.27 ± 0.20	3.32 ± 0.22
Post-test	2.98 ± 0.23	3.05 ± 0.22
Psychomotor skills (hand-eye coordination and finger tapping)		
Hand-eye coordination test (pixels in lnMAE)		
Pre-test	1.69 ± 0.05	1.67 ± 0.04
1 hr	1.56 ± 0.05	1.64 ± 0.04
3 hrs	1.64 ± 0.05	1.63 ± 0.04
Post-test	1.62 ± 0.04	1.55 ± 0.06
Finger tapping test (no. of taps in 30 seconds)		
Pre-test	201 ± 7	203 ± 6
1 hr	205 ± 5	194 ± 6
3 hrs	202 ± 8	196 ± 6
Post-test	198 ± 7	200 ± 6

Table B-19 (Continued): Characteristics and quantitative results for controlled human exposure study of exposure to 1,2,4-TMB in WS. Lammers et al. (2007)

Attention		
Reaction time test (latency, ms)		
Pre-test	251 ± 9	246 ± 8
0.25 hrs	248 ± 10	252 ± 9
1 hr	248 ± 9	254 ± 9
2.25 hrs	253 ± 9	266 ± 12
3 hrs	253 ± 11	257 ± 10
Post-test	258 ± 11	269 ± 13
Color word vigilance test (latency, ms)		
Pre-test	579 ± 28	595 ± 22
1 hr	550 ± 20	569 ± 20
3 hrs	537 ± 17	561 ± 23
Post-test	532 ± 18	557 ± 22

Figure 2. Performance on finger tapping test with the dominant hand at different time points during and after exposure.



Health Effect at LOAEL	NOAEL	LOAEL
n/a	n/a	n/a

Comments: Exposure to 1,2,4-TMB was via WS, which is comprised of additional substances. LOAEL and NOAEL for 1,2,4-TMB alone cannot be extracted from this study because other constituents of the WS mixture may confound results.

Source: Lammers et al. (2007).

Table B-20. Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Lee et al. (2005)

Study (location)	**Outcome assessment**
• A shipyard in Ulsan, Korea	• Various neurobehavioral parameters were measured with computer-based neurobehavioral assessments. • Measured parameters included simple reaction time, symbol digit substitution, and finger tapping speed. • Additional information on occupational history, medical history, age, work duration, education level, alcohol use, and smoking status.
POPULATION CHARACTERISTICS	
Exposed population	**Referent or control description**
• 180 shipyard workers exposed to mixed organic solvents. • Workers were exposed generally during painting activities within the shipyard.	• 60 Shipyard workers that were *not* exposed to mixed organic solvents were used as the referent group
Exposure assessment	**Statistical analysis**
• Data on exposure was collected from 61 workers who wore passive dosimeters on 3 work days. • Average Exposure duration: 16.5±9 years in exposed workers.	• A cumulative exposure index was calculated for each worker. • Student *t*-test was used to determine statistical significance of results in exposed workers compared to non-exposed workers.

Table B-20 (Continued): Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Lee et al. (2005)

RESULTS						
Exposure Subgroup						
• Exposed workers showed significant alterations to symbol digit distribution, dominant hand finger tap rate, and non-dominant hand finger tap rate. • Work duration was also found to influence symbol digit substitution						
	Results of Neurobehavioral Test of Study Subjects					
	Unadjusted Mean ±Std Dev			**Adjusted[a] Mean (S.E.)**		
Observation	**Painters**	**Controls**	**p-value**	**Painters**	**Controls**	**p-value**
Simple Reaction Time	297.2±70.0	292.2±95.0	0.671	296.0 (5.9)	295.8 (10.9)	0.992
Symbol Digit Substitution	3233.2±998.9	2,693.8±711.8	0.000	3,156.6 (67.7)	2,691.6 (124.3)	0.000
Finger tap speed DH[b]	62.6±8.2	66.4±9.7	0.000	63.0 (0.6)	65.5 (1.2)	0.046
Finger tap speed NDH[c]	55.9±8.0	60.2±9.7	0.000	56.1 (0.7)	60.3 (1.2)	0.003

	Neurobehavioral Test Results by Duration of Work, Adjusted for Age and Education		
Observation	**<10 Working Years (S.E.) n = 48**	**10-20 Working Years (S.E.) n = 41**	**>20 Working Years (S.E.) n = 91**
Simple Reation Time	297.8 (20.4)	297.9 (11.2)	292.3 (11.6)
Symbol Digit Substitution	2,972.1 (282.5)	3,033.8 (155.1)	3,452.4 (160.7)*
Finger Tap Speed DH	64.8 (2.3)	63.9 (1.3)	61.3 (1.3)**
Finger Tap Speed NDH	57.6 (2.4)	56.3 (1.3)	55.2 (1.3)

[a]Adjusted for age and education
[b]Finger tapping speed of dominant hand
[c]Finger tapping speed of non-dominant hand
*, ** $p < 0.05$, $p = 0.052$
Source: Lee et al. (2005).

Table B-21. Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB; Norseth et al. (1991)

Study (location)	**Outcome assessment**
• Norway	• Symptoms were recorded via a standard questionnaire on the last day of monitoring. • Monitoring of organic compounds was conducted for 5 days in workers who were divided into subsets based on their level of exposure. • Asphalt, weather, and traffic density data was recorded daily.
POPULATION CHARACTERISTICS	
Exposed population	**Referent or control description**
• In the first group, 79 workers were divided into groups of 5 or 6 based on their exposure level. • A second group of 254 (of which the initial group of 79 was representative) workers completed questionnaires about symptoms.	• A group of 247 maintenance workers who were not exposed to asphalt. The group was given a questionnaire similar to the exposed group.
Exposure assessment	**Statistical analysis**
• Mean concentration of 1,2,4-TMB was 0.015 ppm (0.074 mg/m^3), with range between 0 and 0.122 (0 – 0.60 mg/m^3) ppm. • Mean concentration of 1,3,5-TMB was 0.0014 ppm (0.0069 mg/m^3), with range between 0 and 0.011 (0 – 0.054 mg/m^3) ppm. • Exposure duration: Not reported; measurements represent the means of five days of monitoring.	• Exact two-sided Fisher-Irving test was used to analyze differences in symptom frequency. • Mean difference between groups calculated via two-sided Wilcoxon rank-sum test with a significance level of 5%. • Spearman's correlation coefficient used to estimate correlation between symptoms and possible confounders.

Table B-21 (Continued): Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB; Norseth et al. (1991)

RESULTS				
Exposure subgroup				
• An increase in number of several symptoms was associated with asphalt exposure when asphalt-exposed road workers were compared with workers not exposed to asphalt. • 1,2,4-TMB was found to increase number of symptoms, while no similar correlation was found for 1,3,5-TMB.				
Effect estimates[a]				
Observation	**Symptoms associated with asphalt exposure in exposed and non-exposed groups of workers***			
	Days with symptom	**Asphalt workers (n = 79)**	**Asphalt workers (n = 254)**	**Non-asphalt workers (n = 247)**
	Symptoms of asphalt exposure			
Abnormal fatigue	None	64.6	75.2	84.6
	1–2	21.5	14.6	9.7
	3–5	13.9	10.2	5.7
Reduced appetite	None	86.1	89.8	95.1
	1–2	12.7	7.5	4.1
	3–5	1.3	2.8	0.8
Laryngeal/pharyngeal irritation	None	63.3	74.0	83.0
	1–2	21.5	15.4	11.7
	3–5	15.2	10.6	5.3
Eye irritation	None	54.4	68.9	85.4
	1–2	22.8	22.4	10.5
	3–5	22.8	8.7	4.1
Other, unspecified symptom	None	91.1	85.4	92.3
	1–5	8.9	14.6	7.7

[a]For correlation between symptom sum and 1,2,4-TMB exposure, r = 0.31, $p<0.01$.
*All differences between asphalt workers (n = 254) and non-asphalt workers (n = 247) were statistically significant ($p<0.05$).
Source: Norseth et al. (1991)

Table B-22. Characteristics and quantitative results for epidemiologic cross-sectional study of exposure to 1,2,4-TMB Sulkowski et al. (2002)

Study (location)	**Outcome assessment**
• A factory in which paints and varnishes are produced	• Hearing examinations were carried out in an "audiobus," a motor vehicle equipped with soundproof cabin and diagnostic tools. • Several tests were conducted on subjects, including air and bone pure tone audiometry, impedance audiometry with tympanometry, acoustic reflex threshold measurement, and otoacoustic emissions. • Electronystagmographic tests were conducted in an outpatient clinical setting.
POPULATION CHARACTERISTICS	
Exposed population	**Referent or control description**
• 61 factory workers in direct contact with solvent vapors. • Job titles included resin synthesis analyzers, dry component mixers, mill operators, dispenser operators, colorists, and product packers.	• 40 non-exposed workers from the same factory.
Exposure assessment	**Statistical analysis**
• Data on exposure was collected from 61 workers who wore passive dosimeters on 3 work days. • Average Exposure duration: 15.8±9.1 years.	• Statistical methods utilized included student *t*-test, calculation of means, and linear regression analysis.
RESULTS	
Exposure Subgroup	
• 47.5% of exposed individuals and 5% of the control population exhibited symptoms of vestibular dysfunction, as indicated by decreased duration, amplitude, and slow-phase angular velocity of induced nystagmus. • High frequency hearing loss as indicated by pure tone audiometry was detected in 42% of exposed individuals versus 5% of the control population.	

B.5. ANIMAL TOXICOLOGY STUDIES

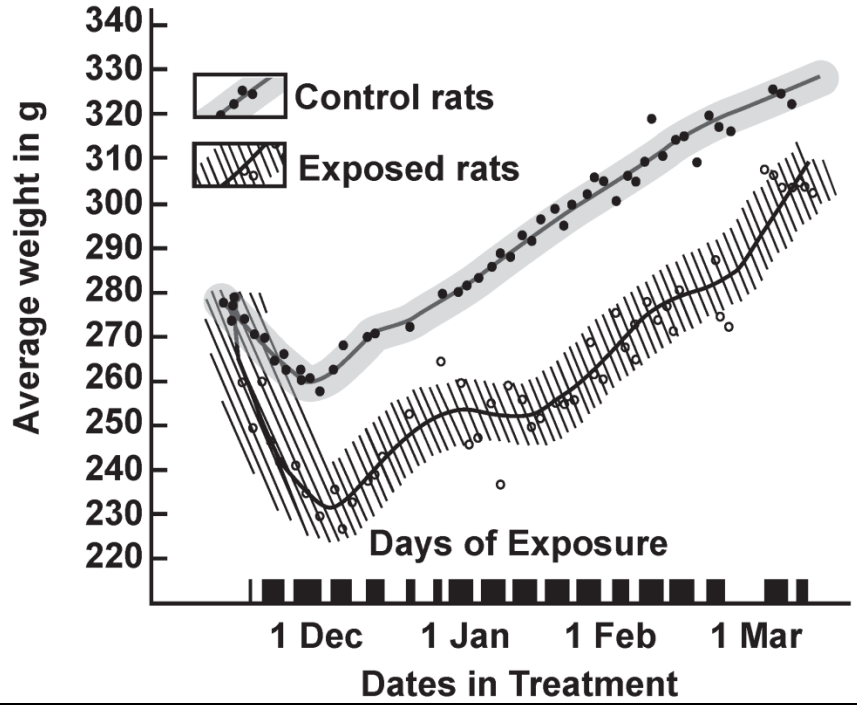
Table B-23. Characteristics and quantitative results for Baettig et al. (1958)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Rats	M	8 rats per dose	i.p. injection	0, 200, 500, and 1,700 ppm (0, 984, 2,460, 8,364 mg/m^3) TMB mixture.	4 mos; 8 hrs/day, 5/weeks

Additional study details

- Mixture of 1,2,4-, 1,2,3-, and 1,3,5-TMB were tested for their effects on growth, (as measured by body weight), behavior, food intake, red blood cell count, and hemoglobin concentration, and various histological parameters.
- Rat behavior was assessed qualitatively.
- TMB mixture (i.e., Fleet-X DV-99) was the same as assessed in the occupational exposure study.
- Study was translated from German to English prior to receipt by EPA.

Figure 2. Effect of long-term exposure to trimethylbenzene (about 1,700 ppm [8,364 mg/m^3]) on the growth of rats. Open circles: Average body weights of the exposed rats. Closed circles: Average weights of the control rats. Hatched [and dotted] area[s]: Double square deviation from the mean values plotted.



Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Table B-23 (Continued): Characteristics and quantitative results for Baettig et al. (1958)

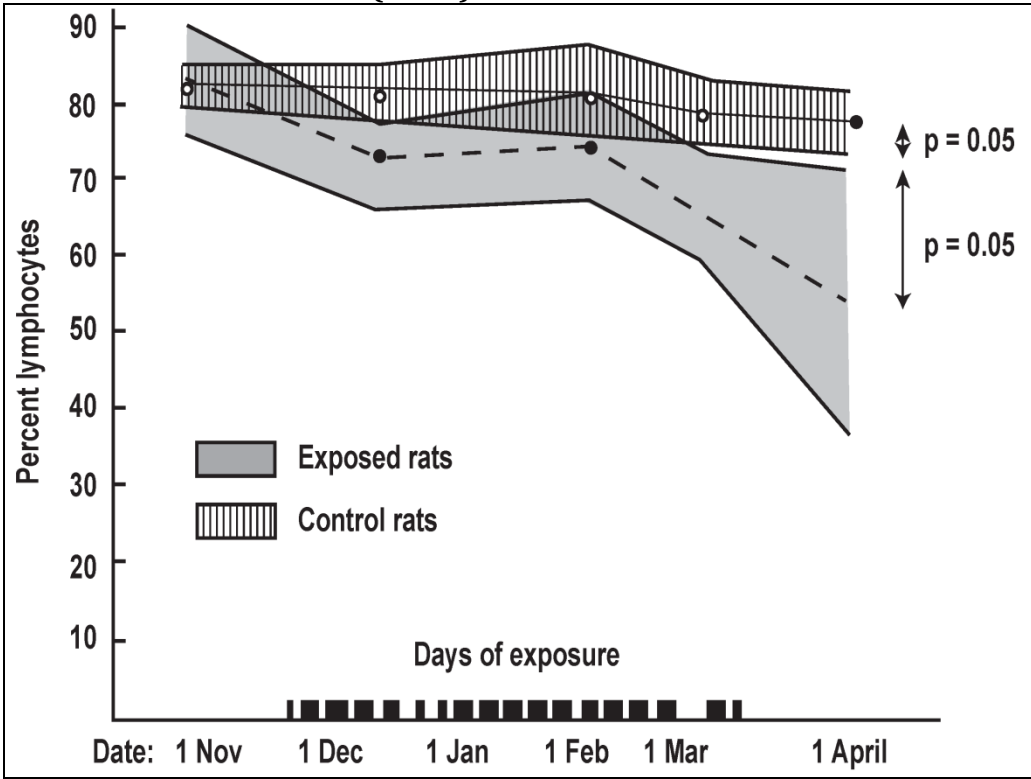


Figure 3. Behavior of the relative number of lymphocytes in trimethylbenzene-exposed rats (exposure: about 1,700 ppm [8,364 mg/m^3]).

Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Month	Number of days exposed per month	Average daily food intake (g/100g bw per month)		Difference (absolute)	Difference (%)
		Control Rats	Exposed Rats		
November	5	5.32	2.42	-3.10	-56.13
December	14	5.46	5.07	-0.93	-7.16
January	20	5.19	6.16	+0.97	+15.60
February	17	4.80	5.46	+0.66	+12.09
March	15	4.73	4.80	+0.07	+1.46
April	13		4.32		

Table 1. Average intake of food by the rats during experimental exposure to TMB mixture

Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Table B-23 (Continued): Characteristics and quantitative results for Baettig et al. (1958)

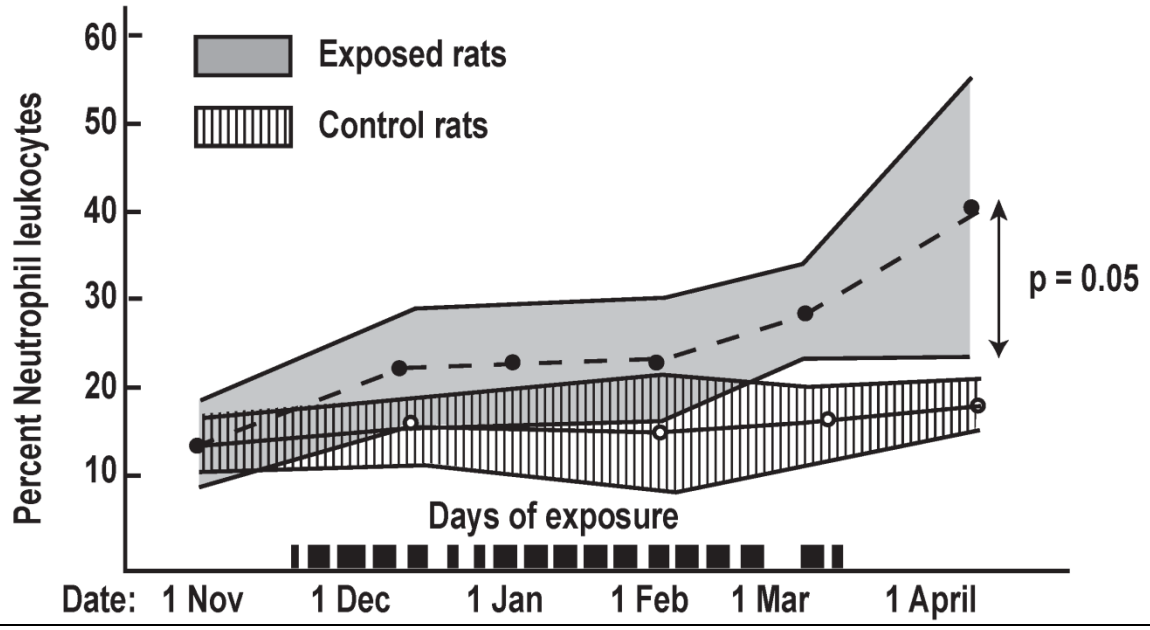


Figure 4. Behavior of the relative number of neutrophil leukocytes in trimethylbenzene exposed rats (exposure: about 1,700 ppm [8,364 mg/m^3]).

Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Month	Number of days exposed per month	Average intake of drinking water (g/100g bw rat/month)		Difference (absolute)	Difference (%)
		Control rats	Exposed rats		
November	5	9.21	10.55	+1.34	+12.70
December	14	9.71	17.18	+7.47	+43.47
January	20	9.38	22.31	+12.93	+57.91
February	17	7.78	15.92	+8.14	+51.13
March	15	7.12	14.16	+7.04	+49.70
April	13		15.66		

Table 2. Average intake of drinking water by rats during experimental exposure to TMB.

Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Table B-23 (Continued): Characteristics and quantitative results for Baettig et al. (1958)

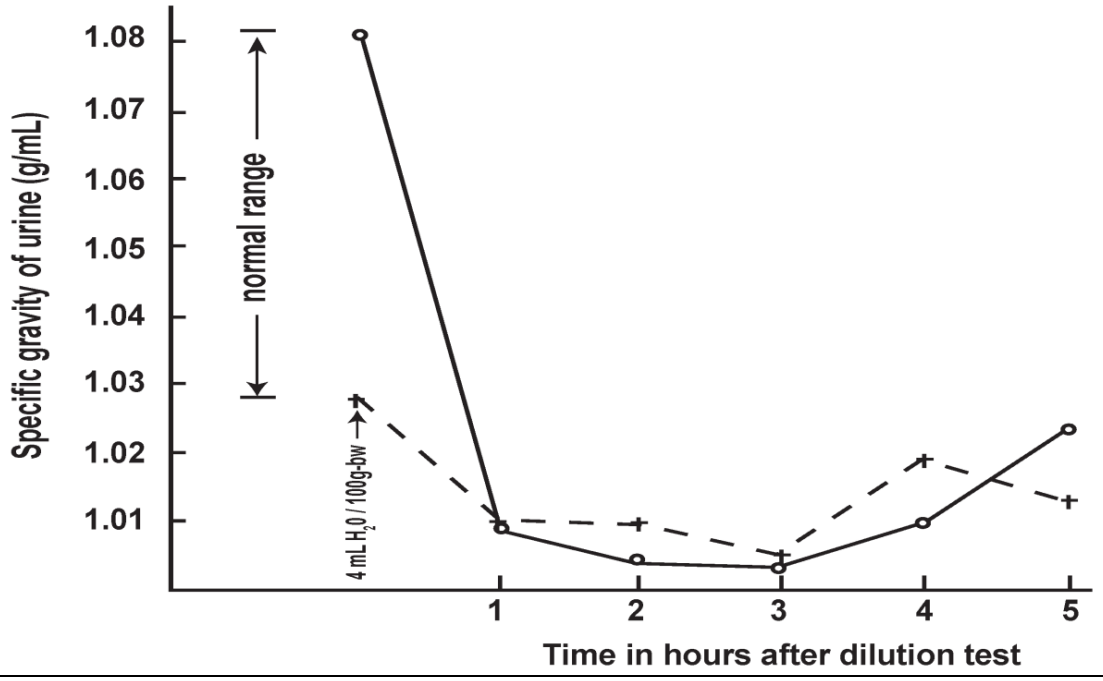


Figure 5. Specific gravity of spontaneous and dilution urines in TMB-exposed rats (exposure: about 1,700 ppm [8,364 mg/m^3]).

Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Table B-23 (Continued): Characteristics and quantitative results for Baettig et al. (1958)

Urinary phenol fraction	Intensity of exposure (ppm)	Duration of exposure (days)	Duration of exposure, in days to significant increase of phenol excretion	Time in days to normalization of phenol excretion after discontinuation of exposure
Total	1700	15	4	10
Free	1700	15	8	3
Bound	1700	15	4	9
Total	500	21	8	6
Free	500	21	8	1
Bound	500	21	21	1
Total	200	10	10	1
Free	200	10	10	1
Bound	200	10	Not increased	-

Table 3. Effect of TMB inhalation on urinary phenol excretion in the rat.

Source: Reproduced with permission of Springer-Verlag (Bättig et al., 1958)

Health Effect at LOAEL	NOAEL	LOAEL
Increased urinary excretion of free and total phenols	0 ppm	200 ppm (984 mg/m^3)

Comments: Battig et al. (1956) is published in German. However, Baettig et al. (1958) presents an English-translation of the results originally presented in Battig et al. (1956). As such, a separate study summary table is not provided for Battig et al. (1956). or of the eight rats in the long-term inhalation experiment died and were subsequently replaced within the first 2 weeks. Behavioral changes were assessed qualitatively. The substance to which rats were exposed was comprised of a mixture of all three TMB structural isomers and may have also contained methylethylbenzene structural isomers. Authors make a statement implying that dose was not consistent throughout experiment.

Table B-24. Characteristics and quantitative results for Gralewicz et al. (1997b)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	15 rats per dose	Inhalation (6 hr/day, 5 days/week)	0, 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,4-TMB	4 weeks

Additional study details

- Animals were exposed to 1,2,4-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/day, 5 days/week for 4 weeks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Rats were tested with a variety of behavioral tests, including radial maze performance, open field activity, passive avoidance, active two-way avoidance, and shock-induced changes in pain sensitivity.
- Tests were performed on days 14–54 following exposure.
- Rats displayed decreased performance on several tests at the 100 ppm and 250 ppm (492 and 1,230 mg/m^3) exposure levels.

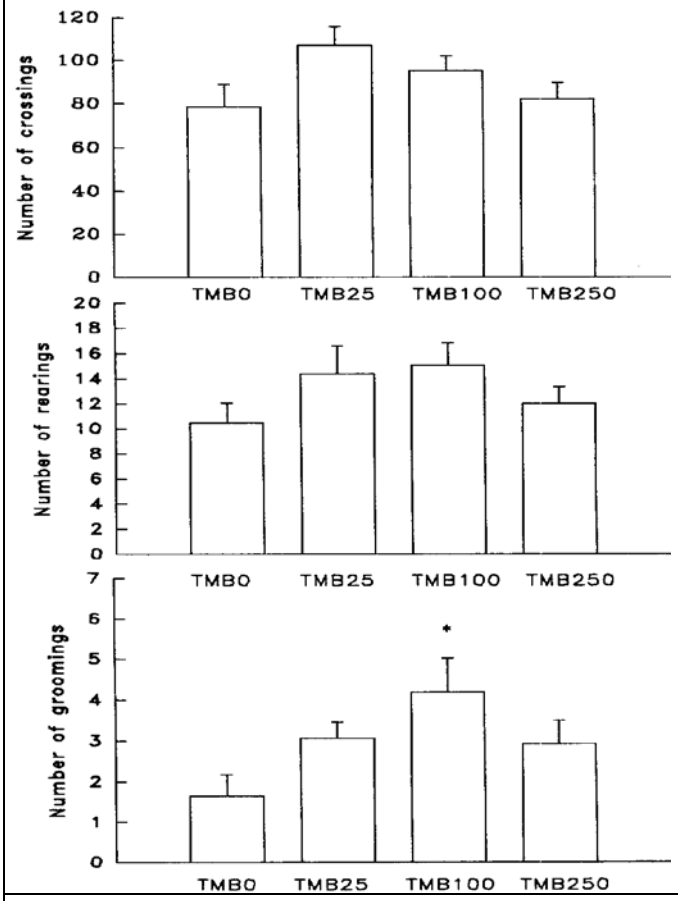


Figure 1. A comparison of spontaneous locomotor (upper diagram), exploratory (middle diagram, and grooming (lower diagram) activity of rats in an open field during a 5-min observation period.

The test was performed 25 days after a 4-week exposure to TMB. The bars represent group means and SE (n = 15 for each group). *p<0.05 compared with TMB0 group (0 ppm control group).

Source: Gralewicz et al. (1997b)

Table B-24 (Continued): Characteristics and quantitative results for Gralewicz et al. (1997b)

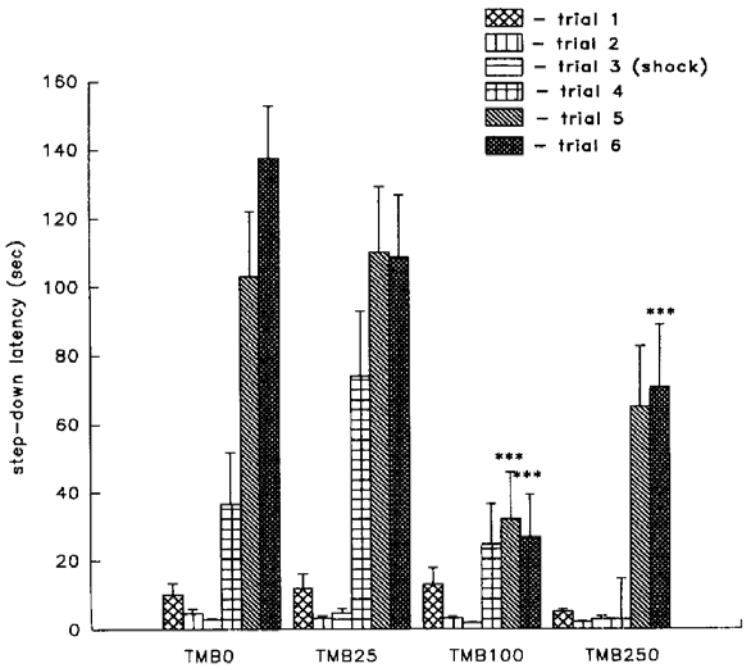


Figure 2. Diagrams illustrating the effect of a 4-week exposure to 1,2,4-TMB on the step-down passive avoidance learning in rats.

The test was performed on days 35–45 after exposure. Trials 1, 2, and 3 were performed at 24-hr intervals. The step-down response was punished by a 10-s foot shock only in trial 3. Trials 4, 5, and 6 were performed 24 hr, 3 days, and 7 days after trial 3, respectively. The maximum step-down latency was 180 s. The bars represent group means and SE (n = 15 for each group). ***p<0.001 compared with respective data from group TMB0 (0 ppm control group).

Source: Gralewicz et al. (1997b)

Table B-24 (Continued): Characteristics and quantitative results for Gralewicz et al. (1997b)

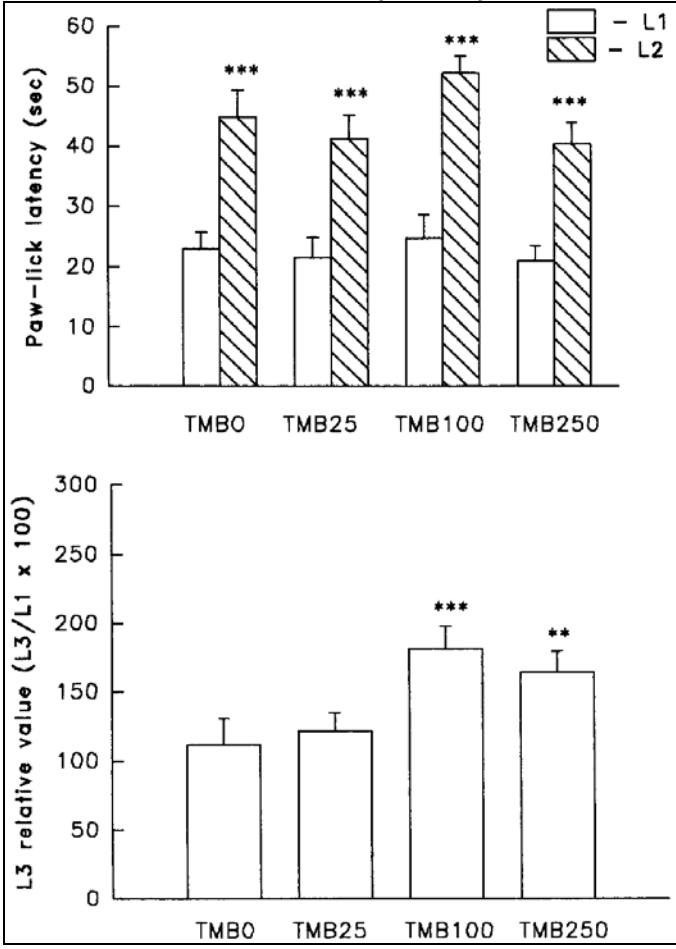


Figure 3. Hot plate behavior tested in rats on day 50 (trials 1 and 2) and day 51 (trial 3) after 4-week exposure to 1,2,4-TMB. Bars represent group means and SE (n = 15 for each group).

Upper diagram: a comparison of the latency of the paw-lick response to a thermal stimulus (54.5°C) on day 50. L1: paw-lick latency in trial 1 performed before a 2 min intermittent foot shock. L2: paw-lick latency in trial 2 performed several seconds after the foot shock. ***p<0.001 compared with L1 in the same group.

Lower diagram: A comparison of the change in the paw-lick latency noted 24 hrs after foot shock (trial 3). ***p<0.001, **p<0.01 when compared to TMB0 (0 ppm control group).

Source: Gralewicz et al. (1997b)

Table B-24 (Continued): Characteristics and quantitative results for Gralewicz et al. (1997b)

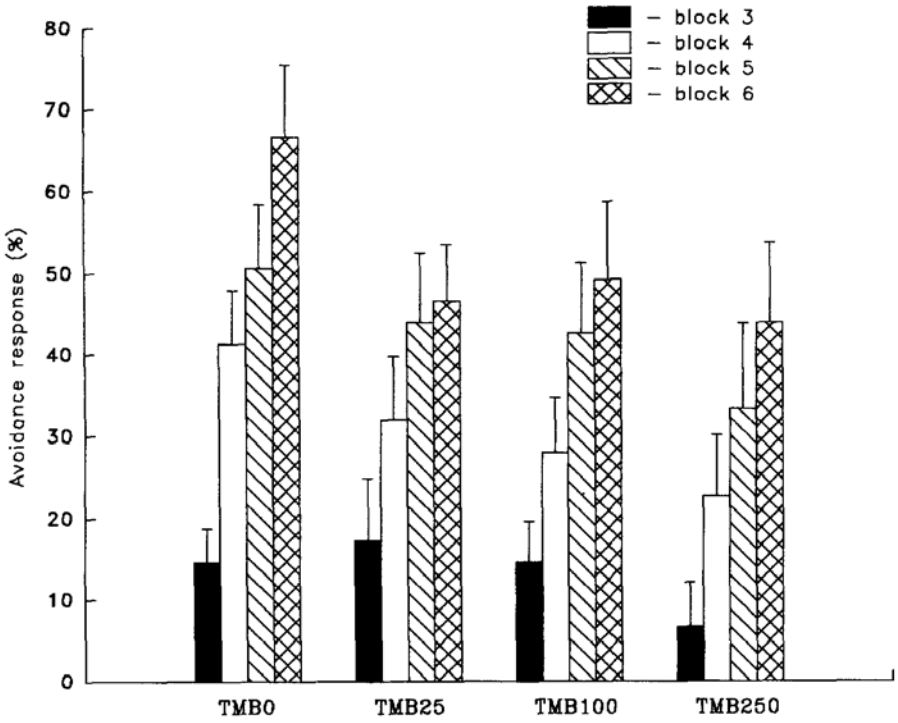


Figure 4. A comparison of the active avoidance performance increment during a single 30-trial training session in consecutive groups of rats.

The testing was performed on day 54 after 4-week exposure to 1,2,4-TMB. Bars represent the percentage (group mean and SE, n = 15 for each group) of avoidance response in successive five-trial blocks. No avoidance response was noted in any group during the first 10 trials and therefore blocks 1 and 2 were omitted in the analysis.

Source: Gralewicz et al. (1997b)

Health Effect at LOAEL	NOAEL	LOAEL
Open field grooming significantly increased, lower than expected step down latency	25 ppm (123 mg/m^3)	100 ppm (492 mg/m^3)

Comments: CNS disturbances were observed up to 2 months after termination of exposure, indicating the persistence of effects after the metabolic clearance of 1,2,4-TMB from the test animals. Duration of exposure only 4 weeks. Generally, short-term exposure studies have limited utility in quantitation of human health reference values.

Source: Gralewicz et al. (1997b)

Table B-25. Characteristics and quantitative results for Gralewicz et al. (1997a)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	9 rats per dose	Inhalation (6 hr/day, 5 days/week)	0, 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,4-TMB	4 weeks

Additional study details

- Animals were exposed to 1,2,4-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/day, 5 days/week for 4 weeks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Rats were tested to determine whether exposure to 1,2,4-TMB altered the pattern of occurrence of spike wave discharges (SWD).
- Rats exposed to 1,2,4-TMB at 100 or 250 ppm (492 or 1,230 mg/m^3) did not show an increase in SWD activity. Rats exposed to 0 or 25 ppm (0 or 123 mg/m^3) 1,2,4-TMB showed progressively decreasing levels of SWD activity.

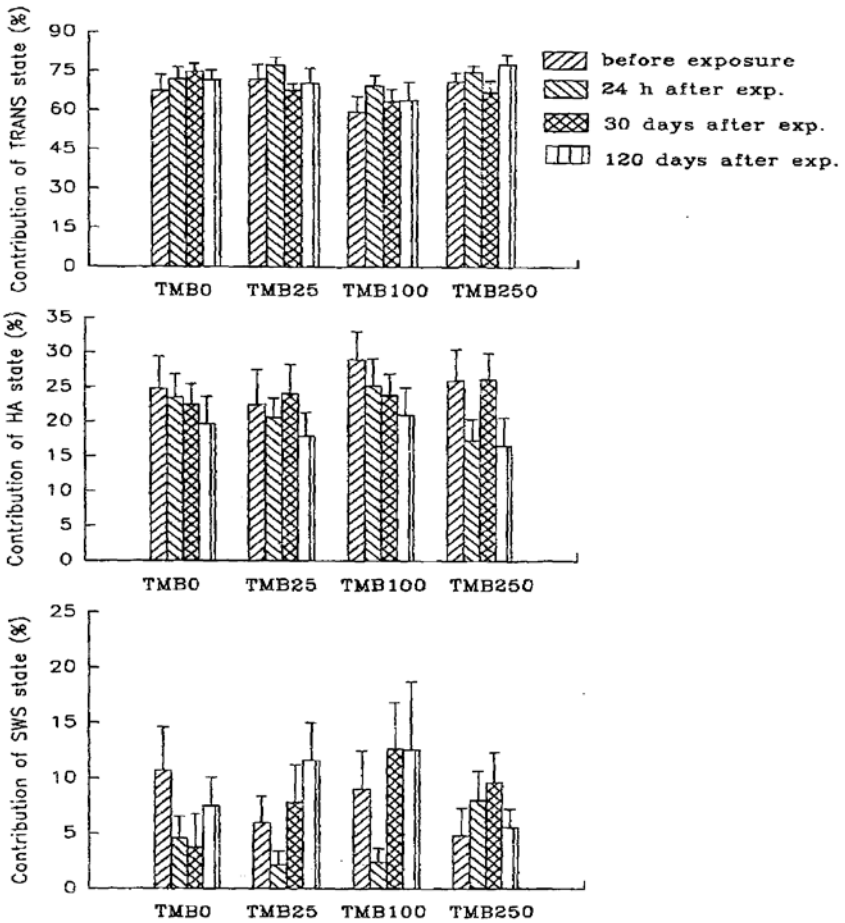


Figure 1. Diagrams showing the effect of a 4-week inhalation exposure to 1,2,4-TMB on the contribution of transitional (upper diagram, high arousal (middle diagram), and slow-wave sleep (lower diagram)) states in the rat EEG during successive 1-hour recording periods.

The bars represent group means and SE.

Source: Gralewicz et al. (1997a)

Table B-25 (Continued): Characteristics and quantitative results for Gralewicz et al. (1997a)

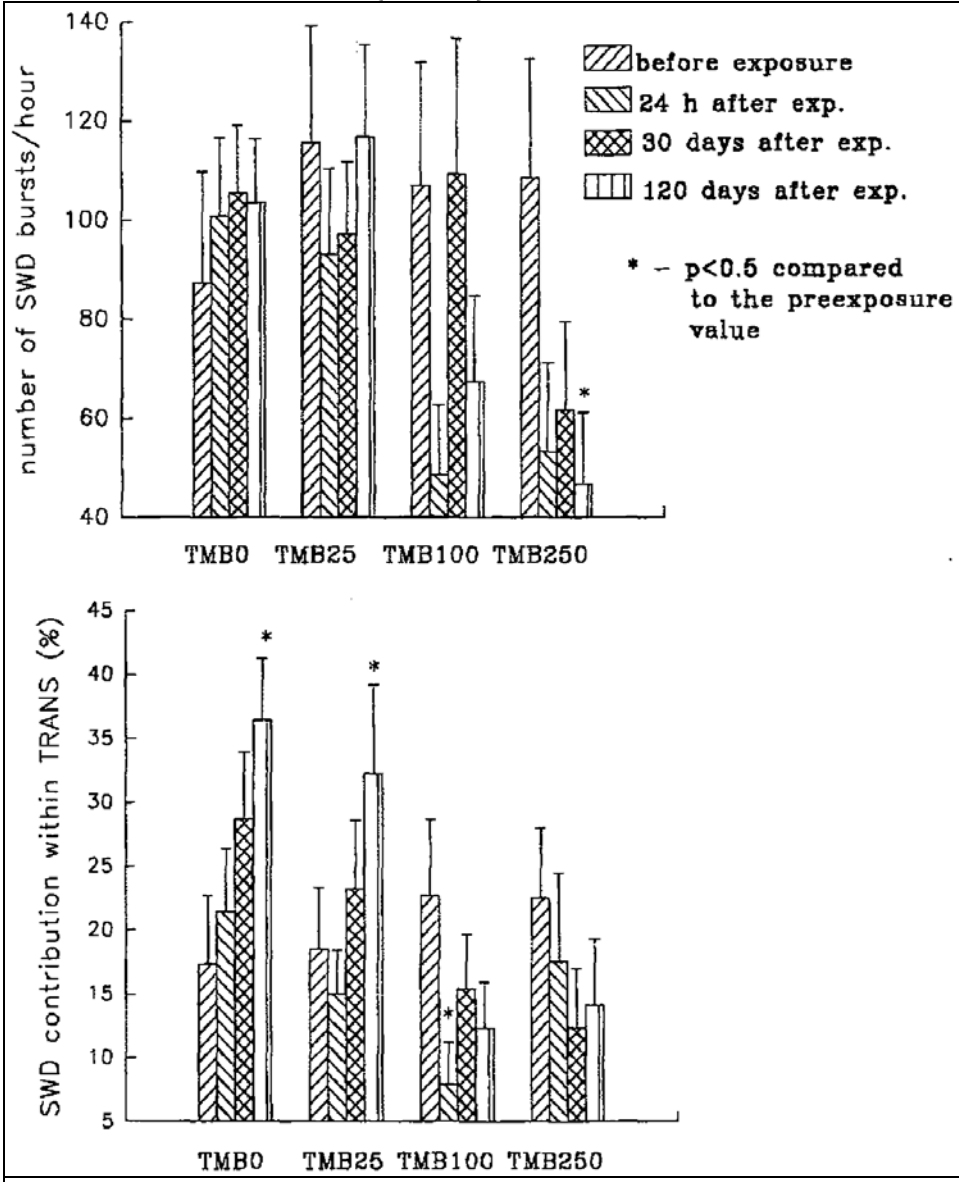


Figure 2. Diagram showing the effect of a 4-week inhalation exposure to 1,2,4-TMB on the SWD burst occurrence (upper diagram) and on the percent contribution of SWD activity within TRANS state (lower diagram) during successive 1-hour recording periods.

The bars represent group means and SE. * denotes $p<0.05$ in comparison to the preexposure value in the same group.

Source: Gralewicz et al. (1997a)

Health Effect at LOAEL	NOAEL	LOAEL
Decreased spike-wave discharges	25 ppm (123 mg/m^3)	100 ppm (492 mg/m^3)

Comments: CNS disturbances were observed up to 4 months after termination of exposure, indicating the persistence of effects after the metabolic clearance of 1,2,4-TMB from the test animals. Duration of exposure only 4 weeks. Generally, short-term exposure studies have limited utility in quantitation of human health reference values.
Source: Gralewicz et al. (1997a)

Table B-26. Characteristics and quantitative results for Gralewicz and Wiaderna (2001)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	10 or 11 rats per dose	Inhalation (6 hr/day, 5 days/week)	0 or 100 ppm (0 or 492 mg/m^3) 1,2,3-, 1,2,4-, or 1,3,5-TMB	4 weeks

Additional study details

- Animals were exposed to 1,2,3-, 1,2,4- or 1,3,5-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/day, 5 days/week for 4 weeks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Rats were tested with a variety of behavioral tests, including radial maze performance, open field activity, passive avoidance, active two-way avoidance, and shock-induced changes in pain sensitivity.
- Tests were performed starting 2 weeks post-exposure.
- 1,2,3-, 1,2,4-, and 1,3,5-TMB-exposed rats showed alterations in performance in spontaneous locomotor activity, passive avoidance learning, and paw-lick latencies.

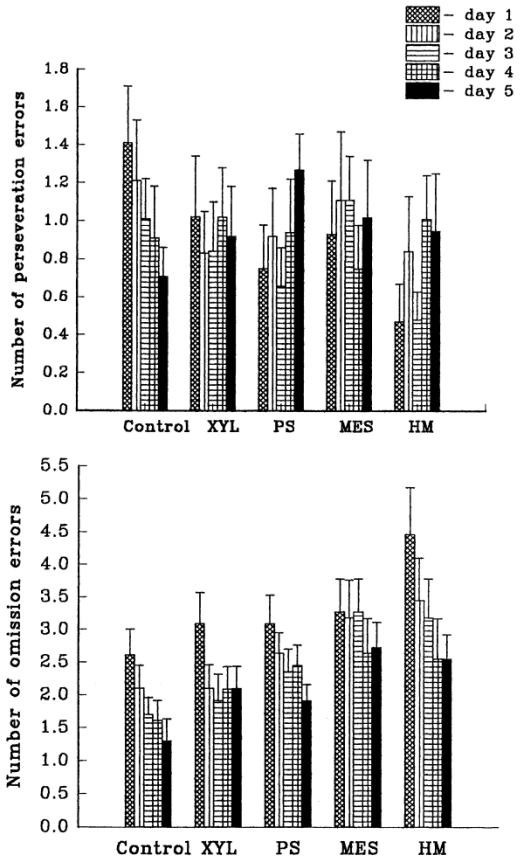


Figure 1. Radial maze performance of rats exposed for 4 weeks to *m*-xylene or a TMB isomer at a concentration of 100 ppm (492 mg/m^3).

The test (one trial a day) was performed on days 14–18 after exposure. The diagrams illustrate the number of perseveration (upper diagram) and omission (lower diagram) errors in successive daily trials.

Denotation:
Control- sham exposed group (n=10),
XYL- *m*-xylene exposed group (n=11),
PS- 1,2,4-TMB exposed group (n=11),
MES- 1,2,3-TMB exposed group (n=11),
HM- hemimellitene exposed group (n=11).
Bars represent group means and SE.

Source: Gralewicz and Wiaderna (2001)

Table B-26 (Continued): Characteristics and quantitative results for Gralewicz and Wiaderna (2001)

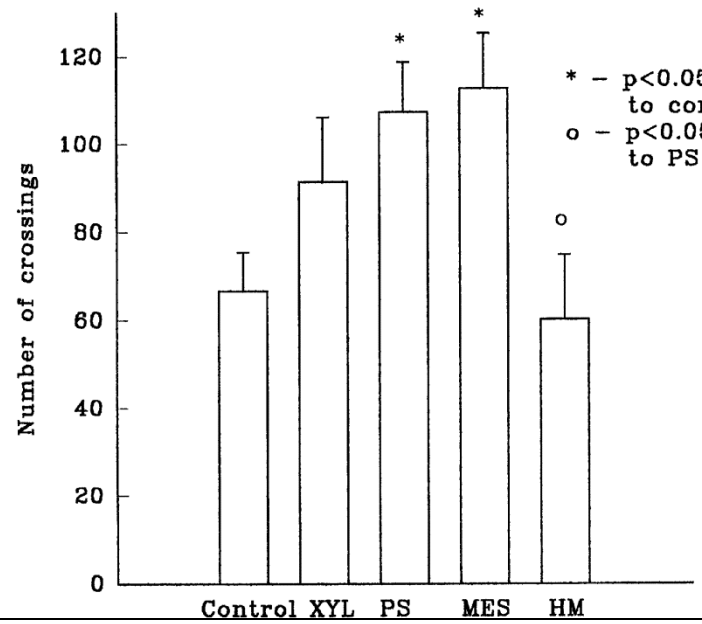
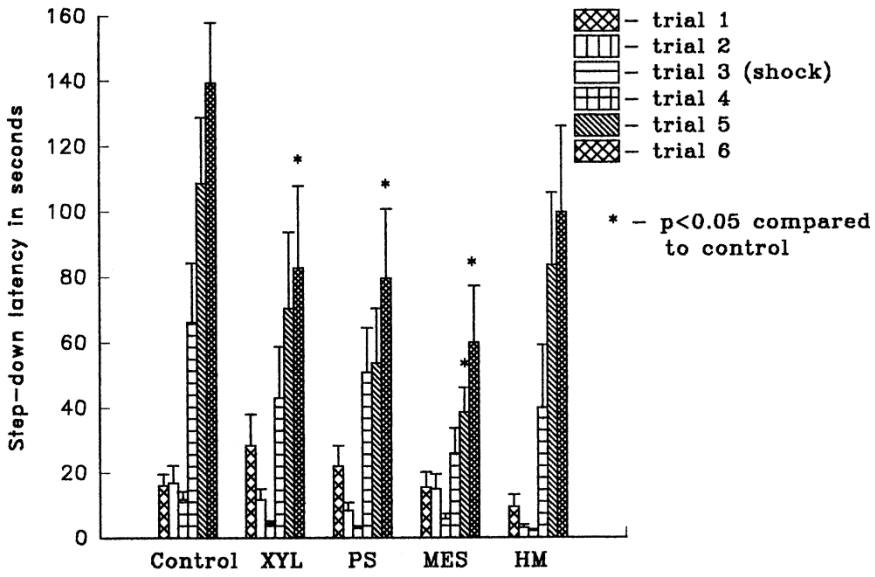


Figure 2. A comparison of open-field locomotor activity in sham-exposed and solvent-exposed rats. The test was performed on day 25 after a 4-week exposure to *m*-xylene or a TMB isomer at concentration of 100 ppm (492 mg/m^3).

Bars represent group means and SE.

Source: Gralewicz and Wiaderna (2001)

Figure 3. Diagram illustrating the effect of a 4-week inhalation exposure to *m*-xylene or a TMB isomer at concentration of 100 ppm (492 mg/m^3) on the step-down response latency in the passive avoidance test.



The test was performed on days 39–48 after exposure. Trials 1, 2, and 3 were performed at 24 hr intervals. The step-down response was punished by a 10 s footshock in trial 3 only. Trials 4, 5, and 6 were performed 24 hr, 3 days, and 7 days after trial 3, respectively. The maximum time of staying on the platform was 180 s. Bars represent means and SE.

Source: Gralewicz and Wiaderna (2001)

Table B-26 (Continued): Characteristics and quantitative results for Gralewicz and Wiaderna (2001)

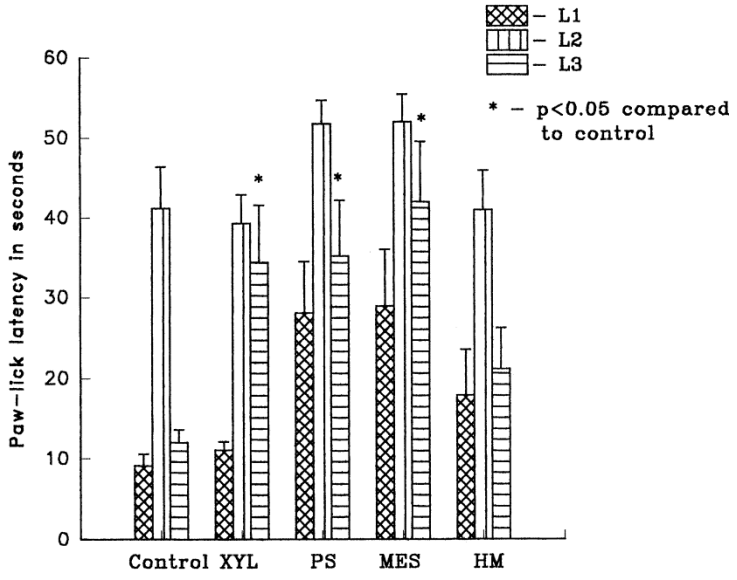


Figure 4. A comparison of sham-exposed and solvent-exposed rats with respect to the latency of the paw-lick response to heat (54.5°C) before (L1), several seconds after (L2), and 24 hr after a 2 min intermittent footshock.

The test was performed on days 50 and 51 after a 4-week inhalation exposure to *m*-xylene or a TMB isomer at a concentration of 100 ppm (492 mg/m^3). Bars represent group means and SE.

Source: Gralewicz and Wiaderna (2001)

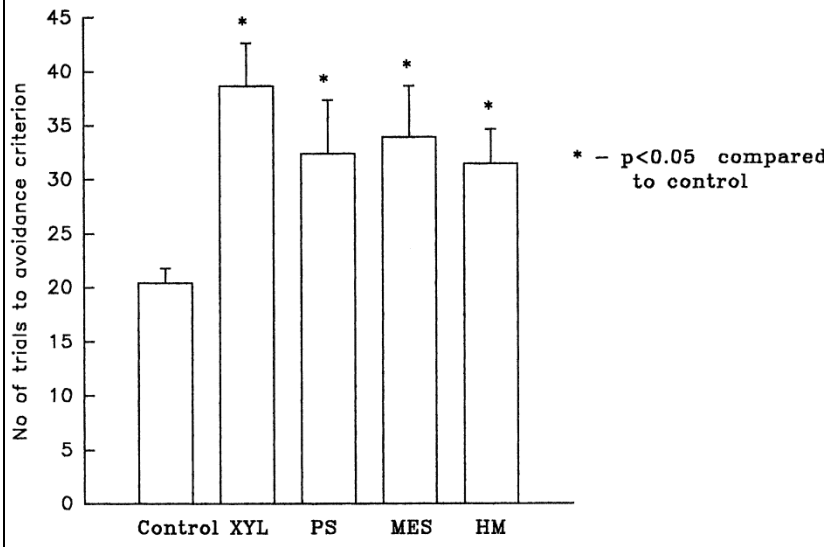


Figure 5. A comparison of sham-exposed and solvent-exposed rats with respect to the latency of the paw-lick response to heat (54.5°C) before (L1), several seconds after (L2), and 24 hr after a 2 min intermittent footshock.

The test was performed on days 50 and 51 after a 4-week inhalation exposure to *m*-xylene or a TMB isomer at a concentration of 100 ppm (492 mg/m^3). Bars represent group means and SE.

Source: Gralewicz and Wiaderna (2001)

Health Effect at LOAEL	NOAEL	LOAEL
Deleterious effects on locomotor activity, passive avoidance learning, and paw-lick latencies	n/a	100 ppm (492 mg/m^3) 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB

Comments: CNS disturbances were observed up to 2 months after termination of exposure, indicating the persistence of effects after the metabolic clearance of 1,2,4-TMB from the test animals. Duration of exposure only 4 weeks. Generally, short-term exposure studies have limited utility in quantitation of human health reference values.
Source: Gralewicz and Wiaderna (2001)

Table B-27. Characteristics and quantitative results for Janik-Speichowicz et al. (1998)

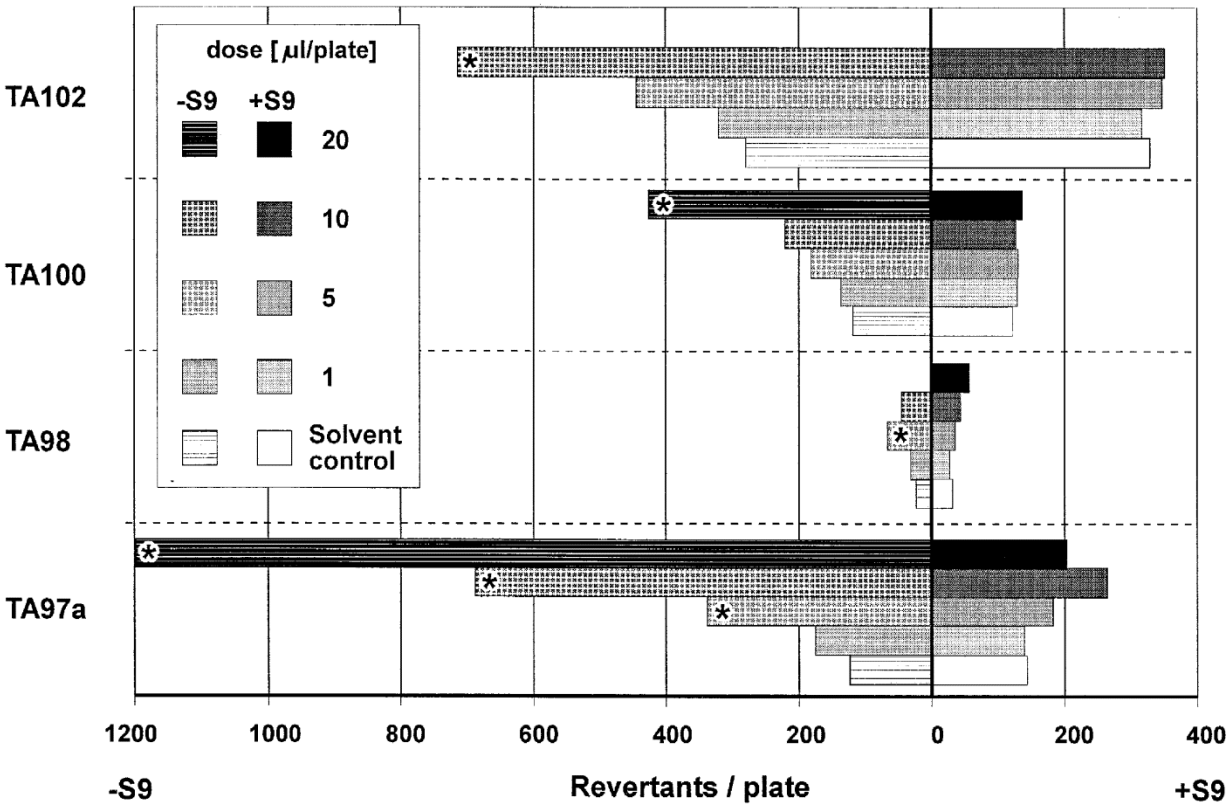
Study Design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Balb/c Mice	M & F	4 or 5 mice/ dose group	i.p. injection	0, 1470, 2160, and 2940 mg/kg body weight	Single exposure, or 2 i.p. injections spaced out over 24 hours

Additional study details

- Animals were given one or two injections of i.p. injections of 1,2,3-TMB.
- Animals were randomized and assigned to the experimental groups.
- Most deaths occurred within the first 2 days following single injections.
- LD_{50} was determined to be 3,670 mg/kg for males and 2,700 mg/kg for females.
- Micronuclei and chromatid exchange assays were conducted on extracted bone marrow to assess genotoxicity.
- Multiple indicators of genotoxicity were used, giving adequate evidence to assess the genotoxic potential of acute exposure to 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB .

Table B-27 (Continued): Characteristics and quantitative results for Janik-Speichowicz et al. (1998)

Figure 2. Dose-related increase in the number of His+ revertants for 1,2,3-TMB in *S. typhimurium* strains



* - mutagenic effect (a 2-fold or greater increase in the number of revertants per plate, as compared with the solvent control number)

Spontaneous revertants: TA97a 129±10 (-S9); 141±17 (+S9);
TA98 23±2 (-S9); 35±6 (+S9);
TA100 126±4 (-S9); 119±5 (+S9);
TA102 282±33 (-S9); 315±32 (+S9)

Source: Janik-Speichowicz et al. (1998)

Observation	Exposure to 1,2,4-TMB (µg or µL)						
	0	100 (Solvent control)	1	5	10	20	30
TA97a (-S9)	121±7	126±13	148±23	158±10	165±8	141±25	115±3
TA97a (+S9)	145±5	141±12	152±7	168±8	176±21	155±20	106±7
TA98 (-S9)	24±3	23±3	24±3	29±5	41±7	27±8	TOX[a]
TA98 (+S9)	31±3	31±5	35±4	28±1	29±4	30±3	29±6
TA100(-S9)	123±71	125±41	138±15	148±18	143±9	124±7	118±4
TA100(+S9)	25±4	21±10	126±62	125±5	112±4	108±3	110±4
TA102(-S9)	258±6	280±12	290±33	262±16	273±20	214±8	TOX
TA102(+S9)	294±11	315±14	279±24	276±11	276±11	236±32	TOX

Table B-27 (Continued): Characteristics and quantitative results for Janik-Speichowicz et al. (1998)

	Exposure to 1,3,5-TMB (µg or µL)							
Observation	**0**	**100 (Solvent control)**	**1**	**5**	**10**	**20**	**30**	**40**
TA97a (-S9)	127±15	131±10	141±13	149±29	139±17	129±13	125±8	NT[b]
TA97a (+S9)	183±6	157±19	180±26	196±16	155±30	137±29	138±20	128±11
TA98 (-S9)	22±4	22±4	27±3	28±5	25±2	37±5	23±5	TOX
TA98 (+S9)	30±3	32±5	31±4	35±5	31±2	39±5	28±2	31±1
TA100(-S9)	138±13	143±15	143±4	152±8	140±26	154±14	130±7	TOX
TA100(+S9)	142±10	138±82	137±3	147±29	139±16	131±10	108±11	115±6
TA102(-S9)	263±23	60±12	268±17	280±19	261±25	238±5	198±2	NT
TA102(+S9)	337±13	336±23	347±34	334±30	353±11	340±37	324±10	NT

	Exposure to 1,2,3-TMB (mg/kg body weight)			
Observation	**0**	**1470**	**2160**	**2940**
	% of Polychromatic Erythrocytes with Micronuclei (± SD)			
Males 30 hr harvest time	--	0.17±0.06	--	0.22±0.07
Males 48 hr harvest time	0.18±009	0.17±0.05	--	0.22±0.10
Males 72 hr harvest time	--	0.17±0.05	--	0.21±0.11
Females 30 hr harvest time	--	--	0.22±0.09	--
Females 48 hr harvest time	0.20±0.08	--	0.20±0.08	--
Females 72 hr harvest time	--	--	0.20±0.14	--
	Ratio of polychromatic to normochromatic erythrocytes			
Males 30 hr harvest time	--	0.82	--	0.85
Males 48 hr harvest time	0.81	0.45	--	0.72
Males 72 hr harvest time	--	0.50	--	0.62
Females 30 hr harvest time	--	--	0.90	--
Females 48 hr harvest time	0.95	--	0.84	--
Females 72 hr harvest time	--	--	0.78	--

	Exposure to 1,2,4-TMB (mg/kg body weight)			
Observation	**0**	**2000**	**3280**	**4000**
	% of Polychromatic Erythrocytes with Micronuclei (± SD)			
Males 30 hr harvest time	--	0.15±0.10	--	0.23±0.10
Males 48 hr harvest time	0.18±0.07	0.18±0.10	--	0.16±0.8
Males 72 hr harvest time	--	0.20±0.08	--	0.16±0.07
Females 30 hr harvest time	--	--	0.23±0.5	--
Females 48 hr harvest time	0.23±0.05	--	0.18±0.05	--
Females 72 hr harvest time	--	--	0.13±0.05	--

Table B-27 (Continued): Characteristics and quantitative results for Janik-Speichowicz et al. (1998)

	Ratio of polychromatic to normochromatic erythrocytes			
Males 30 hr harvest time	--	1.18	--	1.16
Males 48 hr harvest time	0.95	1.02	--	0.74
Males 72 hr harvest time	--	1.02	--	0.68*
Females 30 hr harvest time	--	--	0.98	--
Females 48 hr harvest time	0.95	--	1.01	--
Females 72 hr harvest time	--	--	0.85	--
	Exposure to 1,3,5-TMB (mg/kg body weight)			
Observation	**0**	**1800**	**2960**	**3600**
	% of Polychromatic Erythrocytes with Micronuclei (± SD)			
Males 30 hr harvest time	--	0.20±0.00	--	0.24±0.11
Males 48 hr harvest time	0.21±0.08	0.17±0.09	--	0.17±0.05
Males 72 hr harvest time	--	0.17±0.09	--	0.14±0.05
Females 30 hr harvest time	--	--	0.17±0.09	--
Females 48 hr harvest time	0.20±0.08	--	0.20±0.00	--
Females 72 hr harvest time	--	--	0.22±0.05	--
	Ratio of polychromatic to normochromatic erythrocytes			
Males 30 hr harvest time	--	0.62	--	0.40*
Males 48 hr harvest time	0.61	0.56	--	0.33
Males 72 hr harvest time	--	0.58	--	0.42*
Females 30 hr harvest time	--	--	0.51	--
Females 48 hr harvest time	0.60	--	0.60	--
Females 72 hr harvest time	--	--	0.58	--

Table B-27 (Continued): Characteristics and quantitative results for Janik-Speichowicz et al. (1998)

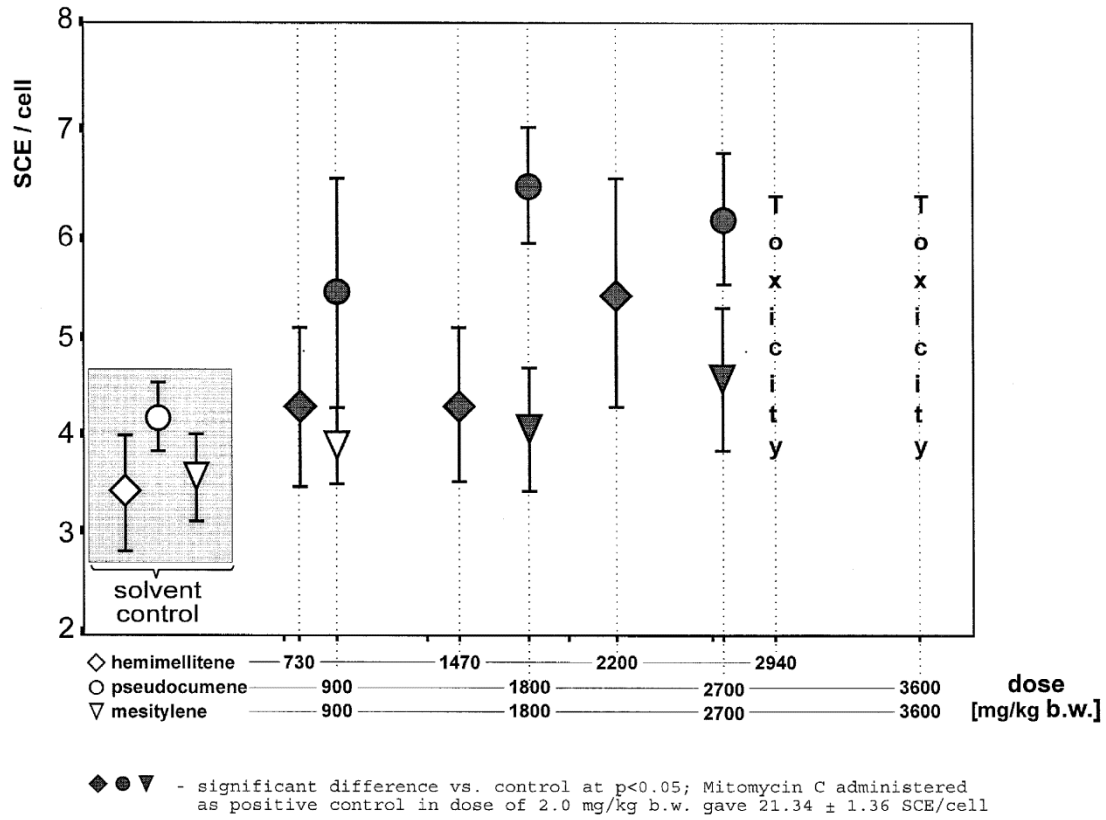


Figure 3. Sister chromatid exchanges induced in bone marrow cells of Imp:Balb/c mice.

Source: Janik-Speichowicz et al. (1998)

Health Effect at LOAEL	NOAEL	LOAEL
Significant increase in SCE induction relative to control	0 mg/kg	730 mg/kg

Comments: Multiple indicators of genotoxicity were investigated, giving adequate evidence to assess the genotoxic potential of acute exposure to 1,2,4-TMB, 1,2,3-TMB, and 1,3,5-TMB. Exposures were acute (occurring within 24 hours) and therefore less germane to study of health effects resulting from chronic exposure. For 1,2,3-TMB, sister chromatid assays were conducted at concentrations differing from the other independent variables (1,2,4- and 1,3,5-TMB). It is also difficult to establish a dose-response relationship for micronucleus formation because there were only two non-control exposure groups in males and only one non-control exposure group in females.

[a]TOX = toxic effects (background growth reduced);
[b]NT = not tested
*Significant difference vs. control at P≤0.05
Source: Janik-Speichowicz et al. (1998)

Table B-28. Characteristics and quantitative results for Koch Industries (1995b)

See Next Page (Table B-28 starts on Next Page)

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

Study Design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Sprague Dawley CD	M/F	20 rats/dose	Oral gavage	0, 50, 200, and 600 mg/kg/day 1,3,5-TMB	90 days

Additional study details

- Rats were treated with 0, 50, 200, or 600 mg/kg/day of 1,3,5-TMB (5 days per week) and were observed daily for adverse clinical signs
- Hematology and serum chemistry was analyzed after 30 days, at the end of the exposure period, and after a 28 day recovery period (in an additional 600 mg/kg/day "recovery" group only).
- No deaths related to 1,3,5-TMB exposure occurred during the study.
- Cumulative weight gain decreased by approximately 11% in the high-dose male group.
- High dose females exhibited an increase in absolute and relative liver weight, while males in the same dose group showed increases in relative liver weight.
- The NOEL was 200 mg/kg

Mean body weight after 90 day 1,3,5-TMB dosing period

	Dose (mg/kg/day)			
Males	**0**	**50**	**200**	**600**
Mean	624	607	602	585
Standard Deviation	48.2	62.0	40.8	66.4
No. of Rats	10	10	9	20
Females				
Mean	327	335	334	330
Standard Deviation	24.8	37.6	21.2	29.3
No. of Rats	10	10	10	20

Mean clinical chemistry parameters, terminal and recovery in males

	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
Na-mean	142.4	142.7	143.0	142.4	141.6
Na-standard deviation	1.49	0.65	1.40	1.32	1.30
Na-number of rats	10	10	9	10	10
K-mean	4.32	4.51	4.37	4.54	4.33
K-standard deviation	0.397	0.339	0.328	0.270	0.240
K-number of rats	10	10	9	10	10
Cl-mean	105.3	105.3	106.0	106.2	104.7

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

	Dose (mg/kg/day)				
	0	**50**	**200**	**600**	**600 (recovery)**
Cl-standard deviation	2.59	2.33	1.72	2.18	0.88
Cl-number of rats	10	10	9	10	10
CK-mean	594	962	934	595	884
CK-standard deviation	340.4	929.8	799.2	389.1	353.4
CK-number of rats	10	10	9	10	10
ALK P-mean	107	112	121	156*	77
ALK P-standard deviation	28.1	26.5	33.7	56.2	20.5
ALK P-number of rats	10	10	9	10	10
ALT-mean	29	30	25	33	25
ALT-standard deviation	6.4	9.8	7.0	9.1	4.4
ALT-number of rats	10	10	9	10	10
AST-mean	72	91	86	85	89
AST-standard deviation	18.9	31.9	25.5	25.0	16.7
AST-number of rats	10	10	9	10	10
GGT-mean	3	2	2	2	1
GGT-standard deviation	0.9	0.9	1.0	1.0	1.5
GGT-number of rats	10	10	9	10	10
BUN-mean	11.8	12.3	12.3	11.5	13.5
BUN-standard deviation	1.45	1.87	1.22	1.30	1.53
BUN-number of rats	10	10	9	10	10
CREA-mean	0.42	0.43	0.42	0.47	0.48
CREA-standard deviation	0.092	0.079	0.110	0.065	0.067
CREA-number of rats	10	10	9	10	10
T PRO-mean	6.0	5.9	6.0	6.1	6.0
T PRO-standard deviation	0.38	0.24	0.31	0.42	0.25

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

T PRO-number of rats	10	10	9	10	10
ALB-mean	3.6	3.6	3.7	3.8	3.7
ALB-standard deviation	0.23	0.19	0.19	0.22	0.09
ALB-number of rats	10	10	9	10	10
GLOB-mean	2.4	2.3	2.3	2.3	2.3
GLOB-standard deviation	0.27	0.18	0.16	0.24	0.24
GLOB-number of rats	10	10	9	10	10
A/G Ratio-mean	1.6	1.6	1.6	1.7	1.7
A/G Ratio-standard deviation	0.19	0.17	0.11	0.15	0.17
A/G Ratio-number of rats	10	10	9	10	10
GLU-mean	1.02	134.6	136.9	121.1*	168.4
GLU-standard deviation	22.80	15.11	15.76	13.14	26.39
GLU-number of rats	10	10	9	10	10
CHOL-mean	38.2	33.1	31.6	45.3	35.3
CHOL-standard deviation	6.83	9.13	9.93	15.99	10.10
CHOL-number of rats	10	10	9	10	10
Ca-mean	10.2	10.2	10.2	10.2	9.9
Ca-standard deviation	0.22	0.29	0.37	0.23	0.24
Ca-number of rats	10	10	9	10	10
PHOS-mean	6.5	6.7	7.0	7.6*	5.8
PHOS-standard deviation	0.64	0.80	0.68	0.58	0.59
PHOS-number of rats	10	10	9	10	10
TBIL-mean	0.4	0.4	0.5	0.5	0.5
TBIL-standard deviation	0.12	0.10	0.09	0.14	0.09
TBIL-number of rats	10	10	9	10	10

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

Mean clinical chemistry parameters, terminal and recovery in females					
	Dose (mg/kg/day)				
Parameter	0	50	200	600	600 (recovery)
Na-mean	142.1	141.6	141.7	138.9*	140.9
Na-standard deviation	1.10	0.96	2.07	2.83	1.47
Na-number of rats	10	10	10	10	10
K-mean	3.94	4.13	4.01	3.86	4.06
K-standard deviation	0.195	0.200	0.119	0.292	0.259
K-number of rats	10	10	10	10	10
Cl-mean	105.9	106.2	106.1	103.0*	107.0
Cl-standard deviation	2.32	1.63	1.05	3.81	1.68
Cl-number of rats	10	10	10	10	10
CK-mean	404	574	381	362	532
CK-standard deviation	172.6	346.4	228.3	242.5	369.7
CK-number of rats	10	10	10	10	10
ALK P-mean	59	57	55	78	38
ALK P-standard deviation	14.8	10.3	14.9	24.5	10.1
ALK P-number of rats	10	10	10	10	10
ALT-mean	21	22	23	24	27
ALT-standard deviation	2.3	4.0	7.3	4.1	7.1
ALT-number of rats	10	10	10	10	10
AST-mean	60	75	62	60	77
AST-standard deviation	16.5	18.6	15.2	15.0	21.4
AST-number of rats	10	10	10	10	10
GGT-mean	2	3	3	3	2
GGT-standard deviation	1.1	1.6	1.0	1.4	1.4
GGT-number of rats	10	10	10	10	10
BUN-mean	14.5	14.0	11.9	13.5	16.2
BUN-standard deviation	1.34	2.57	1.49	4.61	2.31

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

BUN-number of rats	10	10	10	10	10
CREA-mean	0.53	0.51	0.53	0.56	0.55
CREA-standard deviation	0.106	0.085	0.099	0.110	0.099
CREA-number of rats	10	10	10	10	10
T PRO-mean	6.2	6.3	6.6	6.5	6.3
T PRO-standard deviation	0.44	0.41	0.69	0.68	0.66
T PRO-number of rats	10	10	10	10	10
ALB-mean	4.1	4.3	4.5	4.5	4.3
ALB-standard deviation	0.29	0.36	0.58	0.56	0.51
ALB-number of rats	10	10	10	10	10
GLB-mean	2.1	2.0	2.1	2.1	2.0
GLB-standard deviation	0.21	0.17	0.19	0.20	0.18
GLB-number of rats	10	10	10	10	10
A/G Ratio-mean	2.0	2.1	2.1	2.1	2.1
A/G Ratio-standard deviation	0.16	0.22	0.26	0.23	0.18
A/G Ratio-number of rats	10	10	10	10	10
GLU-mean	131.8	136.4	140.1	132.8	150.7
GLU-standard deviation	7.65	11.72	14.48	15.91	19.18
GLU-number of rats	10	10	10	10	10
CHOL-mean	36.2	35.2	38.8	51.2*	28.7
CHOL-standard deviation	8.83	6.64	6.24	17.84	12.93
CHOL-number of rats	10	10	10	10	10
Ca-mean	10.1	10.2	10.4	10.5	10.0
Ca-standard deviation	0.35	0.24	0.42	0.63	0.36
Ca-number of rats	10	10	10	10	10
PHOS-mean	6.1	6.1	6.4	7.5*	5.3
PHOS-standard deviation	1.08	1.27	1.18	1.24	0.80

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

PHOS-number of rats	10	10	10	10	10
TBIL-mean	0.5	0.5	0.4	0.5	0.5
TBIL-standard deviation	0.08	0.10	0.08	0.07	0.07
TBIL-number of rats	10	10	10	10	10
Mean Male Hematology Parameters Terminal and Recovery					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
WBC-mean	9.1	8.1	8.1	7.7	7.8
WBC-standard deviation	2.70	2.50	1.74	1.76	1.24
WBC-number of rats	10	10	9	10	10
RBC-mean	8.94	8.50	8.98	8.72	8.51
RBC-standard deviation	0.375	0.483	0.565	0.275	0.423
RBC-number of rats	10	10	9	10	10
HGB-mean	15.6	15.3	15.8	15.4	15.4
HGB-standard deviation	0.52	0.76	0.77	0.53	0.58
HGB-number of rats	10	10	9	10	10
HCT-mean	43.9	42.2	44.1	43.3	41.6
HCT-standard deviation	1.65	2.72	2.12	1.60	1.99
HCT-number of rats	10	10	9	10	10
MCV-mean	49.1	49.7	49.2	49.6	49.0
MCV-standard deviation	1.17	1.09	1.76	1.66	1.62
MCV-number of rats	10	10	9	10	10
MCH-mean	17.5	18.0	17.7	17.7	18.2
MCH-standard deviation	0.45	0.73	0.85	0.68	0.61
MCH- number of rats	10	10	9	10	10
MCHC-mean	35.6	36.3	35.9	35.6	37.1
MCHC-standard deviation	0.67	1.07	0.60	0.67	0.60

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

MCHC-number of rats	10	10	9	10	10
PLT-mean	1092	1098	1041	1125	1083
PLT-standard deviation	134.1	120.8	100.9	145.9	112.6
PLT-number of rats	10	10	9	10	10
Mean Female Hematology Parameters Terminal and Recovery					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
WBC-mean	5.5	5.6	5.4	5.7	4.6
WBC-standard deviation	2.05	1.53	1.64	1.99	1.55
WBC-number of rats	10	10	10	10	10
RBC-mean	7.88	8.01	7.90	8.34	7.70
RBC-standard deviation	0.729	0.354	0.578	0.548	0.423
RBC-number of rats	10	10	10	10	10
HGB-mean	14.8	15.0	15.2	15.3	15.1
HGB-standard deviation	0.88	0.48	0.82	0.78	0.57
HGB-number of rats	10	10	10	10	10
HCT-mean	41.0	41.4	41.9	43.3	39.9
HCT-standard deviation	3.15	1.91	2.93	2.33	1.67
HCT-number of rats	10	10	10	10	10
MCV-mean	52.1	51.7	53.0	52.0	51.9
MCV-standard deviation	1.65	1.18	1.03	1.24	1.33
MCV-number of rats	10	10	10	10	10
MCH-mean	18.9	18.7	19.2	18.4	19.6
MCH-standard deviation	0.89	0.67	0.53	0.68	0.78
MCH- number of rats	10	10	10	10	10
MCHC-mean	36.2	36.2	36.3	35.4	37.7
MCHC-standard deviation	0.79	0.86	0.83	0.54	0.64

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

MCHC-number of rats	10	10	10	10	10
PLT-mean	1094	1089	1011	1053	1008
PLT-standard deviation	153.3	132.0	97.2	125.7	105.7
PLT-number of rats	10	10	10	10	10
Mean Male Absolute Differential White Blood Cell Counts (Terminal and Recovery)					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
NRBC-mean	0	0	0	0	0
NRBC-standard deviation	0	0	0.7	0	0
NRBC-number of rats	10	10	9	10	10
MAT NEU-mean	1.8	1.7	1.4	1.5	1.0
MAT NEU-standard deviation	1.07	1.10	0.36	0.75	0.29
MAT NEU-number of rats	10	10	9	10	10
LYM-mean	7.1	6.2	6.4	6.0	6.6
LYM-standard deviation	2.78	2.16	1.59	2.16	1.23
LYM-number of rats	10	10	9	10	10
MONO-mean	0.1	0.2	0.3*	0.2*	0.2
MONO-standard deviation	0.09	0.09	0.17	0.18	0.10
MONO-number of rats	10	10	9	10	10
EOS-mean	0.1	0.1	0.0	0.0	0.1
EOS-standard deviation	0.06	0.09	0.07	0.05	0.07
EOS-number of rats	10	10	9	10	10
BASO-mean	0	0	0	0	0
BASO-standard deviation	0	0	0	0	0
BASO-number of rats	10	10	9	10	10
IMM NEU-mean	0	0	0	0	0
IMM NEU-standard deviation	0	0	0	0	0

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

IMM NEU-number of rats	10	10	9	10	10
Mean Female Absolute Differential White Blood Cell Counts (Terminal and Recovery)					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
NRBC-mean	0	0	0	0	0
NRBC-standard deviation	0	0	0	0	0
NRBC-number of rats	10	10	10	10	10
MAT NEU-mean	0.8	0.7	0.9	1.0	0.7
MAT NEU-standard deviation	0.48	0.32	0.69	0.39	0.45
MAT NEU-number of rats	10	10	10	10	10
LYM-mean	4.6	4.7	4.2	4.4	3.7
LYM-standard deviation	1.93	1.52	1.52	2.08	1.34
LYM-number of rats	10	10	10	10	10
MONO-mean	0.1	0.1	0.1	0.2	0.2
MONO-standard deviation	0.14	0.10	0.08	0.17	0.11
MONO-number of rats	10	10	10	10	10
EOS-mean	0.1	0.1	0.1	0.1	0
EOS-standard deviation	0.07	0.07	0.09	0.09	0.07
EOS-number of rats	10	10	10	10	10
BASO-mean	0	0	0	0	0
BASO-standard deviation	0	0	0.03	0	0
BASO-number of rats	10	10	10	10	10
IMM NEU-mean	0	0	0	0	0
IMM NEU-standard deviation	0	0	0	0	0
IMM NEU-number of rats	10	10	10	10	10

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

Mean Male Absolute Organ Weights (g)					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
ADR-mean	0.062	0.059	0.058	0.063	0.060
ADR-standard deviation	0.010	0.015	0.011	0.010	0.008
ADR-number of rats	10	10	9	10	10
BRN-mean	2.25	2.28	2.23	2.19	2.24
BRN-standard deviation	0.073	0.090	0.094	0.084	0.112
BRN-number of rats	10	10	9	10	10
KID-mean	3.92	3.95	4.10	4.16	4.05
KID-standard deviation	0.326	0.262	0.610	0.464	0.491
KID-number of rats	10	10	9	10	10
LIV-mean	19.28	18.91	18.38	20.90	17.38
LIV-standard deviation	1.843	3.074	2.885	3.313	2.222
LIV-number of rats	10	10	9	10	10
LNG-mean	2.19	2.19	2.20	2.06	2.04
LNG-standard deviation	0.299	0.292	0.134	0.158	0.229
LNG-number of rats	10	10	9	10	10
TESTES-mean	4.15	3.78	4.04	4.00	3.91
TESTES-standard deviation	0.290	0.595	0.336	0.250	0.612
TESTES-number of rats	10	10	9	10	10
Mean Female Absolute Organ Weights (g)					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
ADR-mean	0.075	0.078	0085	0.082	0.084
ADR-standard deviation	0.007	0.012	0.013	0.015	0.015
ADR-number of rats	10	10	10	10	10
BRN-mean	2.06	2.06	2.11	2.06	2.11
BRN-standard deviation	0.080	0.083	0.094	0.050	0.059

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

BRN-number of rats	10	10	10	10	10
KID-mean	2.34	2.23	2.38	2.51	2.38
KID-standard deviation	0.314	0.228	0.116	0.264	0.248
KID-number of rats	10	10	10	10	10
LIV-mean	9.44	9.13	10.05	11.78*	9.71
LIV-standard deviation	1.601	0.774	0.967	1.444	1.411
LIV-number of rats	10	10	10	10	10
LNG-mean	1.63	1.73	1.66	1.60	1.63
LNG-standard deviation	0.187	0.140	0.106	0.150	0.140
LNG-number of rats	10	10	10	10	10
OVARIES-mean	0.128	0.123	0.122	0.142	0.142
OVARIES-standard deviation	0.023	0.039	0.042	0.058	0.036
OVARIES-number of rats	10	10	10	10	9
Mean Male Relative[a] Organ Weights (g)					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
FBWb-mean	602	584	576	562	595
FBW-standard deviation	46.4	60.4	40.1	52.2	81.8
FBW-number of rats	10	10	9	10	10
ADR-mean	0.011	0.010	0.010	0.011	0.010
ADR-standard deviation	0.002	0.002	0.002	0.001	0.001
ADR-number of rats	10	10	9	10	10
BRN-mean	0.38	0.39	0.39	0.39	0.38
BRN-standard deviation	0.033	0.032	0.035	0.035	0.044
BRN-number of rats	10	10	9	10	10
KID-mean	0.65	0.68	0.71	0.74*	0.68
KID-standard deviation	0.052	0.052	0.082	0.045	0.039
KID-number of rats	10	10	9	10	10
LIV-mean	3.20	3.23	3.19	3.71*	2.93

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

LIV-standard deviation	0.158	0.336	0.402	0.288	0.274
LIV-number of rats	10	10	9	10	10
LNG-mean	0.37	0.38	0.38	0.37	0.34
LNG-standard deviation	0.045	0.052	0.027	0.038	0.042
LNG-number of rats	10	10	9	10	10
TESTES-mean	0.69	0.65	0.71	0.72	0.67
TESTES-standard deviation	0.060	0.101	0.092	0.089	0.136
TESTES-number of rats	10	10	9	10	10
Mean Female Relative[a] Organ Weights (g)					
	Dose (mg/kg/day)				
Parameter	**0**	**50**	**200**	**600**	**600 (recovery)**
FBWb-mean	309	317	316	308	336
FBW-standard deviation	23.4	34.8	20.0	28.2	33.9
FBW-number of rats	10	10	10	10	10
ADR-mean	0.025	0.025	0.027	0.027	0.025
ADR-standard deviation	0.003	0.005	0.005	0.004	0.005
ADR-number of rats	10	10	10	10	10
BRN-mean	0.67	0.66	0.67	0.68	0.63
BRN-standard deviation	0.067	0.075	0.047	0.065	0.059
BRN-number of rats	10	10	10	10	10
KID-mean	0.76	0.71	0.76	0.82	0.71
KID-standard deviation	0.059	0.088	0.051	0.059	0.040
KID-number of rats	10	10	10	10	10
LIV-mean	3.04	2.90	3.19	3.82*	2.88
LIV-standard deviation	0.365	0.330	0.357	0.223	0.207
LIV-number of rats	10	10	10	10	10
LNG-mean	0.53	0.55	0.53	0.52	0.49
LNG-standard deviation	0.071	0.059	0.052	0.047	0.079

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

LNG-number of rats	10		10		10		10		10	
OVARIES-mean	0.041		0.040		0.039		0.046		0.043	
OVARIES-standard deviation	0.006		0.015		0.014		0.018		0.011	
OVARIES-number of rats	10		10		10		10		9	
Summary of gross necropsy observations (count)										
Tissue and Observation	**Dose (mg/kg/day)**									
	0		**50**		**200**		**600**		**600 (recovery)**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
No gross lesions observed	9	8	8	8	7	9	8	10	8	10
Mandibular lymph nodes; enlarged/red	--c	1	--	--	1	--	--	--	1	--
Mandibular lymph nodes; enlarged	1	--	--	--	1	--	--	--	1	--
Tibia; lesion (fracture)	--	1	--	--	--	--	--	--	--	--
Adrenals; small, unilateral	--	--	1	--	--	--	--	--	--	--
Testes; small, white (right)	--	--	1	--	--	--	--	--	--	--
Testes; absent (left)	--	--	--	--	--	--	--	--	1	--
Eye; opaque (left)	--	--	--	1	--	1	--	--	--	--
Thymus; focus, red	--	--	--	1	--	--	--	--	--	--
Thymus; mottled	--	--	--	--	--	--	1	--	--	--
Lung enlarged	--	--	--	--	1d	--	--	--	--	--
Large intestine, cecum; focus, red	--	--	--	--	1	--	--	--	--	--
Liver; pale	--	--	--	--	--	--	1	--	--	--
Comments; 1,3,5- TMB was the only isomer tested in this study. Effects reported in study appeared reversible in the recovery group, which was observed for 28 days following cessation of exposure.										

Table B-28 (Continued): Characteristics and quantitative results for Koch Industries (1995b)

*Significantly different from vehicle control, p≤0.05
[a] Relative organ weight=[Absolute organ weight (g)/Fasted body weight (g)]x100
[b] fasted body weight
[c] zero incidence
[d] animal died due to gavage error (accidental death)
Na = sodium (mE/litter serum); K = potassium (mE/litter serum); Cl = chloride (mE/litter serum); CK = creatine kinase (IU/liter serum); ALK P = alkaline phosphatase (IU/liter serum); ALT = alanine aminotransferase (IU/liter serum); AST = aspartate aminotransferase (IU/liter serum); GGT = gamma glutamyl transpeptidase (IU/liter serum); BUN = urea nitrogen (mg N/dL serum); CREA = creatinine (mg/dL serum); T PRO = total protein (g protein/dL serum); ALB = albumin (g/dL serum); GLOB = globulin (g/dL serum); A/G Ratio = albumin/globulin ratio; GLU = glucose (mg/dL serum); CHOL = cholesterol (mg/dL serum); T BIL = total bilirubin (mg/dL serum); WBC = white blood cell (103/mm3); RBC = red blood cell (106/mm3); HGB = hemoglobin (g/dL blood); HCT = hematocrit (%); MCV = mean corpuscular volume (femoliter); MCH = mean corpuscular hemoglobin (picogram); MCHC = mean corpuscular hemoglobin concentration (%); PLT = platelet (103/mm3); NRBC = nucleated red blood cells (number/100 white blood cells); MAT NEU = mature neutrophils (103/mm3); LYM = lymphocytes (103/mm3); MONO = monocytes (103/mm3); EOS = eosinophils (103/mm3); BASO = basophils (103/mm3); IMM NEU = immature neutrophils (103/mm3); ADR = adrenal glands; BRN = brain; KID = kidneys; LIV = liver; LNG = lung.

Source: Koch Industries (1995b)

Table B-29. Characteristics and quantitative results for Korsak et al. (1995)

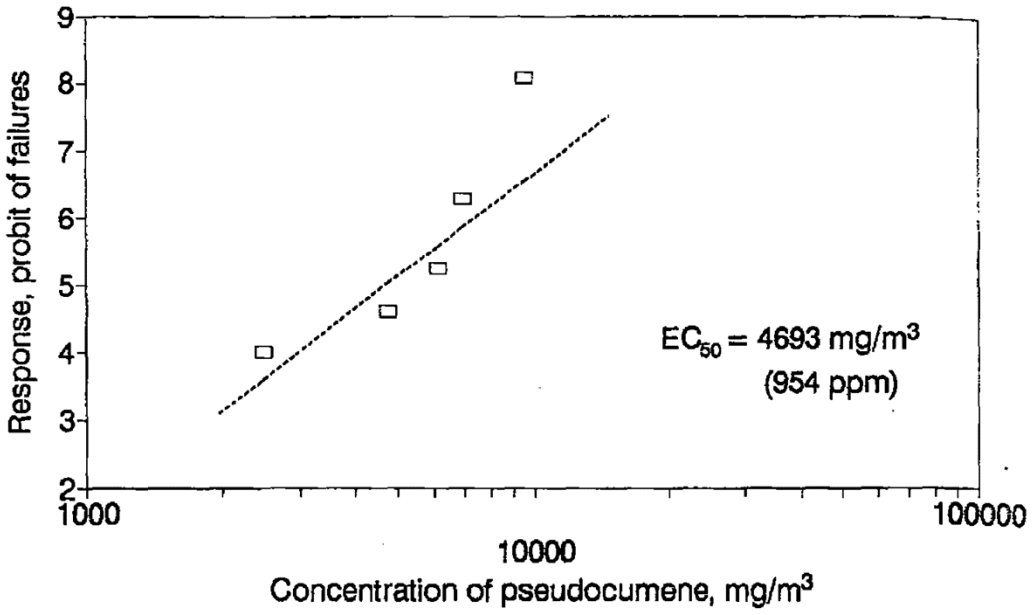
Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
IMP:DAK Wistar rats and Balb/c mice	M	8–10/dose	Inhalation	250–2000 ppm (1,230 – 9840 mg/m^3) 1,2,4-TMB	4 hrs – neurotoxicity tests 6 minutes – respiratory tests

Additional study details

- Animals were exposed to 1,2,4-TMB in a dynamic inhalation chamber (1.3 m^3 volume) with 12–15 air changes/hr.
- Mean initial body weights were 250–300 g for rats and 23–30 g for mice; animals were housed in wire mesh stainless steel cages, with food and water provided ad libitum.
- Animals were randomized and assigned to the experimental groups. Before rotarod experiment, rats were trained, and only rats that balanced for 2 minutes on 10 consecutive days were used.
- Rotarod, hot plate, and respiratory tests were conducted to measure effects on neuromuscular activity, pain sensitivity, and respiratory rate respectively.

Figure 1. Rotarod performance of rats exposed to 1,2,4-TMB (i.e., pseudocumene). Rats were exposed to vapors of solvent for 4 hrs.

Rotarod performance was tested immediately after termination of exposure. Each point represents probit of failures on rotarod in a group of 10 rats.

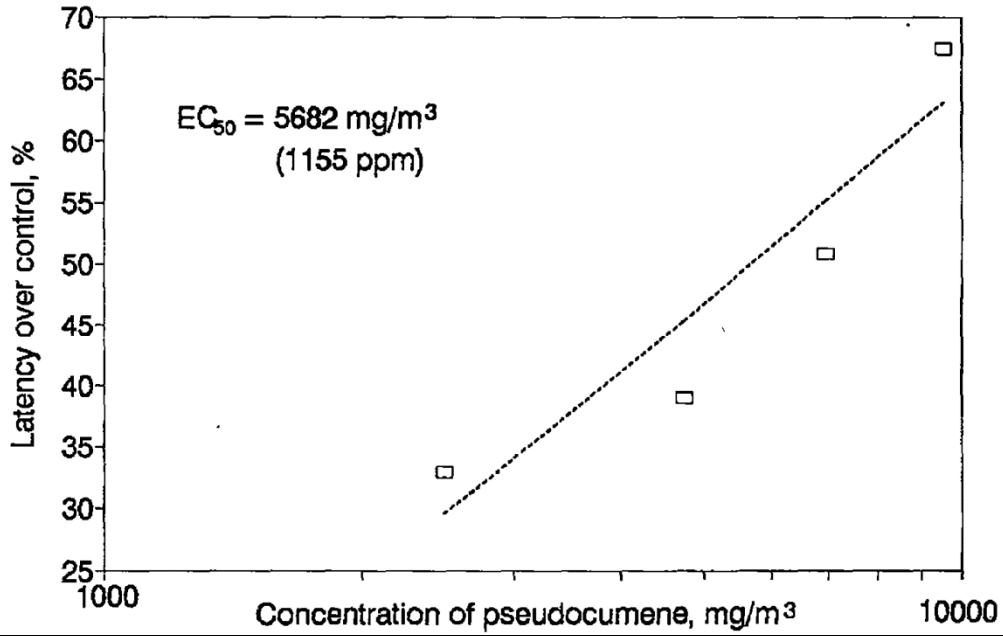


Source: Korsak et al. (1995)

Table B-29 (Continued): Characteristics and quantitative results for Korsak et al. (1995)

Figure 2. Hot-plate behavior in rats exposed to 1,2,4-TMB (i.e., pseudocumene). Rats were exposed to vapors of solvent for 4 hrs.

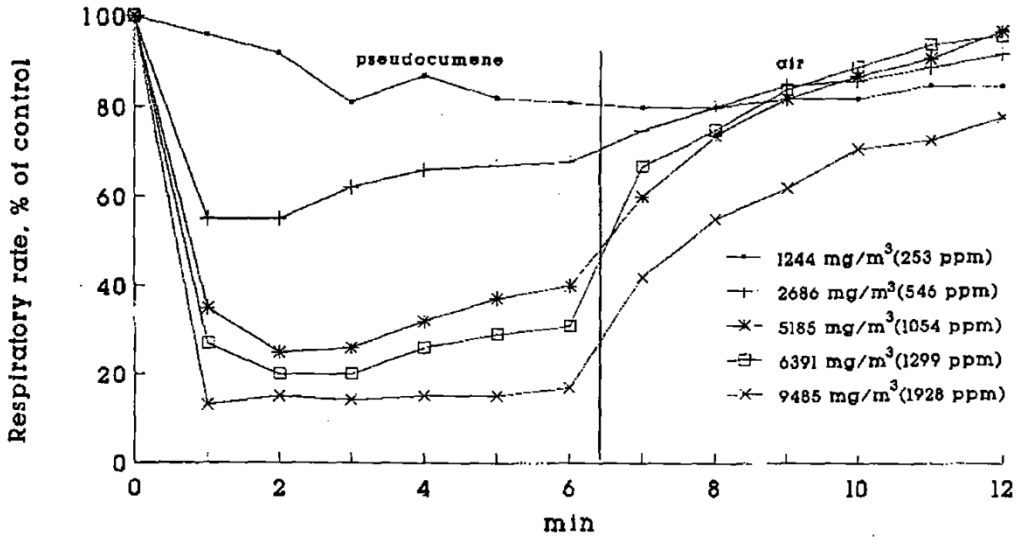
Hot-plate behavior was tested immediately after termination of exposure. Each point represents the mean value of separate measurements of latency over the control in 10 rats.



Source: Korsak et al. (1995)

Figure 3. Time-response relationship for the effect of 1,2,4-TMB (i.e., pseudocumene) on respiratory rate in mice.

Each point represents the mean value in 8–10 mice. After termination of 6 min exposure recovery of respiratory rate was observed.

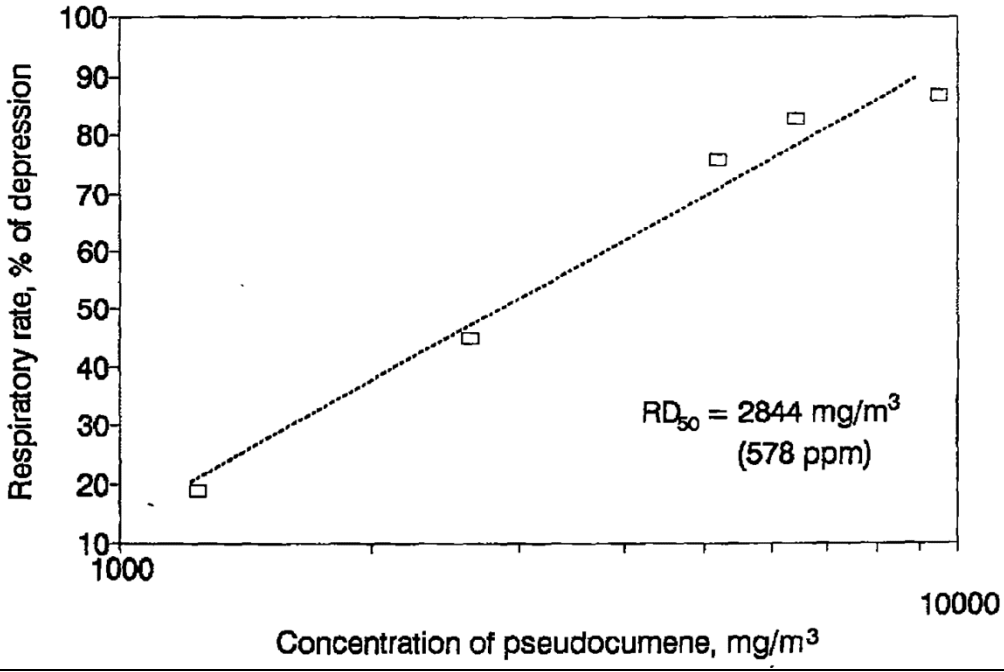


Source: Korsak et al. (1995)

Table B-29 (Continued): Characteristics and quantitative results for Korsak et al. (1995)

Figure 4. Respiratory rate of mice exposed to 1,2,4-TMB (i.e., pseudocumene) in 8–10 mice.

The decrease of respiratory rate observed in the 1st minute of exposure was taken for consideration. The regression line was determined by the least squares procedure.



Source: Korsak et al. (1995)

Health Effect at LOAEL	NOAEL	LOAEL
Decreased respiration rate, impaired rotarod test performance, decreased pain-response time	n/a	n/a

Comments: No values are provided for dose-specific responses, and NOAEL and LOAEL cannot be determined. Exposures were of an acute duration, and therefore not suitable for reference value derivation. However, qualitatively, this study provided evidence of CNS disturbances that, when considered together with short-term and subchronic neurotoxicity studies, demonstrate that TMB isomers perturb the CNS of exposed animals. The respiratory effects in mice also qualitatively support respiratory effects observed in rats exposed subchronically to 1,2,4-TMB and 1,2,3-TMB.

Source: Korsak et al. (1995)

Table B-30. Characteristics and quantitative results for Korsak and Rydzyński (1996)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
IMP: Wistar rats	M	9-10/ dose (1,2,4-TMB) 10-30/ dose (1,2,3-TMB)	Inhalation (4 hrs or 6hr/day, 5 days/week, for 3 mos)	Acute exposure: 250–2,000 ppm 1,230 – 9840 mg/m^3) 1,2,3-, 1,2,4-, or 1,3,5-TMB Subchronic exposure: 0, 123, 492, or 1,230 mg/m^3	4 hrs or 3 mos

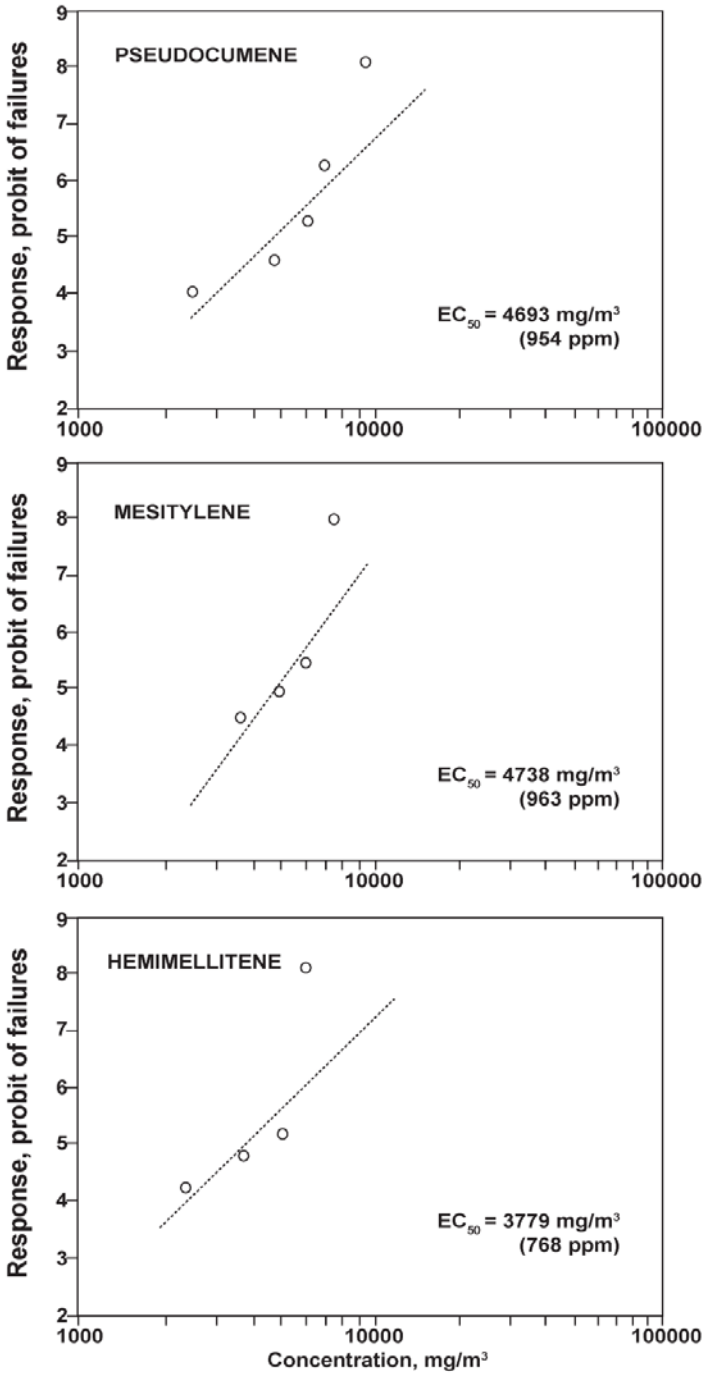
Additional study details

- Animals were exposed to either 1,2,3-, 1,2,4-, or 1,3,5-TMB in a dynamic inhalation chamber (1.3 m^3 volume) with 12 to 15 air changes/hour.
- Mean initial body weights were 250–300 g; rats were housed in wire mesh stainless steel cages, with food and water provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Rotarod and hot plate tests were conducted to measure effects on neuromuscular function and pain sensitivity respectively.
  - Rotarod performance was tested before, and immediately after, termination of exposure.
  - Normal neuromuscular function was indicated by the rats' ability to remain on a rod rotating at 12rpm for 2 minutes.
  - Hot-plate behavior was tested immediately after termination of exposure.
  - Latency of 60 seconds was considered as 100% inhibition of pain sensitivity.
- Authors investigated the effects of exposure to 1,2,3-, 1,2,4- and 1,3,5- TMB on rotarod test performance and pain-sensing response two weeks after the termination of exposure.

Table B-30 (Continued): Characteristics and quantitative results for Korsak and Rydzyński (1996)

Figure 1. Rotarod performance of rats exposed to 1,2,3-TMB (hemimellitene), 1,2,4-TMB (pseudocumene), or 1,3,5-TMB (mesitylene). Rats were exposed to solvent vapors for 4 hrs.

Rotarod performance was tested immediately after termination of exposure. Each point represents probit of failures on rotarod in a group of 10 rats. Normal neuromuscular function was indicated by the rats' ability to remain on a rod rotating at 12 rpm for 2 mins. The rotating rod was suspended 20 cm above metal bars connected to a 80 V/2 mA power source.

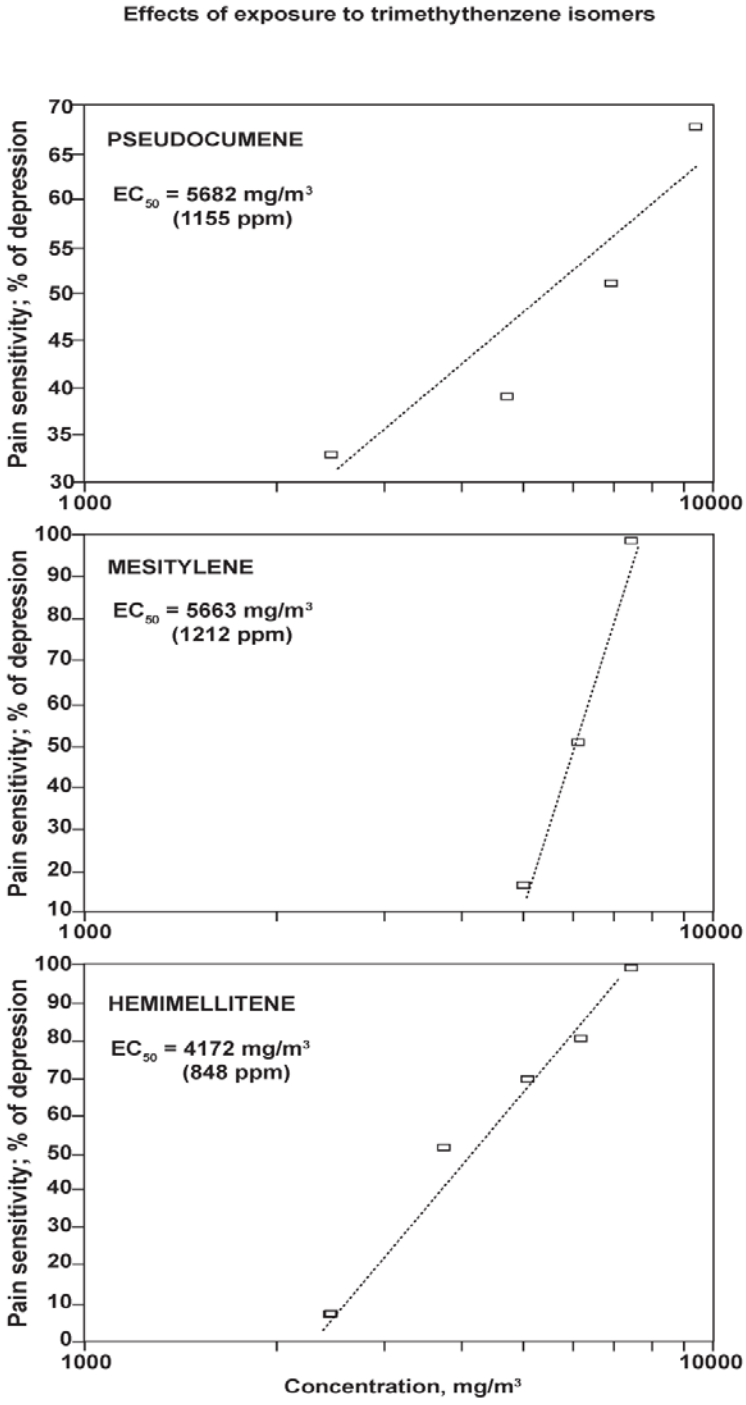


Source: Reproduced from Korsak and Rydzyński (1996)

Table B-30 (Continued): Characteristics and quantitative results for Korsak and Rydzyński (1996)

Figure 2. Hot-plate behaviors in rats exposed to 1,2,3-TMB (hemimellitene), 1,2,4-TMB (pseudocumene), or 1,3,5-TMB (mesitylene). Hot-plate behavior was tested immediately after termination of exposure.

Each point represents the mean value of separate measurements of latency in 10 rats. Latency of 60 sec was considered as 100% inhibition of pain sensitivity.



Source: Reproduced from Korsak and Rydzyński (1996)

Table B-30 (Continued): Characteristics and quantitative results for Korsak and Rydzyński (1996)

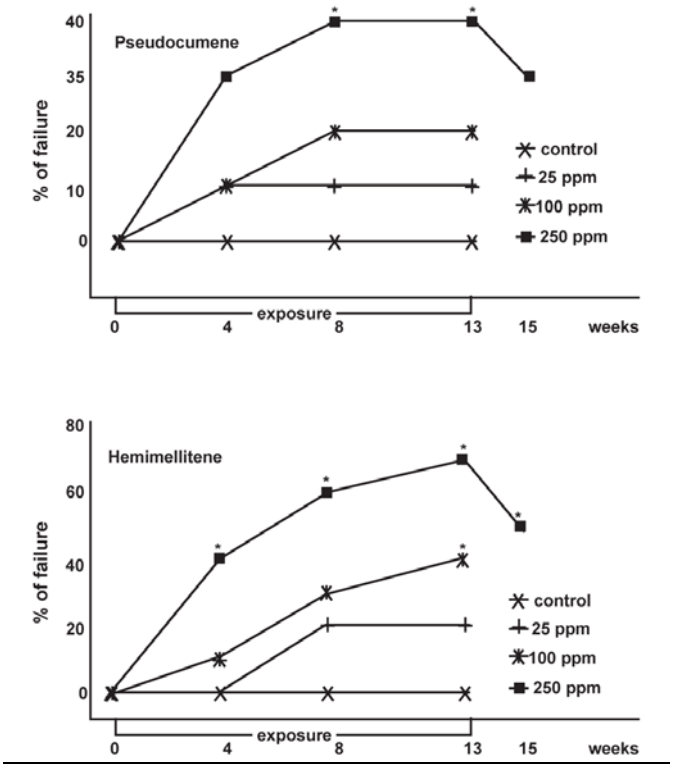


Figure 3. Rotarod performance of rats exposed to 1,2,3-TMB (hemimellitene) or 1,2,4-TMB (pseudocumene) at concentrations of 25, 100, and 250 ppm (123, 492, 1,230 mg/m^3) .

Rats were exposed to vapors of solvents for 6 hr/day, 5 days/week, 3 mos. Statistical significance marked by asterisks, p<0.005.

Source: Reproduced from Korsak and Rydzyński (1996)

	Latency of the paw-lick response, sec	
Observation	**1,2,4-TMB**	**1,2,3-TMB**
Control	15.4 ± 5.8	9.7 ± 2.1
25 ppm (100 mg/m^3)	18.2 ± 5.7	11.8 ± 3.8*
100 ppm (492 mg/m^3)	27.6 ± 3.2**	16.3 ± 6.3***
250 ppm (1,230 mg/m^3)	30.1 ± 7.9**	17.3 ± 3.4**
250 ppm (1,230 mg/m^3) 2 weeks after termination of exposure	17.3 ± 3.9	11.0 ± 2.4
Health Effect at LOAEL	**NOAEL**	**LOAEL**
Decreased pain sensitivity	n/a for 1,2,3-TMB 25 ppm (123 mg/m^3) for 1,2,4-TMB	25 ppm (123 mg/m^3) for 1,2,3-TMB 100 ppm (492 mg/m^3) for 1,2,4-TMB

Comments: Although rotarod data are useful in providing a qualitative description of neuromuscular impairment following 1,2,4-TMB or 1,2,3-TMB exposure, in comparison to effects on pain sensitivity, the data are not considered as robust regarding suitability for derivation of reference values. Namely, data are presented as dichotomized values instead of a continuous measurement of latency. The acute exposures were not suitable for reference value derivation. However, qualitatively, effects observed following acute exposures provided evidence of CNS disturbances that, when considered together with subchronic neurotoxicity tests, demonstrate that TMB isomers perturb the CNS of exposed animals. It is unclear whether the latency to pawlick and rotarod tests were performed sequentially in the same cohort of animals.

*, ** statistically significant from controls at $p \leq 0.05$ and $p \leq 0.01$, respectively.

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

Table B-31. Characteristics and quantitative results for Korsak et al. (1997)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
IMP:DAK Wistar rats and Balb/C mice	M	Acute - 8/dose Subchronic – 6-7/dose	Acute –Inhalation, 6 minutes Subchronic 0 Inhalation,6 hr/day, 5 days/week	Acute – 250–2000 ppm (1,230 – 9840 mg/m^3) 1,2,4-TMB, 1,2,3-TMB, or 1,3,5-TMB Subchronic - 0, 123, 492, 1,230 mg/m^3 1,2,4-TMB	Acute – 6 minutes Subchronic - 90 days

Additional study details

- Animals were exposed to 1,2,4-TMB in a dynamic inhalation chamber (1.3 m^3 volume) with 12–15 air changes/hr.
- Rats weighed 250–300 g and were housed in stainless steel wire mesh cages, with food and water provided ad libitum.
- Rats were anesthetized 24 hrs after termination of exposure, and bronchoalveolar (BAL) fluid was collected from lung lavage.
- All rats exposed to 1,2,4-TMB survived until the end of exposure and no clinical observations of toxicological significance were reported.

Observation	**Exposure concentration (mg/m^3)**			
	0	**123**	**492**	**1,230**
	Body weight (mean ± SD)			
Body weight (g)	411 ± 28	383 ± 25	409 ± 56	416 ± 27
	BAL cell counts (mean ± SD)			
Total cells (10^6/cm^3)	1.93 ± 0.79	5.82 ± 1.32***	5.96 ± 2.80**	4.45 ± 1.58*
Macrophages (10^6/cm^3)	1.83 ± 0.03	3.78 ± 0.8	4.95 ± 0.2**	3.96 ± 0.3**
Polymorphonuclear leucocytes (10^6/cm^3)	0.04 ± 0.02	1.54 ± 0.7	0.52 ± 0.6	0.21 ± 0.3
Lymphocytes (10^6/cm^3)	0.06 ± 0.01	0.5 ± 0.2	0.5 ± 0.4	0.2 ± 0.1
Cell viability (%)	98.0 ± 1.7	95.5 ± 1.6	95.3 ± 3.5	95.3 ± 3.1
	BAL protein levels and enzyme activities (mean ±SD)			
Total protein (mg/mL)[a]	0.19 ± 0.04	0.26 ± 0.07*	0.26 ± 0.06*	0.24 ± 0.08
Mucoproteins (mg/mL)[a]	0.16 ± 0.03	0.14 ± 0.02*	0.13 ± 0.02	0.12 ± 0.02
Lactate dehydrogenase (mU/mL)[a]	34.2 ± 8.52	92.5 ± 37.2***	61.3 ± 22.9*	53.8 ± 28.6
Acid phosphatase mU/mL)[a]	0.87 ± 0.20	1.28 ± 0.37*	1.52 ± 0.42*	1.26 ± 0.22*

Table B-31 (Continued): Characteristics and quantitative results for Korsak et al. (1997)

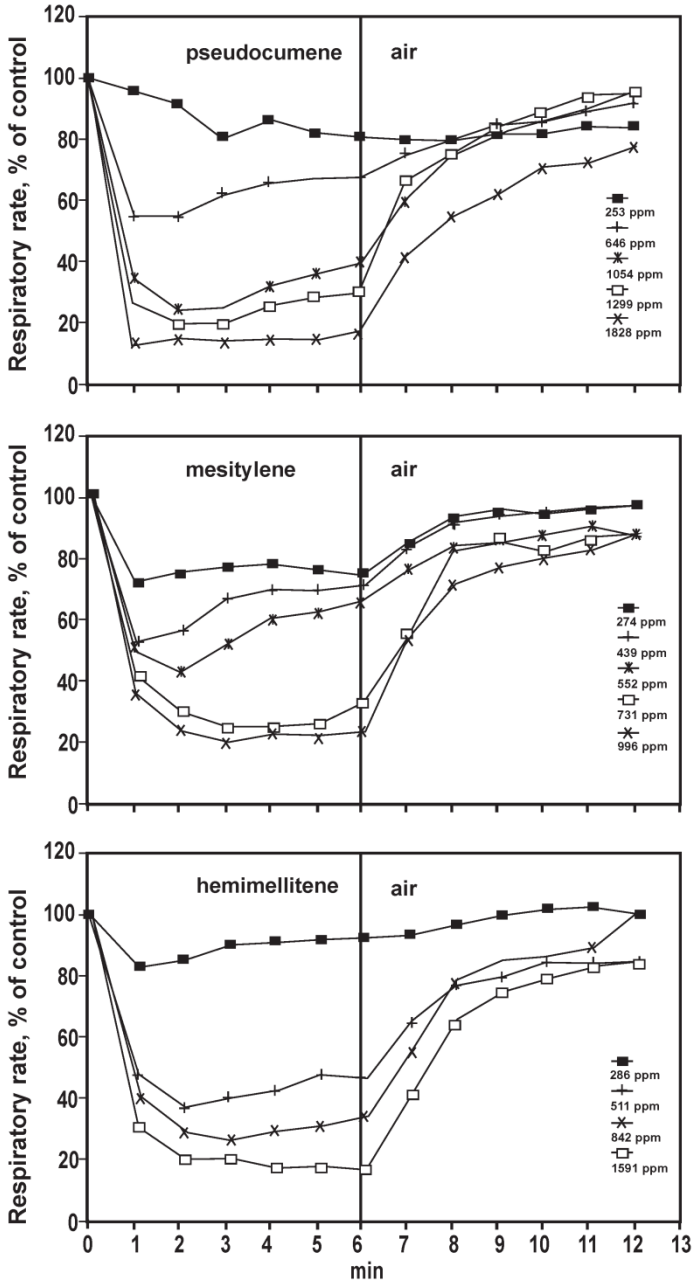


Figure 1. Time-response relationship for the effect of 1,2,4-TMB (i.e., pseudocumene) on respiratory rate in mice.

Each point represents the mean value in 8–10 mice. After termination of 6 min exposure recovery of respiratory rate was observed.

Source: Reproduced from Korsak et al. (1997)

Health Effect at LOAEL	NOAEL	LOAEL
Increased Total BAL cells	n/a	123 mg/m^3

Comments: The observed markers of inflammation are coherent with the observed respiratory irritative effects observed in mice exposed to 1,2,4-TMB acute (i.e., 6 min). The authors did not report at which dose groups the numbers of polymorphonuclear leucocytes and lymphocytes were significantly elevated relative to control.

[a] Jonckheere's test for trend: total protein, $p = 0.0577$; mucroprotein, $p = 0.3949$; lactate dehydrogenase, $p = 0.2805$; acid phosphatase, $p = 0.0164$.

*, **, *** statistically significant from control at $p < 0.05$, 0.01, and 0.001, respectively.

Source: Korsak et al. (1997)

Table B-32. Characteristics and quantitative results for Korsak et al. (2000a)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
IMP: Wistar rats	M and F	10/dose	Inhalation (6 hr/day, 5 days/week)	0, 123, 492, 1,230 mg/m^3	90 days

Additional study details

- Animals were exposed to 1,2,4-TMB in a dynamic inhalation chamber (1.3 m^3 volume) with 16 air changes/hr.
- Mean initial body weights were 213 ± 20 for males and 160 ± 11 for females; rats were housed in polypropylene cages with wire-mesh covers (5 animals/cage), with food and water provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Hematological parameters were evaluated prior to exposure and 1 week prior to termination of exposure, and for the 1230 mg/m3 exposure group, also evaluated two weeks after termination of exposure; blood clinical chemistry parameters were evaluated 18 hrs after termination of exposure (animals were deprived of food for 24 hrs).
- Necropsy was performed on all animals. Pulmonary lesions were graded using an arbitrary scale: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Observation	**Exposure concentration (mg/m^3)**			
	0	**123**	**492**	**1,230**
	Body and Organ weights (mean ± SD)			
	Males			
Terminal body weight (g)	368 ± 22	390 ± 26	399 ± 22	389 ± 29
Absolute organ weight (g)				
Lungs	1.78 ± 0.28	1.83 ± 0.25	2.93 ± 0.26*	1.78 ± 0.36
Liver	10.27 ± 1.82	11.43 ± 1.05	10.78 ± 1.33	10.86 ± 2.04
Spleen	0.68 ± 0.08	0.85 ± 0.19*	0.79 ± 0.09	0.72 ± 0.08
Kidney	2.06 ± 0.13	2.24 ± 0.15	2.14 ± 0.15	2.18 ± 0.16
Adrenals	0.048 ± 0.007	0.046 ± 0.005	054 ± 0.011	0.047 ± 0.005
Testes	3.72 ± 0.35	3.90 ± 0.38	4.03 ± 0.27	3.87 ± 0.24
Heart	0.90 ± 0.04	0.94 ± 0.06	0.94 ± 0.08	0.96 ± 0.07
Relative organ weight (g)				
Lungs	0.496 ± 0.056	0.475 ± 0.056	0.586 ± 0.115	0.477 ± 0.080
Liver	2.896 ± 0.456	2.894 ± 0.427	2.990 ± 0.465	2.901 ± 0.479
Spleen	0.189 ± 0.011	0.220 ± 0.041	0.210 ± 0.018	0.200 ± 0.018
Kidney	0.588 ± 0.029	0.585 ± 0.022	0.587 ± 0.065	0.586 ± 0.040
Adrenals	0.011 ± 0.003	0.010 ± 0.000	0.022 ± 0.024	0.011 ± 0.003
Testes	1.041 ± 0.076	1.020 ± 0.079	1.067 ± 0.102	1.039 ± 0.077
Heart	0.252 ± 0.013	0.239 ± 0.020	0.249 ± 0.014	0.258 ± 0.020

Table B-32 (Continued): Characteristics and quantitative results for Korsak et al. (2000a)

	Females					
Terminal body weight (g)	243 ± 16	243 ± 19	230 ± 14	229 ± 21		
Absolute organ weight (g)						
Lungs	1.29 ± 0.18	1.32 ± 0.12	1.25 ± 0.13	1.23 ± 0.11		
Liver	6.48 ± 1.02	6.54 ± 0.69	5.81 ± 0.83	6.72 ± 1.34		
Spleen	0.59 ± 0.08	0.61 ± 0.11	0.49 ± 0.06*	0.52 ± 0.08		
Kidney	1.55 ± 0.12	1.50 ± 0.14	1.38 ± 0.11*	1.44 ± 0.19		
Adrenals	0.065 ± 0.007	0.070 ± 0.008	0.066 ± 0.010	0.061 ± 0.013		
Ovaries	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.27	0.09 ± 0.02		
Heart	0.66 ± 0.07	0.64 ± 0.05	0.61 ± 0.07	0.63 ± 0.06		
Relative organ weight (g)						
Lungs	0.555 ± 0.058	0.581 ± 0.040	0.596 ± 0.051	0.569 ± 0.053		
Liver	2.770 ± 0.222	2.881 ± 0.309	2.758 ± 0.223	3.078 ± 0.434		
Spleen	0.255 ± 0.025	0.266 ± 0.031	0.237 ± 0.036	0.24 ± 0.033		
Kidney	0.667 ± 0.030	0.661 ± 0.047	0.660 ± 0.042	0.662 ± 0.036		
Adrenals	0.028 ± 0.006	0.031 ± 0.006	0.032 ± 0.006	0.029 ± 0.006		
Ovaries	0.043 ± 0.008	0.041 ± 0.006	0.045 ± 0.013	0.047 ± 0.009		
Heart	0.284 ± 0.023	0.283 ± 0.025	0.291 ± 0.025	0.289 ± 0.015		
	Exposure concentration (mg/m^3)					
Observation	**0**	**123**	**492**	**1,230**	**1,230[a]**	**Trend test[b]**
	Hematological parameters (mean ± SD)					
	Males					
Hematocrit (%)	49.9 ± 1.9	50.4 ± 2.0	50.0 ± 1.9	50.6 ± 1.5	50.1 ± 1.1	0.2993
Hemoglobin (g/dL)	15.1 ± 1.1	15.6 ± 0.9	15.4 ± 0.9	15.4 ± 0.6	16.0 ± 1.0	0.2112
RBCs (× 10^6/mm^3)[c]	9.98 ± 1.68	9.84 ± 1.82	8.50 ± 1.11	7.70 ± 1.38**	7.61 ± 1.6	0.0004
WBCs (× 10^3/mm^3)[d]	8.68 ± 2.89	8.92 ± 3.44	8.30 ± 1.84	15.89 ± 5.74**	7.11 ± 2.1	0.0019
Rod neutrophil (%)	0.0 ± 0.0	0.4 ± 0.5	0.2 ± 0.4	0.9 ± 1.5	0.7 ± 0.8	0.0586
Segmented neutrophil (%)	24.1 ± 9.2	19.7 ± 6.5	20.7 ± 7.7	18.9 ± 10.8	29.4 ± 6.4	0.0730
Eosinophil (%)	1.2 ± 1.7	1.2 ± 1.0	0.4 ± 0.6	1.7 ± 1.4	1.5 ± 1.5	0.2950
Lymphocyte (%)	73.5 ± 10.3	76.2 ± 7.1	76.3 ± 8.5	75.8 ± 16.0	65.4 ± 8.9	0.1297
Monocyte (%)	1.1 ± 1.3	2.5 ± 2.1	2.3 ± 2.2	1.8 ± 2.5	2.7 ± 2.5	0.3818
Lymphoblast (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 1.3	0.3 ± 0.9	0.1387
Myelocyte (%)	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.4046
Erythroblase (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5000
Reticulocyte (%)	3.1 ± 2.3	2.3 ± 1.4	2.8 ± 2.1	3.1 ± 2.5	6.4 ± 3.2	0.4900
Platelet (× 10^3/mm^3)	294 ± 46	293 ± 73	359 ± 46	335 ± 80	386 ± 70	0.0741
Clotting time (sec)	43 ± 19	41 ± 17	37 ± 13	33 ± 7	56 ± 21	0.1457

Table B-32 (Continued): Characteristics and quantitative results for Korsak et al. (2000a)

	Females					
Hematocrit (%)	46.0 ± 1.6	46.6 ± 2.7	47.0 ± 2.7	46.5 ± 4.1	45.8 ± 1.3	0.2336
Hemoglobin (g/dL)	14.5 ± 0.9	13.8 ± 1.3	14.4 ± 0.9	14.2 ± 0.9	14.9 ± 0.9	0.3461
RBCs (× 10^6/mm^3)[c]	8.22 ± 1.16	7.93 ± 2.04	8.51 ± 1.13	7.71 ± 1.58	6.99 ± 1.8	0.1891
WBCs (× 10^3/mm^3)[d]	7.50 ± 1.31	6.76 ± 2.95	9.55 ± 4.48	9.83 ± 3.74	7.11 ± 2.4	0.0307
Rod neutrophil (%)	1.4 ± 1.6	0.5 ± 0.7	0.4 ± 0.5	0.4 ± 0.9	0.5 ± 0.7	0.3270
Segmented neutrophil (%)	22.8 ± 6.5	15.5 ± 7.9	20.7 ± 7.5	17.4 ± 9.3	20.5 ± 9.5	0.1868
Eosinophil (%)	1.2 ± 0.6	1.6 ± 1.6	1.1 ± 1.7	1.2 ± 2.1	2.0 ± 1.7	0.1051
Lymphocyte (%)	73.2 ± 7.9	79.4 ± 8.4	75.5 ± 7.4	78.8 ± 11.6	74.1 ± 9.5	0.2140
Monocyte (%)	1.2 ± 1.3	2.6 ± 2.8	1.3 ± 1.7	1.5 ± 0.8	1.5 ± 1.4	0.4156
Lymphoblast (%)	0.0 ± 0.0	0.1 ± 0.3	0.5 ± 1.5	0.7 ± 1.1	0.8 ± 1.3	0.1361
Myelocyte (%)	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.5	0.1 ± 0.3	0.1 ± 0.3	0.3189
Erythroblase (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5000
Reticulocyte (%)	3.5 ± 2.6	1.7 ± 2.0	1.8 ± 0.9	1.0 ± 0.6*	5.8 ± 3.6	0.0137
Platelet (× 10^3/mm^3)	306 ± 34	234 ± 50*	303 ± 48	325 ± 57	349 ± 77	0.1542
Clotting time (sec)	30 ± 10	23 ± 4	19 ± 5**	22 ± 7*	48 ± 19	0.0034
	Exposure concentration (mg/m^3)					
Observation	**0**	**123**	**492**	**1,230**		**Trend test[b]**
	Clinical chemistry parameters (mean ± SD)					
	Males					
AST (U/dL)[e]	138.7 ± 20.6	141.3 ± 21.0	134.5 ± 27.0	138.4 ± 35.0		0.2223
ALT (U/dL)[f]	51.7 ± 5.9	48.3 ± 7.8	49.7 ± 9.1	46.8 ± 5.1		0.0637
ALP (U/dL)[g]	80.4 ± 12.0	86.2 ± 22.0	84.9 ± 21.0	90.5 ± 19.0		0.1518
SDH (U/dL)[h]	6.6 ± 1.4	8.1 ± 0.8**	7.8 ± 1.0*	8.0 ± 1.1**		0.0083
GGT (µU/ml)[i]	0.22 ± 0.44	0.20 ± 0.42	0.20 ± 0.42	0.20 ± 0.42		0.4700
Bilirubin (mg/dL)	1.027 ± 0.193	0.974 ± 0.338	1.106 ± 0.289	0.932 ± 0.175		0.2594
Total cholesterol (mg/dL)	63.6 ± 13.0	69.1 ± 12.0	72.4 ± 14.9	70.6 ± 19.5		0.0920
Glucose (mg/dL)	141.9 ± 23.9	163.8 ± 29.7	157.9 ± 23.2	162.2 ± 28.9		0.0876
Total protein (g)	5.43 ± 1.00	5.47 ± 1.39	5.34 ± 1.29	5.82 ± 1.49		0.3242
Albumin (g)	3.25 ± 0.60	3.45 ± 0.56	3.41 ± 0.83	3.53 ± 0.66		0.2279
Creatinine (mg/dL)	0.506 ± 0.099	0.437 ± 0.138	0.510 ± 0.150	0.490 ± 0.178		0.3982
Urea (mg/dL)	54.2 ± 8.6	48.8 ± 8.3	47.6 ± 3.4	49.0 ± 8.7		0.1145
Calcium (mg/dL)	10.4 ± 0.5	10.8 ± 0.5	10.7 ± 0.8	10.8 ± 0.7		0.2449
Phosphorus (mg/dL)	6.27 ± 0.49	6.50 ± 0.57	6.49 ± 0.61	6.46 ± 0.78		0.1580
Sodium (mmol/L)	139.0 ± 1.4	1393 ± 1.3	139.6 ± 1.4	139.0 ± 1.4		0.4950
Potassium (mmol/L)	4.87 ± 0.36	4.97 ± 0.34	4.97 ± 0.25	4.83 ± 0.40		0.2907
Chloride (mmol/L)	106.6 ± 1.2	106.1 ± 1.7	106.3 ± 1.5	106.7 ± 1.2		0.4353

Table B-32 (Continued): Characteristics and quantitative results for Korsak et al. (2000a)

	Females				
AST (U/dL)[e]	139.4 ± 16.6	136.7 ± 27.1	145.5 ± 22.7	141.4 ± 15.6	0.2118
ALT (U/dL)[f]	49.8 ± 6.3	51.4 ± 8.2	50.4 ± 9.0	55.1 ± 9.5	0.1844
ALP (U/dL)[g]	41.2 ± 7.8	37.2 ± 6.8	39.8 ± 11.0	49.8 ± 15.5	0.1740
SDH (U/dL)[h]	5.9 ± 1.5	7.3 ± 1.7	7.1 ± 1.8	7.0 ± 1.6	0.0637
GGT (µU/ml)[i]	0.20 ± 0.42	0.30 ± 0.48	0.10 ± 0.32	0.44 ± 0.53	0.2821
Bilirubin (mg/dL)	0.745 ± 0.342	0.690 ± 0.396	0.743 ± 0.248	0.642 ± 0.257	0.3092
Total cholesterol (mg/dL)	64.5 ± 11.9	65.7 ± 12.8	64.1 ± 10.8	62.5 ± 7.6	0.4775
Glucose (mg/dL)	118.2 ± 28.8	138.8 ± 38.5	104.5 ± 23.8	129.9 ± 39.7	0.4838
Total protein (g)	6.91 ± 0.53	7.44 ± 0.89	7.08 ± 0.35	6.94 ± 0.64	0.4036
Albumin (g)	3.42 ± 0.24	3.46 ± 0.27	3.61 ± 0.26	3.42 ± 0.15	0.2408
Creatinine (mg/dL)	0.655 ± 0.135	0.553 ± 0.104	0.629 ± 0.153	0.577 ± 0.133	0.1641
Urea (mg/dL)	52.7 ± 7.8	49.6 ± 6.7	52.8 ± 10.5	52.2 ± 11.8	0.4718
Calcium (mg/dL)	10.5 ± 0.6	10.8 ± 0.8	10.6 ± 0.5	10.8 ± 0.6	0.3011
Phosphorus (mg/dL)	4.75 ± 0.54	5.05 ± 0.70	5.34 ± 0.74	4.90 ± 1.01	0.4050
Sodium (mmol/L)	137.9 ± 1.7	138.0 ± 1.8	137.8 ± 2.5	138.2 ± 2.2	0.3628
Potassium (mmol/L)	4.54 ± 0.22	4.39 ± 0.61	4.51 ± 0.26	4.46 ± 0.25	0.4108
Chloride (mmol/L)	104.9 ± 2.0	105.5 ± 1.3	105.9 ± 1.6	106.4 ± 1.8	0.0601
	Exposure concentration (mg/m^3) [Dose Group ID]				
Observation	**0 [1]**	**123 [2]**	**492 [3]**	**1,230 [4]**	**Comparison to controls[j]**	**Trend test[b]**
	Males				
Proliferation of peribronchial lymphatic tissue (0–4)[k]	16.0[l]	15.6	30.6	17.4	1–3*	0.13
Formation of lymphoepithelium in bronchii (0–4)	18.1	15.6	27.9	18.2		22
Bronchitis and bronchopneumonia (0–4)	19.0	18.3	26.1	16.5		0.49
Interstitial lymphocytic infiltration (0–3)	14.8	18.4	26.9	19.4	1–3*	0.12
Alveolar macrophages (0–3)	14.1	14.8	24.1	26.4	1–4*	0.002
Cumulative score of all individuals	13.9	15.1	29.1	21.3	1–3*	0.02

Table B-32 (Continued): Characteristics and quantitative results for Korsak et al. (2000a)

	Females					
Proliferation of peribronchial lymphatic tissue (0–4)[k]	19.4	21.7	21.2	17.5		0.36
Formation of lymphoepithelium in bronchii (0–4)	18.3	20.1	25.1	16.1		0.48
Bronchitis and bronchopneumonia (0–4)	19.0	22.9	19.0	19.0		0.48
Interstitial lymphocytic infiltration (0–3)	15.8	14.5	21.5	29.2	1–4*	0.0017
Alveolar macrophages (0–3)	19.7	14.9	16.6	29.8	ns	0.03
Cumulative score of all individuals	16.8	15.3	21.3	27.3	ns	0.01

Health Effect at LOAEL	**NOAEL**	**LOAEL**
Increased pulmonary lesions, decreased RBCs, and increased WBCs in males	123 mg/m^3	492 mg/m^3

Comments: The observed inflammatory lesions are coherent with observations of increased inflammatory cell populations in bronchoalveolar lavage fluid in Korsak et al. (1997). The authors did not report the incidences of pulmonary lesions, but rather the results of the Kruskall-Wallis test. This makes it difficult to interpret the dose-response relationship and limits analysis of these endpoints to the NOAEL/LOAEL method for determining a POD, rather than using BMD modeling.

[a]Effects measured in rats exposed to 1,230 mg/m^3 2 weeks after termination of exposure.
[b]p-value reported from Jonckheere's trend test
[c]red blood cells,
[d]white blood cells,
[e]aspartate aminotransferase,
[f]alanine aminotransferase,
[g]alkaline phosphatase,
[h] sorbitol dehydrogenase,
[i]γ-glutamyltransferase,
[j]Reports the results of pair-wise statistical significance of exposure groups compared to controls (i.e., 1-3 would indicate that the 492 mg/m^3 was statistically significantly different from controls)
[k]grading system (0–4, 0–3; see Additional study details above)
[l] results presented as ranges of the Kruskal-Willis test.
*, ** Statistically significant from controls at $p < 0.05$ and 0.01, respectively.
Source: Korsak et al. (2000a)

Table B-33. Characteristics and quantitative results for Korsak et al. (2000b)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Concentration range**	**Exposure duration**
IMP: Wistar rats	M & F	10/dose, 20 in the 1,230 mg/m^3 group	Inhalation (6 hr/day, 5 days/week)	0, 123, 492, 1,230 mg/m^3 1,2,3-TMB	90 days

Additional study details

- Animals were exposed to 1,2,3-TMB in a dynamic inhalation chamber (1.3 m^3 volume) with 16 air changes/hr.
- Mean initial body weights were 290 ± 25 g for males and 215 ± 13 g for females; rats were housed in polypropylene cages with wire-mesh covers (5 animals/cage), with food and water provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Hematological parameters were evaluated prior to exposure and 1 week prior to termination of exposure, and for the 1230 mg/m^3 exposure group, also evaluated two weeks after termination of exposure; blood clinical chemistry parameters were evaluated 18 hrs after termination of exposure (animals were deprived of food for 24 hrs).
- Necropsy was performed on all animals.
- Pulmonary effects were graded using an arbitrary scale: 0 = normal status, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

	Exposure concentration (mg/m^3)			
Observation	**0**	**123**	**492**	**1,230**
	Body and organ weights (mean ± SD)			
	Males			
Terminal Body weight (g)	390 ± 35	408 ± 50	404 ± 33	413 ± 46
Absolute organ weight (g)				
Lungs	1.90 ± 0.22	1.86 ± 0.26	1.99 ± 0.37	1.88 ± 0.34
Liver	8.28 ± 0.97	8.83 ± 1.40	9.05 ± 0.99	9.54 ± 1.50
Spleen	0.71 ± 0.06	0.12 ± 0.10	0.82 ± 0.11	0.79 ± 0.20
Kidney	2.34 ± 0.27	2.29 ± 0.23	2.48 ± 0.25	2.50 ± 0.25
Adrenals	0.059 ± 0.012	0.061 ±0.016	0.061 ± 0.013	0.061 ± 0.012
Testes	3.78 ± 0.44	3.69 ± 0.24	3.71 ± 0.36	3.91 ± 0.12
Heart	1.04 ± 0.13	0.98 ± 0.11	1.08 ± 0.13	1.15 ± 0.19
Relative organ weight (g)				
Lungs	0.510 ± 0.071	0.479 ± 0.026	0.504 ± 0.082	0.468 ± 0.073
Liver	2.208 ± 0.163	2.271 ± 0.129	2.287 ± 0.115	2.414 ± 0.214*
Spleen	0.190 ± 0.019	0.187 ± 0.015	0.207 ± 0.021	0.203 ± 0.058
Kidney	0.623 ± 0.049	0.594 ± 0.029	0.629 ± 0.033	0.637 ± 0.060
Adrenals	0.016 ± 0.003	0.016 ± 0.003	0.015 ± 0.003	0.016 ± 0.003
Testes	1.014 ± 0.087	0.961 ± 0.091	0.941 ± 0.063	1.002 ± 0.106
Heart	0.277 ± 0.027	0.252 ± 0.018	0.274 ± 0.032	0.284 ± 0.026

Table B-33 (Continued): Characteristics and quantitative results for Korsak et al. (2000b)

	Females					
Terminal Body weight (g)	268 ± 18	262 ± 21	263 ± 14	259 ± 23		
Absolute organ weight (g)						
Lungs	1.62 ± 0.15	1.55 ± 0.33	1.47 ± 0.18	1.51 ± 0.16		
Liver	6.05 ± 0.42	5.85 ± 0.47	5.94 ± 0.51	6.05 ± 0.44		
Spleen	0.63 ± 0.05	0.61 ± 0.10	0.57 ± 0.05*	0.56 ± 0.06*		
Kidney	1.58 ± 0.16	1.53 ± 0.12	1.54 ± 0.10	1.62 ± 0.16		
Adrenals	0.080 ± 0.014	0.082 ± 0.010	0.083 ± 0.011	0.075 ± 0.015		
Ovaries	0.12 ± 0.03	0.12 ± 0.03	0.13 ± 0.02	0.14 ± 0.04		
Heart	0.74 ± 0.05	0.71 ± 0.50	0.75 ± 0.06	0.73 ± 0.08		
Relative organ weight (g)						
Lungs	0.651 ± 0.053	0.637 ± 0.122	0.604 ± 0.049	0.639 ± 0.076		
Liver	2.434 ± 0.143	2.400 ± 0.088	2.448 ± 0.190	2.555 ± 0.214		
Spleen	0.257 ± 0.027	0.249 ± 0.032	0.234 ± 0.19	0.237 ± 0.022		
Kidney	0.639 ± 0.076	0.628 ± 0.024	0.638 ± 0.032	0.686 ± 0.058		
Adrenals	0.032 ± 0.005	0.034 ± 0.004	0.034 ± 0.005	0.032 ± 0.008		
Ovaries	0.051 ± 0.014	0.050 ± 0.014	0.056 ± 0.006	0.060 ± 0.018		
Heart	0.298 ± 0.016	0.291 ± 0.012	0.309 ± 0.024	0.307 ± 0.026		
	Exposure concentration (mg/m^3)					
Observation	**0**	**123**	**492**	**1,230**	**1230[a]**	**Trend test[b]**
	Hematological parameters (mean ± SD)					
Hematocrit (%) Males	46.4 ± 1.6	45.8 ± 2.6	45.7 ± 1.3	45.5 ± 2.1	43.5 ± 26	0.1615
Hematocrit (%) Females	42.7 ± 2.2	45.0 ± 2.4	41.8 ± 1.6	41.5 ± 24	41.7 ± 20	0.0198
Hemoglobin (g/dL) Males	16.4 ± 1.0	17.6 ± 1.6	17.6 ± 0.8	15.0 ± 1.2	ND	0.0688
Hemoglobin (g/dL) Females	13.9 ± 0.7	15.1 ± 1.0*	14.6 ± 0.6	14.7 ± 0.9	ND	0.0748
RBCs (× 10^6/mm^3)[c] Males	9.49 ± 2.03	10.25 ± 1.29	10.11 ± 1.27	8.05 ± 1.38*	8.6 ± 1.5	0.0011
RBCs (× 10^6/mm^3)[c] Females	8.03 ± 1.11	8.73 ± 1.24	7.79 ± 1.57	7.27 ± 1.32	6.6 ± 1.8	0.0185
WBCs (× 10^3/mm^3)[d] Males	10.09 ± 2.23	9.38 ± 3.29	7.71 ± 3.45	9.03 ± 275	6.3 ± 4.6	0.1661
WBCs (× 10^3/mm^3)[d] Females	10.71 ± 4.28	9.54 ± 2.37	13.02 ± 3.07	13.01 ± 4.53	62 ± 2.5	0.0189
Rod neutrophil (%) Males	0.8 ± 1.0	1.0 ± 1.1	0.4 ± 0.5	0.5 ± 0.6	5.2 ± 3.0	0.1878
Rod neutrophil (%) Females	0.4 ± 0.8	0.6 ± 0.6	1.1 ± 1.4	0.4 ± 0.8	1.8 ± 2.2	0.4711
Segmented neutrophil (%) Males	24.8 ± 4.5	25.4 ± 5.8	20.7 ± 5.8	17.7 ± 8.3*	27.5 ± 9.2	0.0032
Segmented neutrophil (%) Females	23.1 ± 6.1	19.7 ± 3.4	16.4 ± 4.2*	11.9 ± 7.1**	19.6 ± 8.3	0.0000
Eosinophil (%) Males	1.3 ± 1.4	0.8 ± 1.0	0.8 ± 1.1	0.6 ± 0.8	0.6 ± 0.6	0.1439
Eosinophil (%) Females	1.4 ± 1.0	0.6 ± 0.6	0.7 ± 0.8	0.8 ± 0.9	0.7 ± 0.8	0.2778
Lymphocyte (%) Males	71.2 ± 5.0	71.6 ± 6.8	75.4 ± 4.7	79.3 ± 78.0**	63.7 ± 11.3	0.0015
Lymphocyte (%) Females	73.2 ± 7.9	77.5 ± 4.9	80.4 ± 5.1	84.0 ± 78.0**	75.7 ± 9.9	0.0003

Table B-33 (Continued): Characteristics and quantitative results for Korsak et al. (2000b)

Monocyte (%) Males	1.9 ± 1.6	1.3 ± 1.4	2.3 ± 20	1.6 ± 22	3.1 ± 3.7	0.3014
Monocyte (%) Females	2.0 ± 2.0	1.6 ± 1.6	1.1 ± 1.3	2.1 ± 1.7	1.3 ± 1.8	0.2426
Lymphoblast (%) Males	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.6	0.2 ± 0.6	0.0 ± 0.0	0.2911
Lymphoblast (%) Females	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.3 ± 0.7	0.0 ± 0.0	0.1403
Myelocyte (%) Males	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5000
Myelocyte (%) Females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	0.3963
Erythroblast (%) Males	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5000
Erythroblast (%) Females	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.2995
Reticulocyte (%) Males	2.8 ± 1.3	2.1 ± 1.7	3.8 ± 2.1	4.5 ± 1.8*	6.9 ± 3.1**	0.0017
Reticulocyte (%) Females	2.6 ± 0.9	4.6 ± 2.5*	5.2 ± .50*	4.4 ± 3.0	6.8 ± 3.5**	0.0459
Platelet (× 10^3/mm^3) Males	262 ± 51	266 ± 70	257 ± 81	242 ± 76	277 ± 80	0.1708
Platelet (× 10^3/mm^3) Females	224 ± 68	290 ± 70	249 ± 53	204 ± 44	258 ± 45	0.0329
Clotting time (sec) Males	29.7 ± 8.6	23.0 ± 10.0	37.9 ± 9.9	29.2 ± 15.6	21.7 ± 5.4	0.4650
Clotting time (sec) Females	27.2 ± 2.8	25.0 ± 9.4	23.8 ± 9.5	25.1 ± 12.1	25.9 ± 8.0	0.3479
	Exposure concentration (mg/m^3)					
Observation	**0**	**123**	**492**	**1,230**		**Trend test[b]**
	Clinical chemistry parameters (mean ± SD)					
AST (U/dL)[e] Males	107.8 ± 14.2	102.9 ± 15.1	103.6 ± 14.5	119.6 ± 27.3		0.2223
AST (U/dL)[e] Females	96.1 ± 9.4	96.9 ± 9.9	117.1 ± 23.9	104.6 ± 15.7		0.2118
ALT (U/dL)[f] Males	41.3 ± 2.0	40.7 ± 3.1	41.5 ± 5.5	45.5 ± 5.6		0.0637
ALT (U/dL)[f] Females	39.7 ± 3.5	39.5 ± 6.4	36.2 ± 3.3	30.5 ± 9.9**		0.1844
ALP (U/dL)[g] Males	70.5 ± 15.2	70.6 ± 11.7	66.5 ± 10.8	63.7 ± 15.7		0.1518
ALP (U/dL)[g] Females	21.5 ± 2.7	25.8 ± 8.4	31.1 ± 8.6*	30.5 ± 9.9*		0.1740
SDH (U/dL)[h] Males	1.6 ± 0.7	2.3 ± 1.3	2.5 ± 0.9	2.7 ± 0.7*		0.0083
SDH (U/dL)[h] Females	1.7 ± 0.7	1.9 ± 0.9	1.5 ± 0.7	1.8 ± 1.0		0.0637
GGT (µU/ml)[i] Males	0.77 ± 0.66	0.77 ± 0.97	0.40 ± 0.51	0.50 ± 0.75		0.4700
GGT (µU/ml)[i] Females	0.55 ± 0.72	0.44 ± 1.01	0.66 ± 1.11	0.30 ± 0.48		0.2821
Bilirubin (mg/dL) Males	0.600 ± 0.516	0.600 ± 0.516	0.800 ± 0.422	0.625 ± 0.518		0.2594
Bilirubin (mg/dL) Females	0.911 ± 0.348	1.161 ± 0.469	0.930 ± 0.463	0.976 ± 0.421		0.3092
Total cholesterol (mg/dL) Males	63.1 ± 10.1	62.2 ± 11.6	64.5 ± 16.2	65.0 ± 9.1		0.0920
Total cholesterol (mg/dL) Females	60.1 ± 12.2	62.4 ± 15.3	62.3 ± 7.7	64.4 ± 14.1		0.4775
Glucose (mg/dL) Males	95.5 ± 13.1	110.8 ± 14.7	100.2 ± 15.2	114.5 ± 20.6		0.0876
Glucose (mg/dL) Females	115.9 ± 8.5	121.0 ± 17.5	109.2 ± 5.8	109.8 ± 10.8		0.4838
Total protein (g) Males	7.84 ± 0.13	8.02 ± 0.50	7.76 ± 0.27	8.04 ± 0.59		0.3242
Total protein (g) Females	8.24 ± 1.24	8.36 ± 1.14	8.65 ± 0.84	8.62 ± 0.96		0.4036
Albumin (g) Males	3.15 ± 0.73	3.15 ± 1.33	3.08 ± 1.30	2.95 ± 1.12		0.2279
Albumin (g) Females	3.22 ± 1.28	3.17 ± 1.03	2.58 ± 1.28	3.60 ± 1.17		0.2408
Creatinine (mg/dL) Males	41.24 ± 8.94	41.35 ± 11.28	40.79 ± 9.30	43.61 ± 13.10		0.3982
Creatinine (mg/dL) Females	62.54 ± 10.66	61.60 ± 7.07	67.11 ± 10.86	59.71 ± 7.51		0.1641

Table B-33 (Continued): Characteristics and quantitative results for Korsak et al. (2000b)

Urea (mg/dL) Males	38.7 ± 4.5	38.1 ± 9.1	36.9 ± 4.1	41.7 ± 7.5	0.1145
Urea (mg/dL) Females	42.0 ± 5.5	43.5 ± 4.4	40.0 ± 4.3	39.0 ± 29	0.4718
Calcium (mg/dL) Males	10.6 ± 0.6	10.7 ± 0.8	10.8 ± 0.7	10.9 ± 0.5	0.2449
Calcium (mg/dL) Females	11.1 ± 0.8	11.7 ± 0.3	11.8 ± 0.2	11.8 ± 0.7	0.3011
Phosphorus (mg/dL) Males	8.60 ± 0.95	8.26 ± 0.60	9.19 ± 0.88	9.41 ± 0.55	0.1580
Phosphorus (mg/dL) Females	6.56 ± 0.70	6.25 ± 1.17	6.41 ± 1.02	7.18 ± 1.09	0.4050
Sodium (mmol/L) Males	143.9 ± 2.1	144.1 ± 1.5	143.9 ± 25	144.8 ± 24	0.4950
Sodium (mmol/L) Females	144.0 ± 1.5	143.8 ± 1.3	142.7 ± 1.3	143.8 ± 1.4	0.3628
Potassium (mmol/L) Males	4.70 ± 0.35	4.45 ± 0.28	4.75 ± 0.37	4.97 ± 0.56	0.2907
Potassium (mmol/L) Females	4.52 ± 0.41	4.51 ± 0.43	4.28 ± 0.41	4.37 ± 0.34	0.4108
Chloride (mmol/L) Males	107.3 ± 2.3	107.7 ± 4.3	106.8 ± 1.8	106.5 ± 1.9	0.4353
Chloride (mmol/L) Females	108.1 ± 3.2	108.1 ± 1.5	107.1 ± 1.3	107.2 ± 23	0.0601

	Exposure concentration (mg/m^3) [Dose group ID]					
Observation	**0 [1]**	**123 [2]**	**492 [3]**	**1230 [4]**	**Comparison to controls[j]**	**Trend test[b]**
Proliferation of peribronchial lymphatic tissue (0–3)[k]Males	2.0[l] (23.4)[m]	1.2 (11.5)	1.8 (22.0)	2.0 (23.5)	1–2*	$p = 0.2$
Proliferation of peribronchial lymphatic tissue (0–3)Females	2.4 (22.8)	1.3 (12.1)	1.5 (16.4)	1.3 (22.3)	1–2**; 1–3	$p = 0.2$
Formation of lymphoepithelium in bronchii (0–3) Males	1.5 (23.9)	0.9 (14.9)	1.0 (16.0)	1.5 (25.7)	1–3*; 1–4**	$p = 0.3$
Formation of lymphoepithelium in bronchii (0–3) Females	1.8 (27.9)	0.7 (11.1)	1.1 (16.9)	1.5 (23.8)		$p = 0.3$
Goblet cells (0–3) Males	1.8 (18.6)	1.5 (14.5)	2.5 (28.5)	1.8 (18.2)		$p = 0.18$
Goblet cells (0–3) Females	1.3 (11.9)	1.6 (16.9)	2.0 (23.1)	2.4 (28.4)	1–3*; 1–4**	$p = 0.001$
Interstitial lymphocytic infiltration (0–3) Males	0.4 (18.0)	0.1 (14.1)	0.4 (18.0)	1.5 (31.0)	1–4*	$p = 0.006$
Interstitial lymphocytic infiltration (0–3) Females	1.2 (23.7)	0.6 (15.3)	0.8 (17.9)	1.1 (22.9)		$p = 0.4$
Alveolar macrophages (0–3) Males	0.9 (17.9)	0.9 (17.9)	1.2 (22.6)	1.2 (21.7)		$p = 0.15$
Alveolar macrophages (0–3) Females	1.5 (26.1)	1.1 (21.1)	0.5 (17.8)	0.7 (14.8)		$p = 0.01$

Table B-33 (Continued): Characteristics and quantitative results for Korsak et al. (2000b)

Bronchitis and broncho-pneumonia (0–4) Males	0.5 (20.1)	0.2 (16.6)	0.8 (23.8)	0.7 (19.5)		$p = 0.3$
Bronchitis and broncho-pneumonia (0–4) Females	0.2 (17.6)	0.4 (22.5)	0.2 (17.5)	0.6 (21.8)		$p = 0.3$
Cumulative score of all individual Males	7.1 (19.8)	4.8 (11.2)	7.7 (24.2)	8.7 (25.8)		$p = 0.01$
Cumulative score of all individual Females	8.4 (24.9)	5.7 (13.5)	6.5 (16.8)	8.2 (24.6)	1–2*	$p = 0.4$
Health Effect at LOAEL	**NOAEL**			**LOAEL**		
Pulmonary lesions	492 mg/m^3			1230 mg/m^3		
Comments: The observed inflammatory lesions are coherent with observations of increased inflammatory cell populations in bronchoalveolar lavage fluid due to 1,2,4-TMB exposure in Korsak et al. (1997). The authors did not report the incidences of pulmonary lesions, but rather the results of the Kruskall-Wallis test. This makes it difficult to interpret the dose-response relationship and limits analysis of these endpoints to the NOAEL/LOAEL method for determining a POD, rather than using BMD modeling.						

[a]Effects measured in rats exposed to 1,230 mg/m^3 2 weeks after termination of exposure.
[b]p-value reported from Jonckheere's trend test
[c]red blood cells,
[d]white blood cells,
[e]aspartate aminotransferase,
[f]alanine aminotransferase,
[g]alkaline phosphatase,
[h] sorbitol dehydrogenase,
[i]γ-glutamyltransferase,
[j] Reports the results of pair-wise statistical significance of exposure groups compared to controls (i.e., 1-3 would indicate that the 492 mg/m^3 was statistically significantly different from controls)
[k] grading system (0–4, 0–3; see Additional study details above)
[l] mean
[m] results presented as ranges of the Kruskal-Willis test.
*, ** Statistically significant from controls at $p < 0.05$ and 0.01, respectively.

Source: Korsak et al. (2000b).

Table B-34. Characteristics and quantitative results for Lammers et al. (2007)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
WAG/RijCR/BR Wistar rats	M	8 /group	Inhalation (8 hr/day for 3 consecutive days)	0, 600, 2,400, or 4,800 mg/m^3 1,2,4-TMB (as a constituent of WS)	3 days

Additional study details

- Rats were exposed to 1,2,4-TMB as a constituent of WS at concentrations of 0, 600, 2,400, or 4,800 mg/m^3 for 3 days. Several tests were conducted to evaluate impact of WS on CNS. These included tests of observation, spontaneous motor activity and learned visual discrimination.
- White spirit was found to affect performance and learned behavior in rats.

Observation	**Functional observations and physiological parameters in rats following exposure to WS (exposure concentration mg/m^3)**			
	0	**600**	**2,400**	**4,800**
Functional observation battery (mean ± SD)				
Gait score[a]				
Before first 8 hr exposure	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
After first 8 hr exposure	1.00 ± 0.00	1.00 ± 0.00	1.13 ± 0.13	1.25 ± 0.16
After third 8 hr exposure	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Click response[b]				
Before first 8 hr exposure	2.13 ± 0.13	2.63 ± 0.18	2.38 ± 0.18	2.50 ± 0.19
After first 8 hr exposure	2.88 ± 0.13	2.50 ± 0.19	2.75 ± 0.37	2.63 ± 0.18
After third 8 hr exposure	2.13 ± 0.13	3.25 ± 0.31*	2.88 ± 0.23	2.75 ± 0.25
Physiological parameters (mean ± SD)				
Body weight (g)				
Before first 8 hr exposure	270.0 ± 2.61	269.2 ± 2.48	273.3 ± 3.52	272.8 ± 2.20
After first 8 hr exposure	279.7 ± 2.53	277.7 ± 3.11	278.0 ± 3.21**	273.8 ± 2.51***
After third 8 hr exposure	280.9 ± 2.68	278.4 ± 2.44	275.9 ± 2.83***	268.5 ± 2.67***
Body temperature (°C)				
Before first 8 hr exposure	37.60 ± 0.34	37.33 ± 0.39	37.49 ± 0.39	37.29 ± 0.37
After first 8 hr exposure	36.41 ± 0.05	36.25 ± 0.12	36.16 ± 0.11	35.95 ± 0.21
After third 8 hr exposure	36.60 ± 0.10	36.44 ± 0.17	36.25 ± 0.05	36.11 ± 0.09**

Table B-34 (Continued): Characteristics and quantitative results for Lammers et al. (2007)

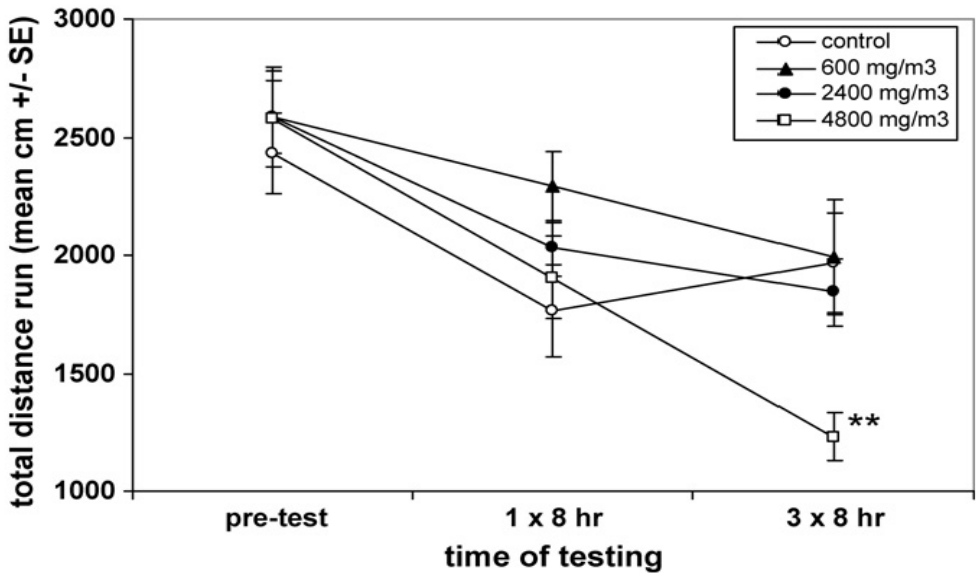


Figure 1. Effects of WS on total distance run during motor activity assessment in rats.

Observation	Visual discrimination performance in rats exposed to WS for 3 consecutive days (exposure concentration in mg/m^3)[c]			
	0	600	2,400	4,800
Lever response latency (sec)				
Before first 8 hr exposure	1.93 ± 0.34	2.09 ± 0.24	1.70 ± 0.15	2.29 ± 0.31**
After first 8 hr exposure	2.44 ± 0.56	2.66 ± 0.29	3.24 ± 0.21	12.00 ± 2.37**
After second 8 hr exposure	2.17 ± 0.41	2.32 ± 0.29	2.10 ± 0.18	4.88 ± 1.53**
After third 8 hr exposure	3.21 ± 1.22	2.68 ± 0.41	3.86 ± 0.65	6.31 ± 1.35**
One day after third 8 hr exposure	2.27 ± 0.52	1.93 ± 0.16	1.88 ± 0.16	2.34 ± 0.31**
Number of lever response latencies <2 sec				
Before first 8 hr exposure	68.00 ± 5.46	67.38 ± 2.58	77.12 ± 4.32***	71.25 ± 4.00**
After first 8 hr exposure	70.38 ± 2.93	61.88 ± 3.92	58.75 ± 2.58***	45.62 ± 4.87**
After second 8 hr exposure	70.62 ± 3.60	68.00 ± 3.81	69.00 ± 2.98***	61.50 ± 5.00**
After third 8 hr exposure	71.50 ± 3.38	66.38 ± 3.34	63.75 ± 5.04***	55.62 ± 5.12**
One day after third 8 hr exposure	72.50 ± 3.58	69.75 ± 2.90	73.38 ± 2.93***	64.88 ± 4.23**

Table B-34 (Continued): Characteristics and quantitative results for Lammers et al. (2007)

Number of lever response latencies >6 sec				
Before first 8 hr exposure	3.88 ± 0.90	5.25 ± 0.84	3.25 ± 0.45*	5.62 ± 0.92**
After first 8 hr exposure	5.00 ± 1.10	7.62 ± 1.83	11.12 ± 0.85*	25.75 ± 5.05**
After second 8 hr exposure	4.38 ± 0.96	5.62 ± 0.78	5.00 ± 0.65*	12.25 ± 3.80**
After third 8 hr exposure	7.38 ± 2.07	6.88 ± 1.16	10.88 ± 1.96*	17.50 ± 2.76**
One day after third 8 hr exposure	4.62 ± 1.31	4.38 ± 1.07	3.75 ± 0.70*	6.50 ± 1.86**
Drink response latency (sec)				
Before first 8 hr exposure	0.35 ± 0.04	0.29 ± 0.03	0.36 ± 0.03	0.32 ± 0.02*
After first 8 hr exposure	0.37 ± 0.04	0.31 ± 0.03	0.39 ± 0.02	0.52 ± 0.04*
After second 8 hr exposure	0.36 ± 0.04	0.28 ± 0.03	0.33 ± 0.02	0.39 ± 0.04*
After third 8 hr exposure	0.38 ± 0.05	0.32 ± 0.04	0.39 ± 0.02	0.43 ± 0.07*
One day after third 8 hr exposure	0.36 ± 0.03	0.31 ± 0.02	0.34 ± 0.02	0.33 ± 0.04*
Health Effect at LOAEL	**NOAEL**		**LOAEL**	
n/a	n/a		n/a	
Comments: Exposure to 1,2,4-TMB was via WS, which is comprised of additional substances. LOAEL and NOAEL cannot be extracted from this study because other constituents of the WS mixture may confound results.				

[a]Gait score indicates the severity of gait changes and is scored as 1 (normal) to 4 (severely abnormal).
[b]Click response was scored as 0 (no reaction) to 5 (exaggerated reaction).
[c]Data for parameters that did not show statistically significant group differences are not shown; statistical analysis: repeated measures ANCOVA + pairwise group comparisons.
*,**,*** Statistically significant from controls at $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively.
Source: Lammers et al. (2007)

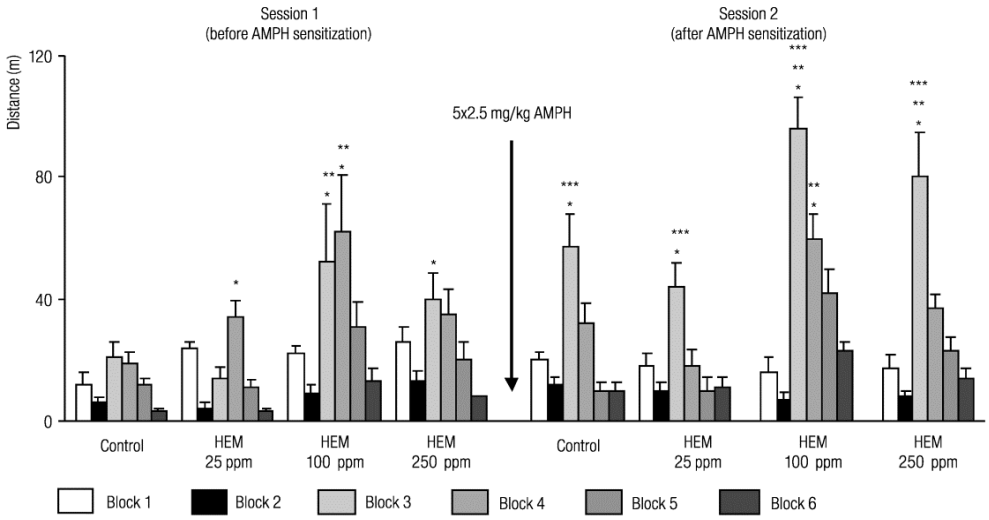
Table B-35. Characteristics and quantitative results for Lutz et al. (2010)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	6–8 rats per dose	Inhalation (6 hr/day, 5 days/week)	0, 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,3- or 1,2,4-TMB	4 weeks

Additional study details

- Animals were exposed to 1,2,3- or 1,2,4-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/day, 5 days/week for 4 weeks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Behavioral sensitivity to amphetamine was measured via test of open-field locomotor activity.
- Differences were observed between 1,2,3- and 1,2,4-TMB exposed rats, with 1,2,3-TMB-exposed rats displaying greater amphetamine sensitization than 1,2,4-TMB exposed rats.

Table B-35 (Continued): Characteristics and quantitative results for Lutz et al. (2010)

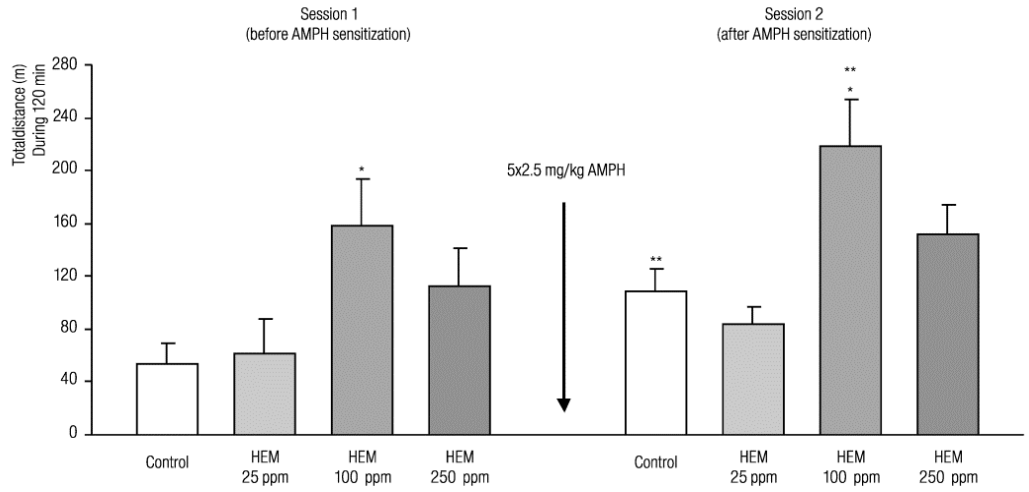


Block 1 — control (preinjection) activity, block 2 — activity after the SAL injection, blocks 3, 4, 5 and 6 — activity during successive 30 min sections after AMPH (0.5 mg/kg) injection.
ANOVA: group effects: F (3.24) =9.80; P = 0.0002; session effects: F (1.24) =34.22; P = 0.0000; interaction: F (3.24) =20.64; P = 0.0000.
* P < 0.05 — compared to post SAL measurement.
** P < 0.05 — compared to control 0 in the same session.
*** P < 0.05 — compared to corresponding measure before sensitization.
The bars represent mean values and SEM of the ambulatory activity (distance in metres) in successive 30 min blocks in the rats exposed to hemimellitene on the locomotor response to AMPH challenge before (session 1) and 14 days after (session 2) a repeated (2.5 mg/kg, 1/day×5 days) AMPH treatment.

Figure 1. Diagram illustrating the effect of prior exposure to 1,2,3-TMB on the locomotor response (all measurements) to the amphetamine challenge before (session 1) and 14 days after (session 2) a repeated (2.5 mg/kg, 1/day × 5 day) amphetamine treatment.

Source: Lutz et al. (2010)

Table B-35 (Continued): Characteristics and quantitative results for Lutz et al. (2010)



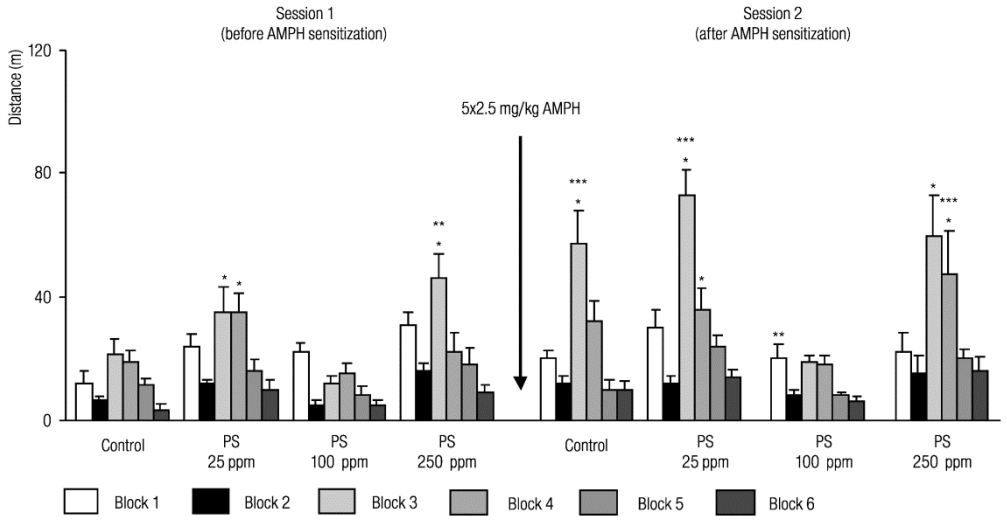
* P < 0.05 — compared to control. ** P < 0.05 — compared to corresponding measure before sensitization.
Bars represent mean values and SEM of the cumulated locomotor activity (distance in metres) during the 2-hour measurement following AMPH (0.5 mg/kg) challenge.

Figure 2. Diagram illustrating the effect of prior exposure to 1,2,3-TMB on the locomotor response (pooled measurements) to the amphetamine challenge before (session 1) and 14 days after (session 2) a repeated amphetamine treatment (2.5 mg/kg, 1/day × 5 days).

Source: Lutz et al. (2010)

Table B-35 (Continued): Characteristics and quantitative results for Lutz et al. (2010)

Figure 3. Diagram illustrating the effect of prior exposure to 1,2,4-TMB on the locomotor response (all measurements) to the amphetamine challenge before (session 1) and 14 days after (session 2) a repeated (2.5 mg/kg, 1/day × 5 days) amphetamine treatment. Remaining notations are the same as in Figure 1.

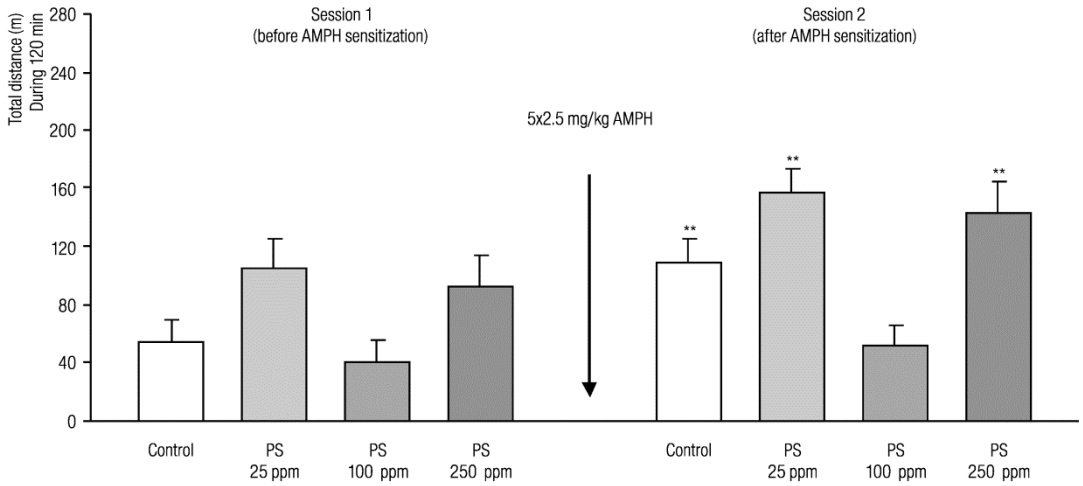


ANOVA: group effects: F (3.25) =8.90; P = 0.004. Session effects: F (1.25) =30.91; P = 0.0000. Interaction: F (3.25) =29.48; P = 0.0000.
* P < 0.05 — compared to post SAL measurement.
** P < 0.05 — compared to control 0 in the same session.
*** P < 0.05 — compared to corresponding measure before sensitization.
The bars represent mean values and SEM of the ambulatory activity (distance in metres) in successive 30 min blocks.

Source: Lutz et al. (2010)

Table B-35 (Continued): Characteristics and quantitative results for Lutz et al. (2010)

Figure 4. Diagram illustrating the effect of prior exposure to 1,2,4-TMB on the locomotor response (pooled measurements) to amphetamine challenge before (session 1) and 14 days after (session 2) a repeated amphetamine treatment (2.5 mg/kg, 1/day × 5 days).



* P < 0.05 — compared to control. ** P < 0.05 — compared to corresponding measure before sensitization.
Bars represent mean values and SEM of the cumulated locomotor activity (distance in metres) during the 2-hour measurement following AMPH (0.5 mg/kg) challenge.

Source: Lutz et al. (2010)

Health Effect at LOAEL	NOAEL	LOAEL
Increased sensitivity to amphetamine as measured by open-field locomotion	0 ppm	25 ppm (123 mg/m^3) 1,2,4-TMB or 1,2,3-TMB

Comment: This study observed increased amphetamine sensitization, particularly in rats exposed to 100 ppm (492 mg/m^3) 1,2,3-TMB, and provided evidence for differences in toxicity between different TMB isomers. Control group for 1,2,4-TMB also showed statistically significant increase in locomotor activity after receiving amphetamine treatment.

Source: Lutz et al. (2010)

Table B-36. Characteristics and quantitative results for Maltoni et al. (1997)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Sprague-Dawley rats: CRC/BT	M	50 males, 50 females per group	Stomach tube (in olive oil)	0 or 800 mg/kg BW 1,2,4-TMB	4 days/week for 104 weeks
Additional study details • Rats were exposed to 1,2,4-TMB for 2 years via stomach tube administration 4 days/week. • Animals were 7 weeks old at start of experiments. • Systematic necropsy was conducted upon animal death. • A slight increase in total number of tumors was detected amongst males and females, and an increase in the number of head cancers in males was also observed.					

	Long-term carcinogenicity of 1,2,4-TMB	
Observation	**0 mg/kg**	**800 mg/kg**
Total number of tumors		
Males		
Total benign and malignant tumors	54.0	62.0
Malignant tumors	24.0	26.0
No. malignant tumors/100 rats	26.0	34.0
Females		
Total benign and malignant tumors	70.0	66.0
Malignant tumors	22.0	24.0
No. malignant tumors/100 rats	22.0	32.0
Both sexes		
Total benign and malignant tumors	62.0	64.0
Malignant tumors	23.0	25.0
No. malignant tumors/100 rats	24.0	33.0

Table B-36 (Continued): Characteristics and quantitative results for Maltoni et al. (1997)

Head cancers		
Males		
Zymbal gland cancer	2.0	4.0
Ear duct cancer	--	2.0
Neuroesthesio-epitheliomas	--	2.0
Oral cavity cancers	--	2.0
Total head cancers	2.0	10.0
Females		
Zymbal gland cancer	2.0	2.0
Ear duct cancer	2.0	--
Neuroesthesioepi-theliomas	--	4.0
Oral cavity cancers	2.0	--
Total head cancers	6.0	6.0
Both sexes		
Zymbal gland cancer	2.0	3.0
Ear duct cancer	1.0	1.0
Neuroesthesio-epitheliomas	--	3.0
Oral cavity cancers	1.0	1.0
Total head cancers	4.0	8.0
Health Effect at LOAEL	**NOAEL**	**LOAEL**
Various malignant and non-malignant cancers	n/a	800 mg/kg

Comments: Neuroesthesioepithelioma is uncommon in Sprague-Dawley rats, although there were increases in the number of neuroesthesioepithelioma in both males and females. Only one dose level was tested (800 mg/kg), making any determination of dose-response impossible. Statistical significance of data not provided, although post-hoc statistical tests performed by EPA failed to observe any statistical increase in tumors.

Source: Maltoni et al. (1997)

Table B-37. Characteristics and quantitative results for McKee et al. (2010)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	8 rats per group	Inhalation	0, 125, 1,250, or 5,000 mg/m^3 1,2,4-TMB	8 hrs/day for 3 consecutive days

Additional study details

- Animals were exposed to 1,2,4-TMB for 8 hrs/day for 3 days in modified H1000 inhalation chambers.
- Animals were randomized and assigned to the experimental groups.
- Test on neurobehavioral effects were conducted prior to, during, and after exposure period.
- Motor activity was affected on the third day of exposure in the highest exposure group, although brain concentrations of 1,2,4-TMB were lower than on previous days.

	Exposure concentration 1,2,4-TMB (mg/m^3)			
Observation	**0**	**125**	**1,250**	**5,000**
Results of functional and motor activity observations				
Forelimb grip strength (g)				
One-day pre-exposure	1,107 ± 41.2	1,065 ± 52.3	1,223 ± 25.9	1,090 ± 47.0
First 8 hr exposure	1,064 ± 39.9	814 ± 91.7*	1,059 ± 59.8	1,023 ± 55.7
Third 8 hr exposure	908 ± 56.1	847 ± 64.3	956 ± 67.7	1,156 ± 68.7*
Total distance traveled (cm)				
One-day pre-exposure	3,773 ± 120	3,598 ± 301	3,543 ± 167	3,575 ± 119
First 8 hr exposure	2,479 ± 110	3,048 ± 257	2,125 ± 171	1,897 ± 200
Third 8 hr exposure	2,459 ± 118	2,740 ± 226	1,967 ± 316	1,172 ± 226*
Number of movements				
One-day pre-exposure	1,054 ± 31	999 ± 80	990 ± 44	998 ± 32
First 8 hr exposure	697 ± 29	848 ± 66	600 ± 48	529 ± 53
Third 8 hr exposure	687 ± 31	744 ± 56	541 ± 82	329 ± 61*
	Exposure concentration 1,2,4-TMB (mg/m^3)			
Observation	**0**	**125**	**1,250**	**5,000**
Visual discrimination performance testing (means ± SD)				
Trials[a]				
One-day pre-exposure	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
First 8 hr exposure	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.13 ± 0.88
Third 8 hr exposure	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
One-day post-exposure	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Percentage reinforcements obtained[b]				
One-day pre-exposure	99.88 ± 0.13	99.88 ± 0.13	99.88 ± 0.13	100 ± 0.0
First 8 hr exposure	100 ± 0.0	100 ± 0.0	99.38 ± 0.63	99.74 ± 0.17
Third 8 hr exposure	99.63 ± 0.26	99.63 ± 0.26	99.63 ± 0.38	100 ± 0.0
One-day post-exposure	99.63 ± 0.26	99.88 ± 0.13	99.88 ± 0.13	100 ± 0.0

Table B-37 (Continued): Characteristics and quantitative results for McKee et al. (2010)

Discrimination ratio[c]				
One-day pre-exposure	0.81 ± 0.84	0.84 ± 0.03	0.83 ± 0.02	0.83 ± 0.03
First 8 hr exposure	0.86 ± 0.02	0.91 ± 0.03	0.91 ± 0.01	0.95 ± 0.01*
Third 8 hr exposure	0.89 ± 0.02	0.88 ± 0.03	0.94 ± 0.01	0.95 ± 0.02
One-day post-exposure	0.87 ± 0.03	0.89 ± 0.03	0.92 ± 0.02	0.88 ± 0.03
Percentage inter-trial intervals responded to[d]				
One-day pre-exposure	12.88 ± 2.00	10.13 ± 1.56	10.75 ± 1.94	10.38 ± 1.84
First 8 hr exposure	12.50 ± 2.12	8.88 ± 2.03	11.50 ± 2.60	10.19 ± 1.28
Third 8 hr exposure	12.00 ± 1.65	8.88 ± 2.24	8.25 ± 1.71	5.75 ± 1.39
One-day post-exposure	10.88 ± 1.39	10.63 ± 1.81	11.25 ± 0.92	8.50 ± 1.40
Repetitive errors[e]				
One-day pre-exposure	8.25 ± 3.71	7.63 ± 1.70	10.75 ± 2.73	7.25 ± 1.75
First 8 hr exposure	2.00 ± 0.50	3.25 ± 1.47	4.63 ± 1.58	1.88 ± 0.67
Third 8 hr exposure	2.63 ± 1.70	4.75 ± 1.81	3.00 ± 0.78	1.25 ± 0.73
One-day post-exposure	4.75 ± 2.81	2.75 ± 1.35	4.63 ± 3.09	4.13 ± 1.38
Repetitive inter-trial responses[f]				
One-day pre-exposure	3.63 ± 1.02	5.88 ± 1.33	7.25 ± 1.93	3.25 ± 1.35
First 8 hr exposure	6.13 ± 1.73	3.88 ± 1.22	5.63 ± 1.97	8.38 ± 2.50
Third 8 hr exposure	7.25 ± 1.24	3.25 ± 0.88	2.25 ± 1.52*	1.63 ± 0.98*
One-day post-exposure	6.63 ± 1.94	2.88 ± 0.83	5.13 ± 1.54	2.63 ± 0.68
Trial response latency[g]				
One-day pre-exposure	1.83 ± 0.18	2.25 ± 0.55	2.06 ± 0.40	2.28 ± 0.43
First 8 hr exposure	1.70 ± 0.18	2.38 ± 0.43	2.52 ± 0.40	3.91 ± 0.73*
Third 8 hr exposure	1.91 ± 0.23	2.69 ± 0.69	2.75 ± 0.94	1.82 ± 0.13
One-day post-exposure	1.68 ± 0.16	2.70 ± 0.60	2.18 ± 0.73	1.45 ± 0.06
Standard deviation of response latency				
One-day pre-exposure	2.16 ± 0.38	3.82 ± 1.57	3.33 ± 1.42	4.65 ± 2.23
First 8 hr exposure	2.06 ± 0.38	3.64 ± 1.32	4.19 ± 1.65	7.33 ± 3.43
Third 8 hr exposure	2.74 ± 0.71	4.03 ± 1.50	5.25 ± 3.04	2.34 ± 0.40
One-day post-exposure	1.84 ± 0.38	5.95 ± 2.40	5.88 ± 4.21	1.81 ± 0.38
Latency <2 sec[h]				
One-day pre-exposure	61.75 ± 4.55	70.13 ± 2.23	67.75 ± 66.88	66.88 ± 3.22
First 8 hr exposure	68.50 ± 3.84	69.75 ± 3.75	65.76 ± 3.13	52.13 ± 3.96
Third 8 hr exposure	70.38 ± 4.34	64.13 ± 4.35	74.88 ± 1.75	79.00 ± 2.32
One-day post-exposure	69.38 ± 2.98	67.63 ± 3.20	78.13 ± 3.05	78.00 ± 2.34
Latency >6 sec[i]				
One-day pre-exposure	3.38 ± 0.71	5.38 ± 1.48	4.63 ± 1.15	4.00 ± 1.05
First 8 hr exposure	3.88 ± 0.58	5.00 ± 1.69	6.00 ± 1.34	10.63 ± 1.80*
Third 8 hr exposure	4.25 ± 0.98	5.63 ± 2.44	5.63 ± 1.92	3.13 ± 0.61
One-day post-exposure	2.13 ± 0.67	6.00 ± 1.68	3.38 ± 1.40	1.88 ± 0.35

Table B-37 (Continued): Characteristics and quantitative results for McKee et al. (2010)

Drink response latency[j]				
One-day pre-exposure	0.29 ± 0.01	0.32 ± 0.02	0.38 ± 0.03*	0.33 ± 0.02
First 8 hr exposure	0.26 ± 0.01	0.30 ± 0.02	0.43 ± 0.03*	0.49 ± 0.03*
Third 8 hr exposure	0.30 ± 0.02	0.32 ± 0.03	0.37 ± 0.02	0.34 ± 0.03
One-day post-exposure	0.27 ± 0.01	0.34 ± 0.03	0.36 ± 0.03	0.30 ± 0.02
Health Effect at LOAEL	**NOAEL**		**LOAEL**	
n/a	n/a		n/a	
Comments: This study observed alterations in a number of parameters, including forelimb grip strength, total distance traveled, number of movements, and several visual discrimination performance tests. LOAEL and NOAEL cannot be determined because a dose-response relationship was not apparent. Statistically significant results occurred in a low exposure group and not others, while forelimb grip was found to be significantly increased in the highest exposure group on day 3. Acute duration of exposure (exposure on 3 consecutive days). Generally, acute exposure studies have limited utility in quantitation of human health reference values.				

[a]Total number of trials completed during each session, maximum = 100.
[b]Number of reinforcements obtained divided by the number of reinforcements delivered (×100).
[c]Number of correct trial responses divided by the number of trial responses.
[d]The number of inter-trial intervals in which at least 1 response was made divided by the total number of ITI (×100).
[e]The total number of incorrect trial responses following an initial incorrect response.
[f]The total number of ITI responses following an initial ITI response.
[g]The latency (seconds) to make a correct trial response.
[h]The number of responses within 2 seconds.
[i]The number of responses taking more than 6 seconds.
[j]The mean latency (seconds) to obtain reinforcement.
*Statistically significant from controls at $p < 0.05$.

Source: McKee et al. (2010)

Table B-38. Characteristics and quantitative results for Saillenfait et al. (2005)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Sprague-Dawley rats	F & M	24 dams per dose	Inhalation (6 hr/day GD6–GD20)	0, 100, 300, 600, 900 ppm (0, 492, 1,476, 2,952, or 4,428 mg/m^3) 1,2,4-TMB; 0, 100, 300, 600, 1,200 ppm (0, 492, 1,476, 2,952, or 5,904 mg/m^3) 1,3,5-TMB	Gestational days GD6–GD20

Additional study details

- Animals were exposed to 1,2,4- or 1,3,5-TMB in 200 L glass/steel inhalation chambers for 6 hrs/day starting on GD6 and ending on GD20.
- Animals were randomized and assigned to the experimental groups.
- After GD20, dams were sacrificed and weighed, as were their uteri and any fetuses.
- Decreases in maternal body weight and fetal toxicity were observed.

	Exposure concentration to 1,3,5-TMB				
Observation	**0 ppm**	**100 ppm (492mg/m^3)**	**300 ppm (1,476mg/m^3)**	**600 ppm (2,952 mg/m^3)**	**1,200 ppm (5,904 mg/m^3)**
Maternal parameters					
No. treated	24	24	24	24	24
No. (%) pregnant at euthanization	21 (87.5)	22 (91.7)	21 (87.5)	17 (70.8)	18 (75.0)
No. deaths	0	0	0	0	0
Body weight (g) on day 6	274 ± 17[g]	273 ± 16	274 ± 21	270 ± 17	275 ± 14
Body weight change (g)					
Days 0–6	31 ± 11	31 ± 8	31 ± 7	29 ± 8	28 ± 8
Days 6–13	25 ± 12	29 ± 4	23 ± 6	16 ± 8**	10 ± 7
Days 13–21	110 ± 14	109 ± 10	95 ± 21*	80 ± 20**	63 ± 26**
Days 6–21	135 ± 15	138 ± 11	118 ± 24*	95 ± 24**	73 ± 28**
Corrected weight gain[a]	29 ± 14	30 ± 9	20 ± 12	7 ± 20**	-12 ± 19**
Food consumption (g/day)					
Days 0–6	22 ± 2	22 ± 3	22 ± 2	22 ± 2	23 ± 2
Days 6–13	22 ± 2	22 ± 2	20 ± 1*	18 ± 2**	17 ± 2**
Days 13–21	26 ± 2	25 ± 2	24 ± 2*	21 ± 3**	19 ± 3**
Days 6–21	24 ± 2	24 ± 2	22 ± 2*	20 ± 2**	18 ± 2**

Table B-38 (Continued): Characteristics and quantitative results for Saillenfait et al. (2005)

Observation	Exposure concentration to 1,3,5-TMB				
	0 ppm	100 ppm (492mg/m^3)	300 ppm (1,476mg/m^3)	600 ppm (2,952 mg/m^3)	1,200 ppm (5,904 mg/m^3)
Gestational parameters					
All litters[b]	21	22	21	17	18
No. of corpora lutea per dam	15.3 ± 1.5[g]	15.4 ± 1.7	15.5 ± 1.7	14.9 ± 2.1	15.2 ± 1.5
Mean no. of implantation sites per litter	14.9 ± 1.5	14.9 ± 1.8	14.5 ± 3.4	13.0 ± 5.1	13.6 ± 3.7
Mean % post-implantation loss per litter[c]	4.8 ± 4.2	3.9 ± 4.3	6.8 ± 8.5	1.6 ± 3.7	4.4 ± 6.9
Mean % dead fetuses per litter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean % resorption sites per litter	4.8 ± 4.2	3.9 ± 4.3	6.3 ± 6.5	1.6 ± 3.7	4.4 ± 6.9
Live litters[d]	21	22	21	17	18
Mean no. of live fetuses per litter	14.1 ± 1.6	14.3 ± 1.7	13.4 ± 3.4	12.8 ± 5.0	13.1 ± 3.7
Mean % male fetuses per litter	49.3 ± 13.5	48.2 ± 16.3	52.1 ± 18.1	51.1 ± 20.9	48.5 ± 18.2
Fetal body weight (g)					
All fetuses	5.64 ± 0.35	5.61 ± 0.24	5.43 ± 0.45	5.36 ± 0.68	4.98 ± 0.56**
Male fetuses	5.80 ± 0.41	5.76 ± 0.27	5.50 ± 0.31	5.39 ± 0.55*	5.10 ± 0.57**
Female fetuses	5.50 ± 0.32	5.47 ± 0.21	5.27 ± 0.47	5.18 ± 0.68	4.81 ± 0.45**
Observation	**Exposure concentration to 1,3,5-TMB**				
	0 ppm	**100 ppm (492mg/m^3)**	**300 ppm (1,476mg/m^3)**	**600 ppm (2,952 mg/m^3)**	**1,200 ppm (5,904 mg/m^3)**
Fetal variations and malformations					
Total no. fetuses examined (litters)					
External	297 (21)	314 (22)	282 (21)	217 (17)	236 (18)
Visceral	149 (21)	157 (22)	141 (20)	109 (15)	118 (18)
Skeletal	148 (21)	157 (22)	141 (21)	108 (17)	118 (18)
Malformations					
Diaphragmatic hernia	0	1 (1)	0	1 (1)	0
Multiple skeletal malformations[e]	1 (1)	0	0	0	0
External variations	0	0	0	0	0
Club foot (bilateral)	0	1 (1)	0	0	0
Visceral variations					
Dilated renal pelvis	2 (2)	0	5 (4)	0	2 (2)
Distended ureter	12 (9)	14 (8)	18 (8)	5 (3)	11 (6)

Table B-38 (Continued): Characteristics and quantitative results for Saillenfait et al. (2005)

Skeletal variations					
Fifth sternebrae incomplete ossification or unossified[f]	2 (2)	2 (2)	7 (4)	7 (5)	12 (7)
Fourth sternebrae, split	0	0	0	0	1 (1)
Cervical rib, rudimentary	2 (2)	0	5 (5)	5 (3)	2 (2)
Fourteenth rib, supernumerary	11 (8)	9 (6)	11 (6)	15 (8)	17 (8)
Thoracic vertebra centra, incomplete ossification	10 (5)	8 (6)	10 (7)	9 (7)	9 (7)
	Exposure concentration to 1,2,4-TMB				
Observation	**0 ppm**	**100 ppm (492mg/m^3)**	**300 ppm (1,476mg/m^3)**	**600 ppm (2,952 mg/m^3)**	**900 ppm (4,428 mg/m^3)**
Maternal parameters					
No. treated	25	24	24	24	24
No. (%) pregnant at euthanization	24 (96.0)	22 (91.7)	22 (91.7)	22 (91.7)	24 (100)
No. deaths	0	0	0	0	0
Body weight (g) on day 6	271 ± 18[g]	272 ± 21	272 ± 22	275 ± 19	269 ± 18
Body weight change (g)					
Days 0–6	27 ± 8	28 ± 6	28 ± 7	28 ± 12	24 ± 8
Days 6–13	27 ± 8	27 ± 6	26 ± 6	19 ± 8**	14 ± 12**
Days 13–21	105 ± 28	98 ± 16	100 ± 20	97 ± 17	82 ± 14**
Days 6–21	131 ± 33	124 ± 18	126 ± 24	116 ± 23	95 ± 19**
Corrected weight gain[a]	29 ± 12	31 ± 14	27 ± 12	15 ± 17**	0 ± 14**
Food consumption (g/day)					
Days 0–6	23 ± 2	23 ± 2	23 ± 2	23 ± 3	23 ± 3
Days 6–13	21 ± 3	20 ± 2	20 ± 2	18 ± 2**	17 ± 2**
Days 13–21	26 ± 3	25 ± 2	24 ± 2	23 ± 3**	22 ± 3**
Days 6–21	24 ± 3	23 ± 2	22 ± 2	21 ± 3**	20 ± 2**
	Exposure concentration to 1,2,4-TMB				
Observation	**0 ppm**	**100 ppm (492mg/m^3)**	**300 ppm (1,476mg/m^3)**	**600 ppm (2,952 mg/m^3)**	**900 ppm (4,428 mg/m^3)**
Gestational parameters					
All litters[b]	24	22	22	22	24
No. of corpora lutea per dam	15.4 ± 2.1[g]	15.2 ± 1.3	15.2 ± 2.1	15.8 ± 1.7	15.7 ± 2.5
Mean no. of implantation sites per litter	14.2 ± 3.3	13.7 ± 2.9	14.1 ± 3.2	14.9 ± 2.4	15.0 ± 2.4
Mean % post-implantation loss per litter[c]	10.0 ± 22.1	8.6 ± 8.9	5.8 ± 6.8	5.0 ± 5.7	5.4 ± 6.7
Mean % dead fetuses per litter	0.0 ± 0.0	0.3 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean % resorption sites per litter	10.0 ± 22.1	8.3 ± 9.1	5.8 ± 6.8	5.0 ± 5.7	6.4 ± 6.7

Table B-38 (Continued): Characteristics and quantitative results for Saillenfait et al. (2005)

Live litters[d]	23	22	22	22	24
Mean no. of live fetuses per litter	13.9 ± 2.5	12.5 ± 3.0	13.3 ± 3.2	14.1 ± 2.3	14.3 ± 2.6
Mean % male fetuses per litter	46.6 ± 17.1	46.0 ± 14.1	49.9 ± 13.4	46.2 ± 15.4	50.4 ± 16.2
Fetal body weight (g)					
All fetuses	5.71 ± 0.34	5.64 ± 0.31	5.56 ± 0.47	5.40 ± 0.39*	5.60 ± 0.40**
Male fetuses	5.86 ± 0.34	5.79 ± 0.30	5.72 ± 0.49	5.55 ± 0.48*	5.20 ± 0.42**
Female fetuses	5.57 ± 0.33	5.51 ± 0.31	5.40 ± 0.45	5.28 ± 0.40*	4.92 ± 0.40**
	Exposure concentrations to 1,2,4-TMB				
Observation	**0 ppm**	**100 ppm (492mg/m^3)**	**300 ppm (1,476mg/m^3)**	**600 ppm (2,952 mg/m^3)**	**900 ppm (4,428 mg/m^3)**
Fetal variations and malformations					
Total no. fetuses examined (litters)					
External	319 (23)	275 (22)	293 (22)	310 (22)	342 (24)
Visceral	160 (23)	137 (22)	147 (22)	155 (22)	171 (24)
Skeletal	159 (23)	138 (22)	146 (22)	155 (22)	171 (24)
Malformations					
Diaphragmatic hernia	0	0	1 (1)	0	1 (1)
Multiple skeletal malformations[e]	0	0	0	1 (1)	0
External variations					
Club foot (bilateral)	3 (3)	0	0	0	0
Visceral variations					
Dilated renal pelvis	3 (3)	3 (3)	3 (3)	3 (3)	3 (2)
Distended ureter	7 (4)	5 (3)	8 (5)	8 (5)	2 (2)

Table B-38 (Continued): Characteristics and quantitative results for Saillenfait et al. (2005)

Skeletal variations					
Third sternebrae, incomplete ossification	0	1 (1)	0	0	0
Fifth sternebrae incomplete ossification or unossified[f]	1 (1)	0	4 (4)	5 (4)	6 (6)
Extra ossification site	0	1 (1)	0	0	0
Cervical rib, rudimentary	1 (1)	2 (2)	0	3 (2)	2 (2)
Fourteenth rib, supernumerary	25 (10)	13 (8)	18 (12)	21 (10)	34 (16)
Thirteenth rib, short (unilateral)	1 (1)	0	0	0	0
Thoracic vertebral centra, incomplete ossification	8 (6)	4 (4)	7 (4)	6 (6)	7 (5)

Health Effect at LOAEL	**NOAEL**	**LOAEL**
Maternal toxicity: decrease in maternal body weight and food consumption Developmental toxicity: significant reduction in fetal body weight	Maternal toxicity: 300 ppm (1,476 mg/m^3) for 1,3,5-TMB and 1,2,4-TMB Fetal toxicity: 300 ppm (1,476 mg/m^3) for 1,2,4- and 1,3,5-TMB	Maternal toxicity: 600 ppm (2,952 mg/m^3) for 1,3,5-TMB and 1,2,4-TMB Fetal toxicity: 600 ppm (2,952 mg/m^3) for 1,2,4- and 1,3,5-TMB

Comments: This study observed alterations in a number of maternal and fetal parameters, including decreased maternal and fetal weight. Values reported by authors can be used to determine NOAEL and LOAEL. There was no investigation of pre-implantation developmental toxicity due to 1,2,4-TMB or 1,3,5-TMB exposure. 1,2,3-TMB maternal or developmental toxicity not investigated.

[a]Body weight gain during GD6–GD21 minus gravid uterine weight.
[b]Includes all animals pregnant at euthanization.
[c]Resorptions plus dead fetuses.
[d]Includes all animals with live fetuses at euthanization.
[e]Runt showing skeletal alterations including missing ribs, missing thoracic vertebrae, incomplete ossification of sternebrae and skull bones.
[f]Unossified = alizarine red S negative.
[g]Mean ± SD.
*, ** Statistically significant from controls at $p < 0.05$ and 0.01, respectively.

Source: Saillenfait et al. (2005)

Table B-39. Characteristics and quantitative results for Tomas et al. (1999a)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
WAG/Rij Rats	M	6 rats per dose	Oral (gavage, in olive oil)	0, 2, 8, or 32 mmol/kg BW (240, 960, 3,840 mg/kg BW). 1,2,3-, 1,2,4-, and 1,3,5-TMB	Acute

Additional study details

- 1,2,3-, 1,2,4-, and 1,3,5-TMB were tested for their effects on electrocortical arousal by an electrocardiogram before and after oral administration (in olive oil) of 0, 0.002, 0.008, or 0.032 mol/kg BW of each isomer.
- Solvent concentration in peripheral blood was determined via head space gas chromatography.
- All three TMB isomers were found to cause a slight increase in locomotor activity.

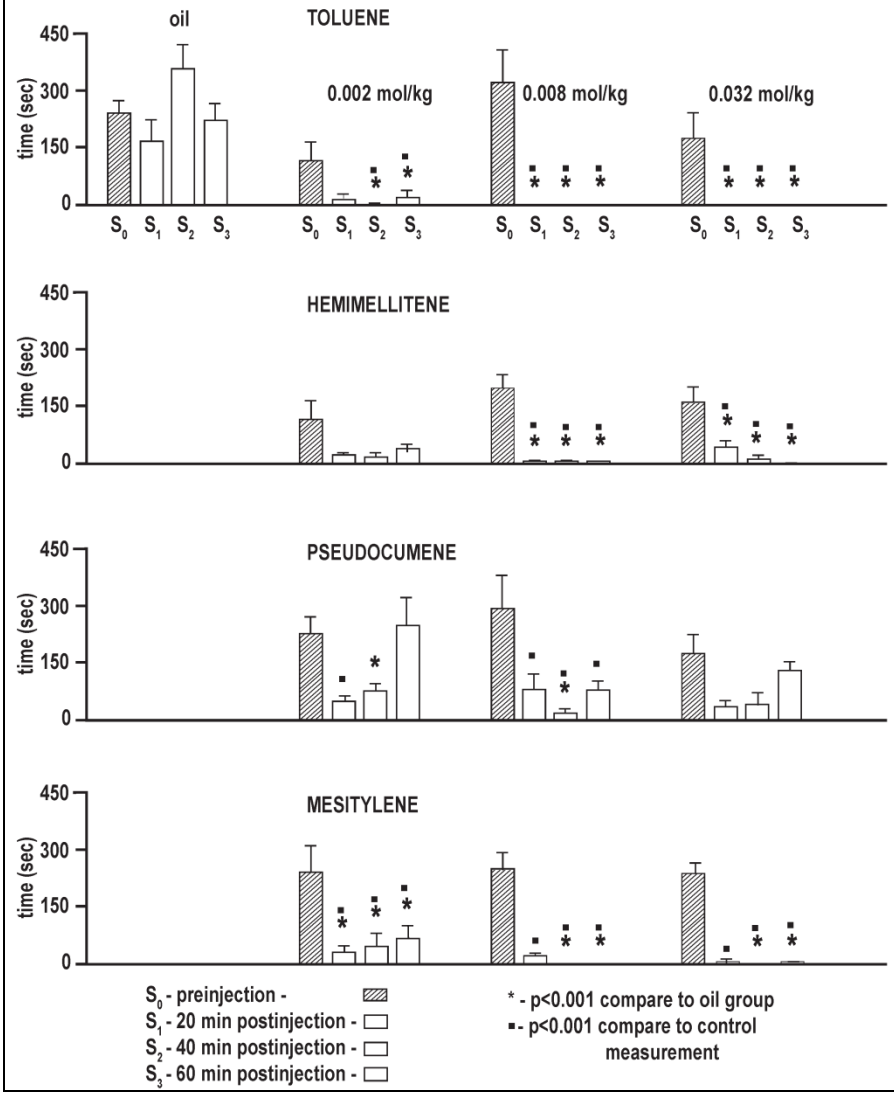


Figure 1. Changes in total duration of high-voltage spindle episodes following acute exposure to toluene and 1,2,3-, 1,2,4-, or 1,3,5-TMB at doses of 0.002, 0.008, and 0.032 mol/kg.

Source: Reproduced from Tomas et al. (1999a)

Table B-39 (Continued): Characteristics and quantitative results for Tomas et al. (1999a)

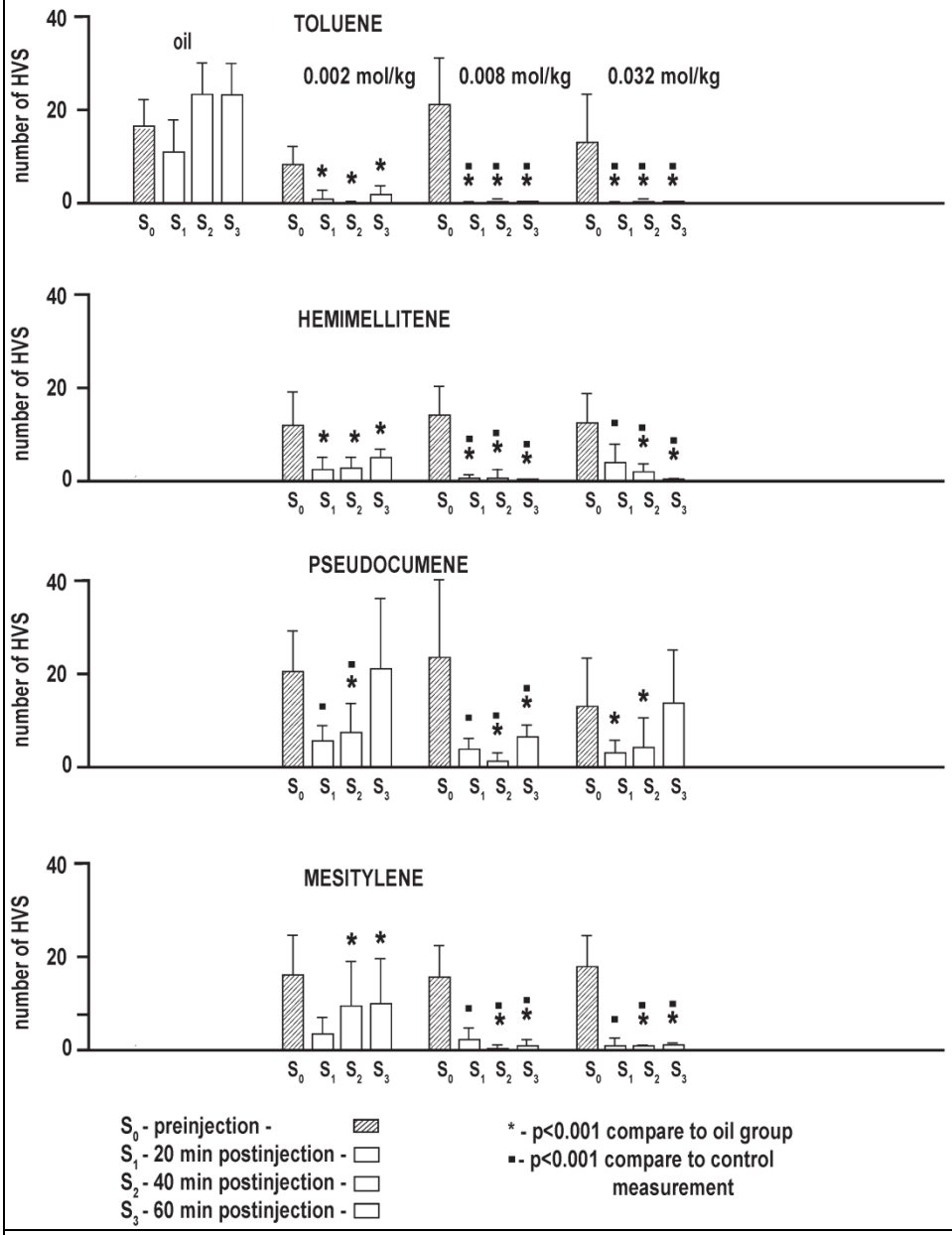


Figure 2. Changes in number of high-voltage spindle episodes following acute exposure to toluene and 1,2,3-, 1,2,4-, and 1,3,5-TMB at doses of 0.002, 0.008, and 0.032 mol/kg.

Source: Reproduced from Tomas et al. (1999a)

Health Effect at LOAEL	NOAEL	LOAEL
Abnormal electrocortical stimulation	n/a	2 mmol/kg 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB

Comments: Exposures were of an acute duration, and therefore not suitable for reference value derivation. However, qualitatively, this study provided evidence of CNS disturbances that, when considered together with short-term and subchronic neurotoxicity studies, demonstrate that TMB isomers perturb the CNS of exposed animals.

Source: Tomas et al. (1999a)

Table B-40. Characteristics and quantitative results for Tomas et al. (1999b)

Study design

Species	Sex	N	Exposure route	Dose range	Exposure duration
WAG/Rij rats	M	10 rats per dose	Oral (in olive oil)	0, 8, 16, or 32 mmol/kg BW (960, 1,920, or 3,850 mg/kg BW) 1,2,4-TMB, 1,2,3-TMB, or 1,3,5-TMB	Acute

Additional study details

- 1,2,3-, 1,2,4-, and 1,3,5-TMB were tested for their effects on locomotor activity by an open field test following oral administration (in olive oil) of 0, 8, 16, or 32 mmol/kg BW of all isomers.
- All three TMB isomers were found to cause a slight increase in locomotor activity.

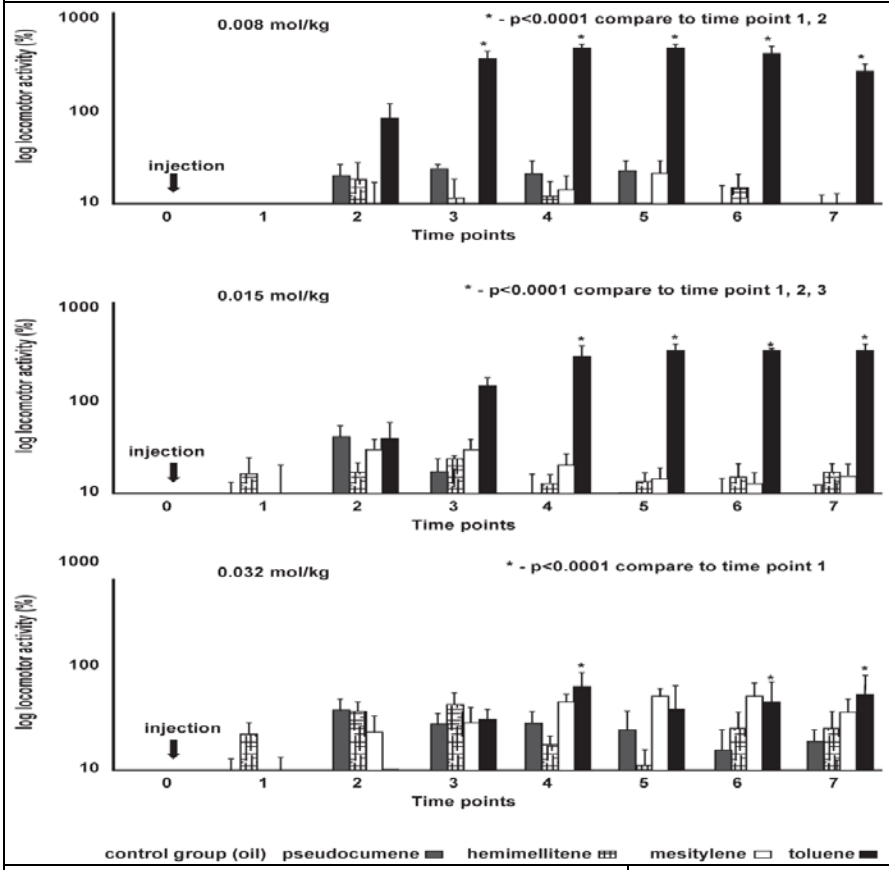


Figure 1. Locomotor activity following acute exposure to toluene and TMB isomers at doses of 0.008 mol/kg, 0.016 mol/kg, and 0.032 mol/kg.

Source: Reproduced from Tomas et al. (1999b)

Health Effect at LOAEL	NOAEL	LOAEL
Increased locomotor activity	16 mmol/kg 1,2,3-TMB 16 mmol/kg 1,2,4-TMB 8 mmol/kg 1,3,5-TMB	32 mmol/kg 1,2,3-TMB 32 mmol/kg 1,2,4-TMB 16 mmol/kg 1,3,5-TMB

Comments: Exposures were of an acute duration, and therefore not suitable for reference value derivation. However, qualitatively, this study provided evidence of CNS disturbances that, when considered together with short-term and subchronic neurotoxicity studies, demonstrate that TMB isomers perturb the CNS of exposed animals.

Source: Tomas et al. (1999b)

Table B-41. Characteristics and quantitative results for Tomas et al. (1999c)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	4 rats per dose	i.p. injection	6.6 mmol/kg BW 1,2,3-, 1,2,4-, and 1,3,5-TMB	Acute

Additional study details

- 1,2,3-, 1,2,4-, and 1,3,5-TMB were tested for their effects on the CNS by monitoring evoked hippocampal and cortical activity following i.p. injection of 6.6 mmol/kg BW of any isomer.
- Solvent concentration in peripheral blood was determined via head space gas chromatography.
- Significant differences in hippocampal and cortical activity occurred following injection.

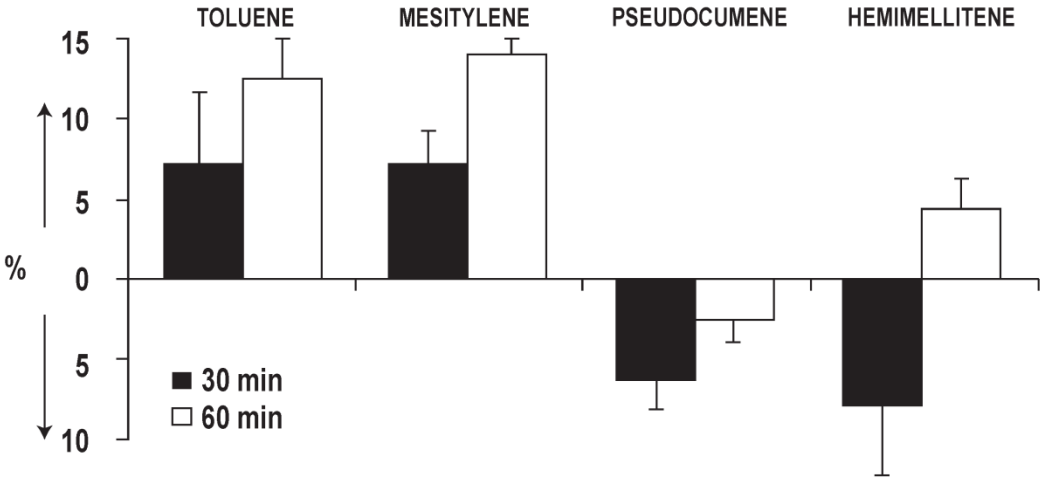


Figure 1. Amplitude abnormalities of the cortical N1 wave 30 and 60 min after i.p. solvent injection.

Source: Reproduced from Tomas et al. (1999c)

Table B-41 (Continued): Characteristics and quantitative results for Tomas et al. (1999c)

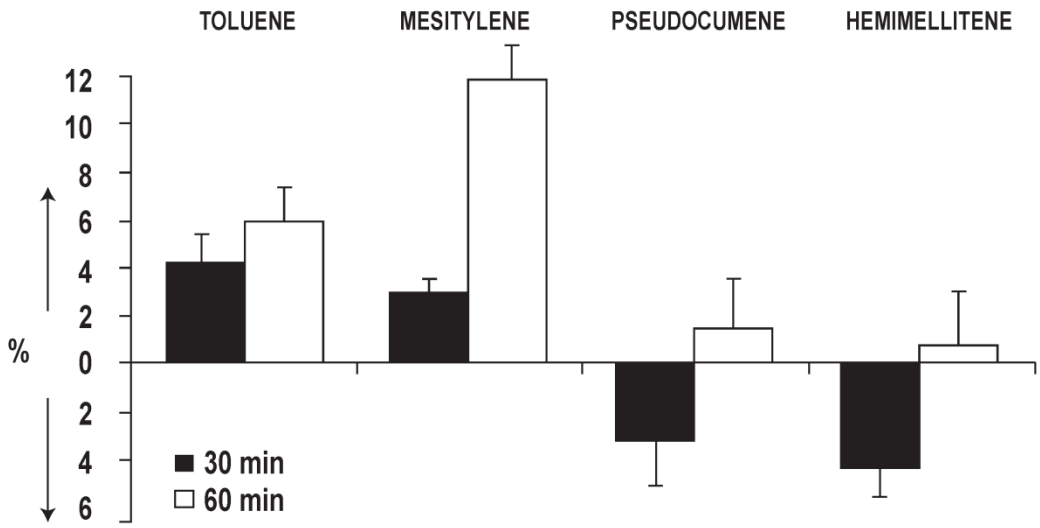


Figure 2. Amplitude abnormalities of the cortical P1–N1 wave 30 and 60 minutes after i.p. solvent injection.

Source: Reproduced from Tomas et al. (1999c)

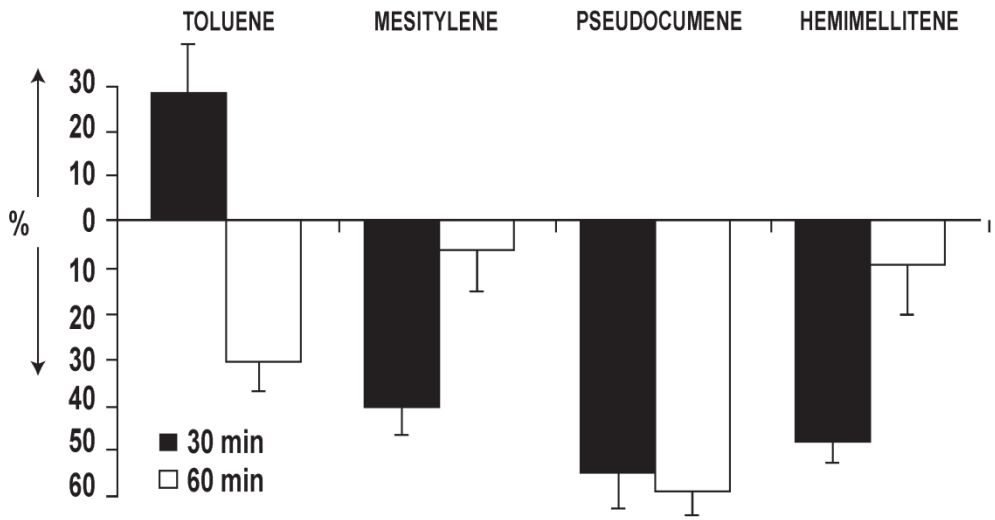


Figure 3. Amplitude abnormalities of the hippocampal N1 wave 30 and 60 min after i.p. solvent injection.

Source: Reproduced from Tomas et al. (1999c)

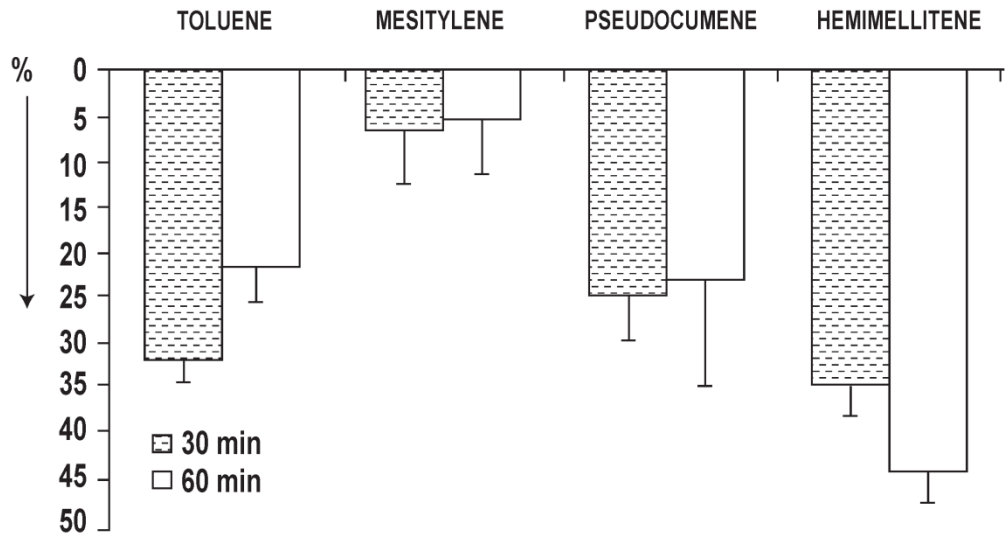


Figure 4. The effect of i.p. solvent injection on the cortical EEG in the 13–20.75 Hz frequency band.

Source: Reproduced from Tomas et al. (1999c)

Table B-41 (Continued): Characteristics and quantitative results for Tomas et al. (1999c)

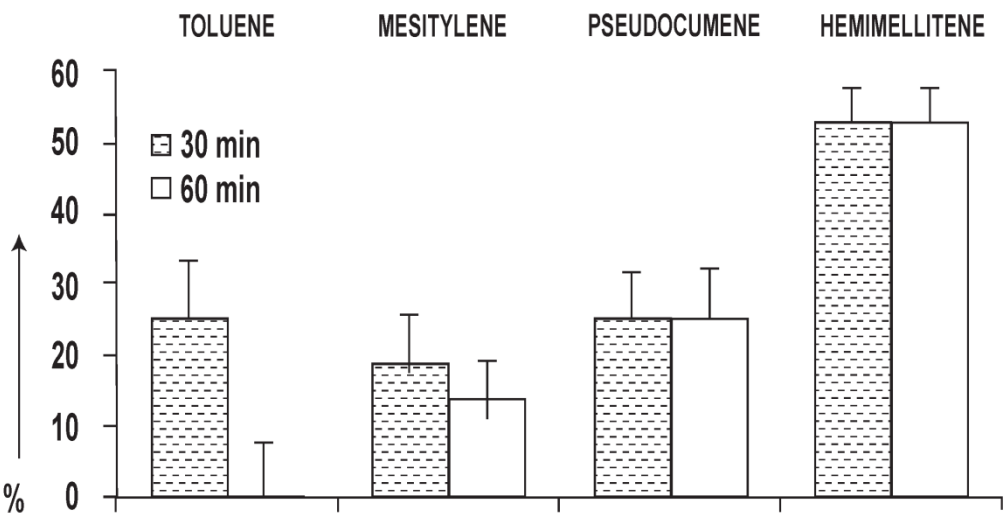


Figure 5. The effect of i.p. solvent injection on the hippocampal EEG in the 1–3.75 Hz frequency band.

Source: Reproduced from Tomas et al. (1999c)

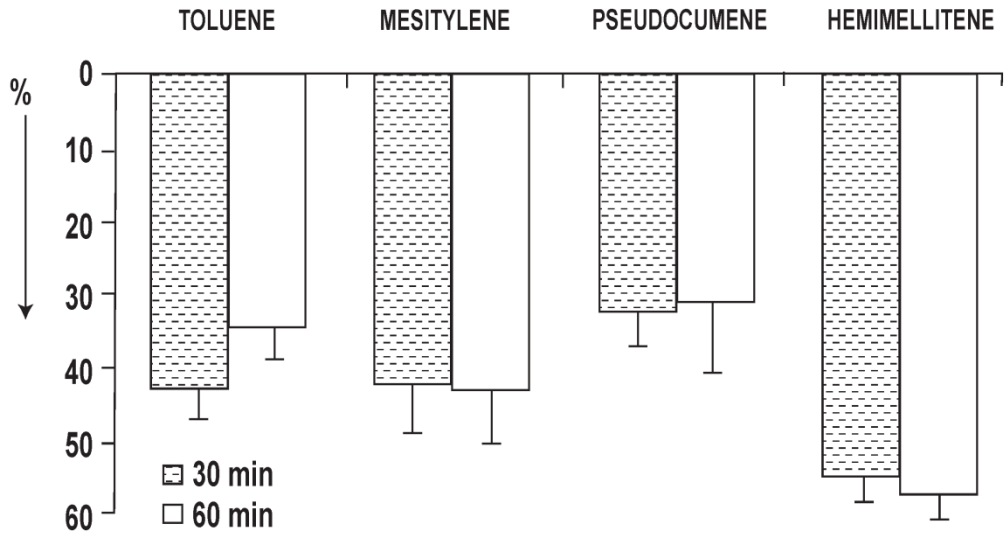


Figure 6. The effect of i.p. solvent injection on the hippocampal EEG in the 7–9.75 Hz frequency band.

Source: Reproduced from Tomas et al. (1999c)

Health Effect at LOAEL	NOAEL	LOAEL
n/a (acute exposure study, one dose level)	n/a	6.6 mmol/kg 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB

Comments: Unable to quantify dose-response relationship from data because only one dose group used. Exposures were of an acute duration, and therefore not suitable for reference value derivation. However, qualitatively, this study provided evidence of CNS disturbances that, when considered together with short-term and subchronic neurotoxicity studies, demonstrate that TMB isomers perturb the CNS of exposed animals.

Source: Tomas et al. (1999c).

Table B-42. Characteristics and quantitative results for Wiaderna et al. (1998)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	13 or 14 rats/ dose	Inhalation (6 hr/day, 5 days/week)	0 or 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,3-TMB	4 weeks

Additional study details

- Animals were exposed to 1,2,3-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/day, 5 days/week for 4 weeks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Rats were tested with a variety of behavioral tests, including radial maze performance, open field activity, passive avoidance, and active two-way avoidance.
- Tests were performed on days 14–18 following exposure.
- Neurobehavioral effects were observed at 25 and 100 ppm (123 and 492 mg/m^3) concentrations, but not at 250 ppm (1,230 mg/m^3).

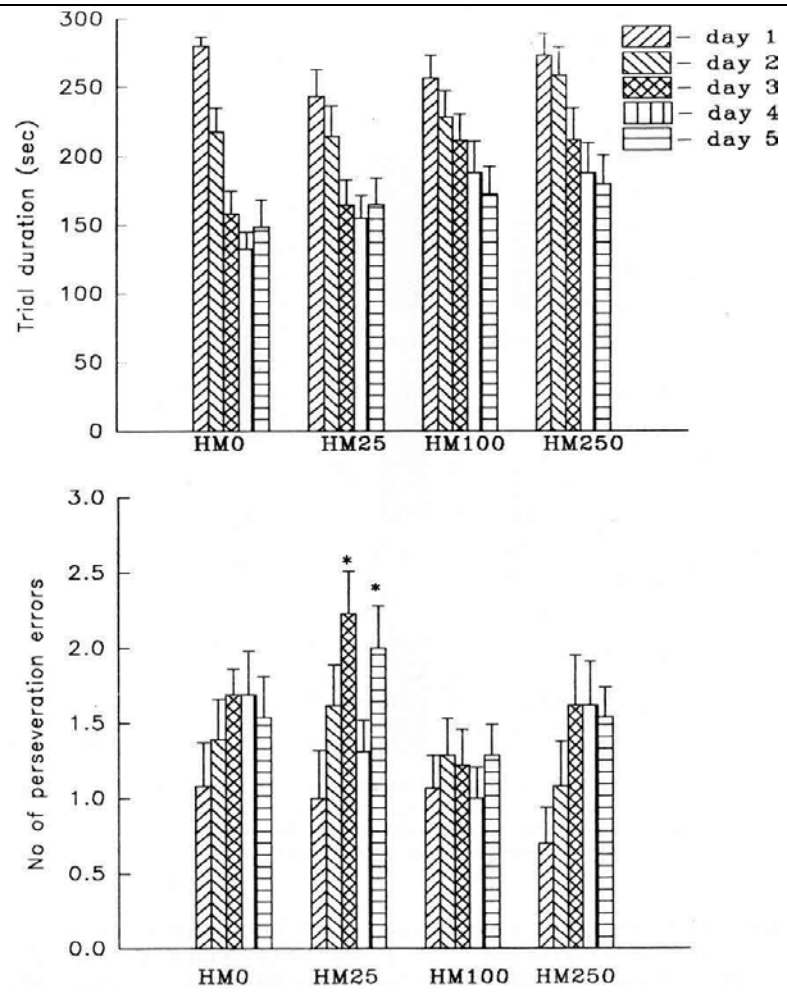


Figure 1. Radial maze performance of rats exposed for 4 weeks to 1,2,3-TMB.

The test (one trial a day) was performed on days 14–18 after exposure. Upper diagram: changes in trial duration, i.e., the time of successive eight arm entries, during successive days of training. Lower diagram: number of perseveration errors in successive daily trials.

Denotation of groups: HM0-sham exposed group (n = 13), HM25, HM100, HM250-groups exposed to 1,2,3-TMB at concentrations of 25 ppm (123 mg/m^3, n = 13), 100 ppm (492 mg/m^3, n = 14), and 250 ppm (1,230 mg/m^3, n = 13) respectively. Bars represent group means and standard error.
* $p < 0.05$ compared to trial 1 in the same group.

Source: Wiaderna et al. (1998)

Table B-42 (Continued): Characteristics and quantitative results for Wiaderna et al. (1998)

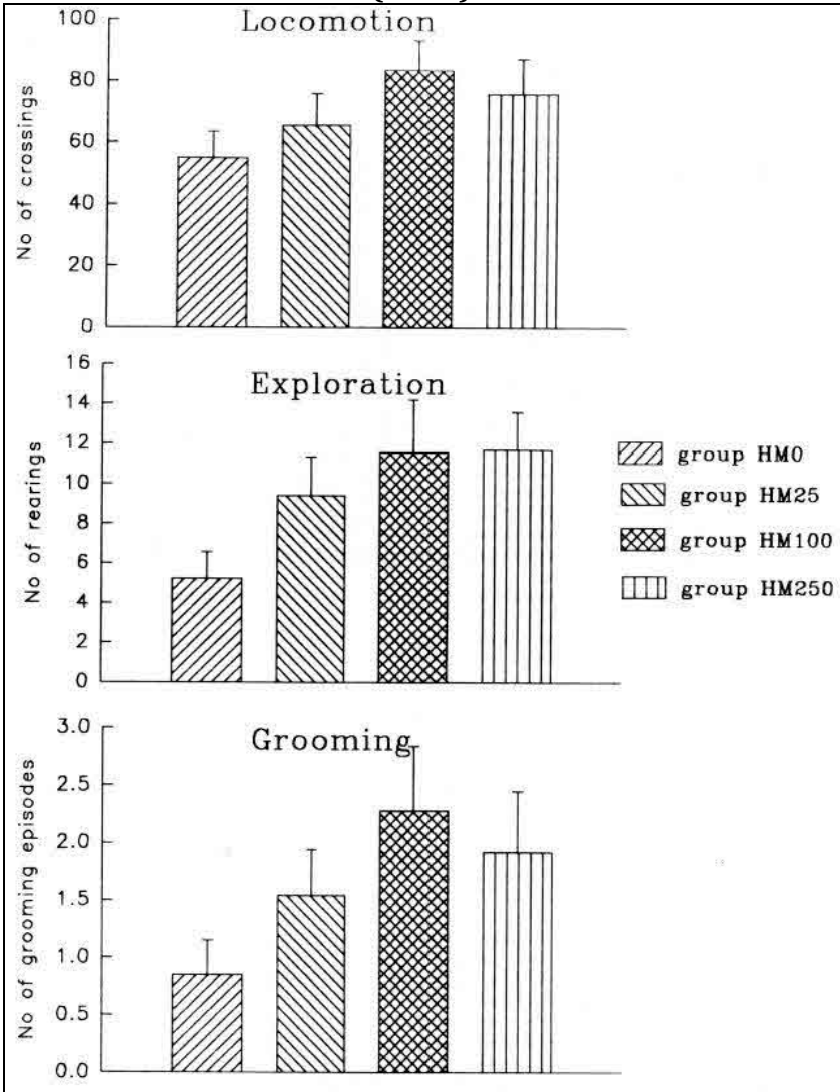


Figure 2. A comparison of spontaneous locomotor (upper diagram), exploratory (middle diagram), and grooming (lower diagram) activity of rats in an open field during a 5-min observation period.

The test was performed 25 days after a 4-week exposure to 1,2,3-TMB. Denotation of groups as in Figure 1 (above). The bars represent group means and SE.
Source: Wiaderna et al. (1998)

Table B-42 (Continued): Characteristics and quantitative results for Wiaderna et al. (1998)

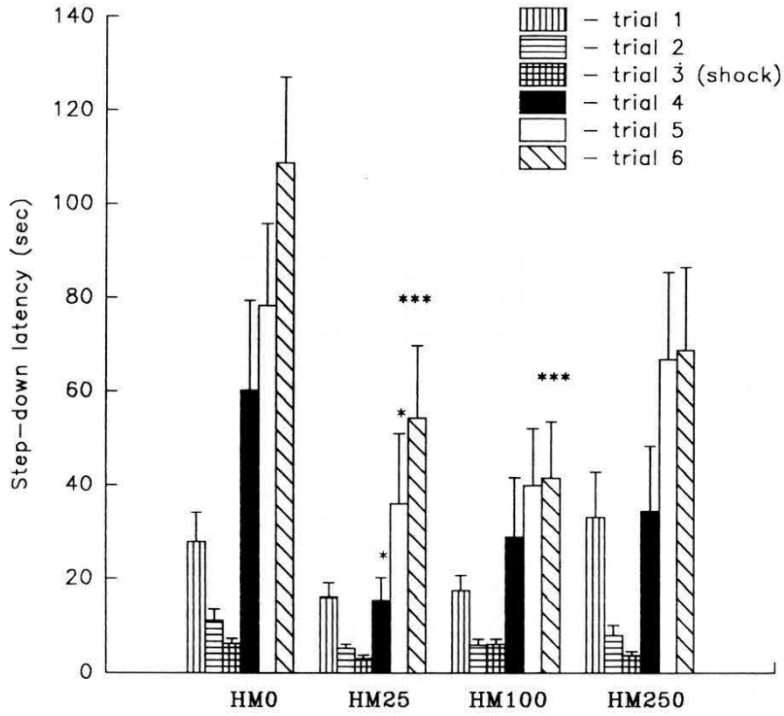


Figure 3. Diagrams illustrating the effect of a 4-week exposure to 1,2,3-TMB on the step-down passive avoidance learning in rats.

The test was performed on days 39–48 after exposure. Trials 1, 2, and 3 were performed at 24 hr intervals. The step-down response was punished by a 10 sec footshock in trial 3 only. Trials 4, 5, and 6 were performed 24 hr, 3 days, and 7 days after trial 3, respectively. The maximum step-down latency was 180 sec. Denotations of groups as in Figure 1 (above). The bars represent group means and SE
*, *** $p < 0.05$ and $p < 0.001$, compared with respective data from control group. Source: Wiaderna et al. (1998)

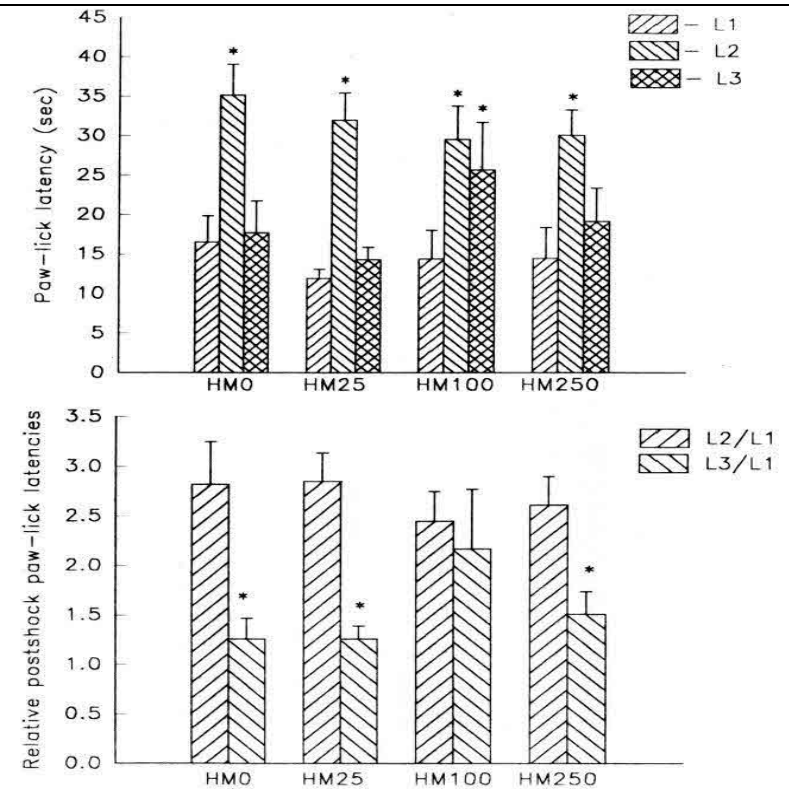


Figure 4. Hot-plate behavior tested in rats on day 50 (trials 1 and 2) and day 51 (trial 3) after a 4-week exposure to 1,2,3-TMB.

Denotation of groups as in Figure 1 (above). The bars represent group means and SE. Upper diagram: A comparison of the latency of the paw-lick response to a thermal stimulus (54.5°C) on day 50. L1-paw-lick latency in trial 1 performed before a 2 min intermittent footshock. L2-paw-lick latency in trial 2 performed several seconds after the footshock. L3-paw-lick latency in trial 3 performed 24 hr after the footshock
* $p < 0.05$ compared to L2/L1 of the same group.
Source: Wiaderna et al. (1998)

Table B-42 (Continued): Characteristics and quantitative results for Wiaderna et al. (1998)

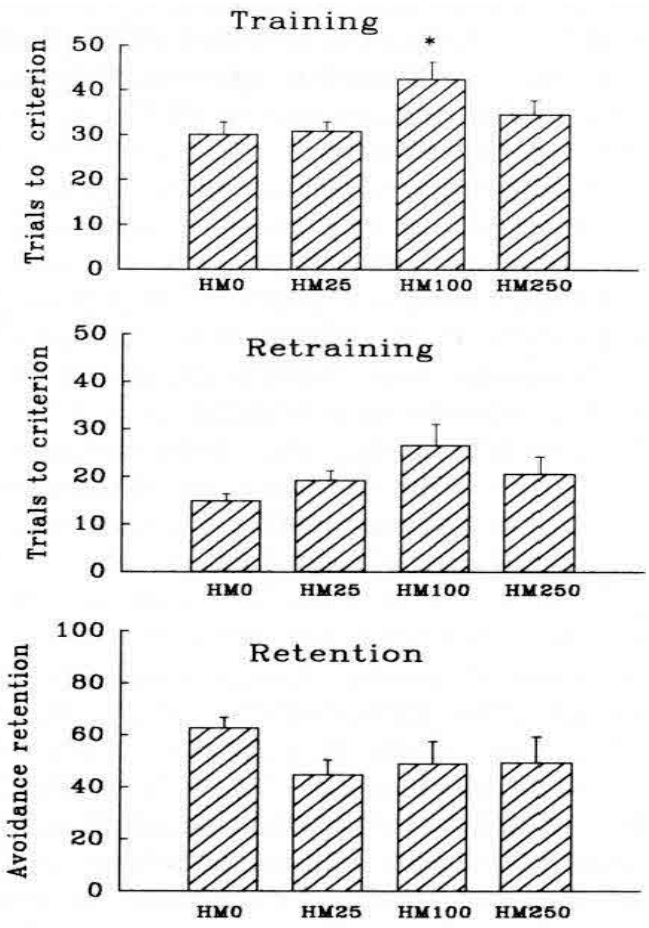


Figure 5. Active avoidance learning and retention in rats after a 4-week exposure to 1,2,3-TMB.

Upper and middle diagrams: comparisons of the number of trials to attain an avoidance criterion (four avoidance responses during five successive trials) during the training (upper diagram and retraining (middle diagram) session). Lower diagram: a retention score calculated according to the formula: %Ret = (1 – Resc/Tesc) × 100, where Resc and Tesc are numbers of escape responses during retraining and training, respectively. Denotation of groups as in Figure 1 (above). The bars represent group means and SE.

* $p < 0.05$ compared to control group.

Source: Wiaderna et al. (1998)

Health Effect at LOAEL	NOAEL	LOAEL
Impaired learning of passive avoidance	n/a	25 ppm (123 mg/m^3)

Comments: CNS disturbances were observed up to 2 months after termination of exposure, indicating the persistence of effects after metabolic clearance of 1,2,3-TMB from the test animals. No effects were observed in the 250 ppm (1,230 mg/m^3) exposure group. Duration of exposure only 4 weeks. Generally, short-term exposure studies have limited utility in quantitation of human health reference values.

Source: Wiaderna et al. (1998)

Table B-43. Characteristics and quantitative results for Wiaderna et al. (2002)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
LOD: Wistar rats	MM	12 rats per dose	Inhalation (6 hr/day, 5 days/week)	0 or 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,3-TMB	4 weeks

Additional study details

- Animals were exposed to 1,3,5-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/day, 5 days/week for 4 weeks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Rats were tested with a variety of behavioral tests, including radial maze performance, open field activity, passive avoidance, active two-way avoidance, and shock-induced changes in pain sensitivity.
- 1,3,5-TMB-exposed rats showed alterations in performance in spontaneous locomotor activity, active and passive avoidance learning, and paw-lick latencies.

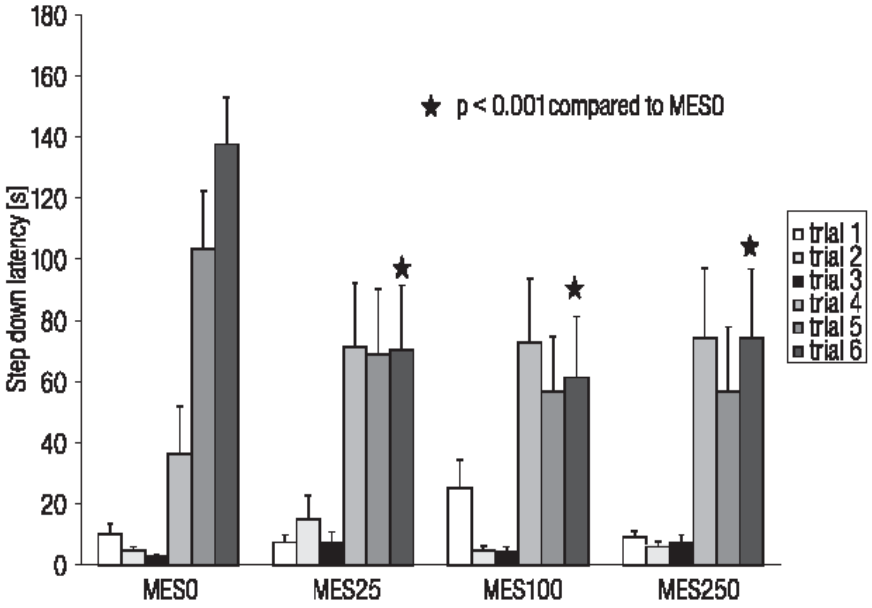


Figure 1. Passive avoidance. The comparison of the time of staying on the platform in the consecutive test trials.

The test was performed between days 35 and 45 after the exposure to 1,3,5-TMB. Leaving the platform in trial 3 was punished by an electric shock. Trials 1, 2, 3, and 4 were performed at 24 hr intervals, while trials 5 and 6 were effected 3 and 7 days after trial 3, respectively.
The bars represent group means and SE.
Source: Wiaderna et al. (2002)

Table B-43 (Continued): Characteristics and quantitative results for Wiaderna et al. (2002)

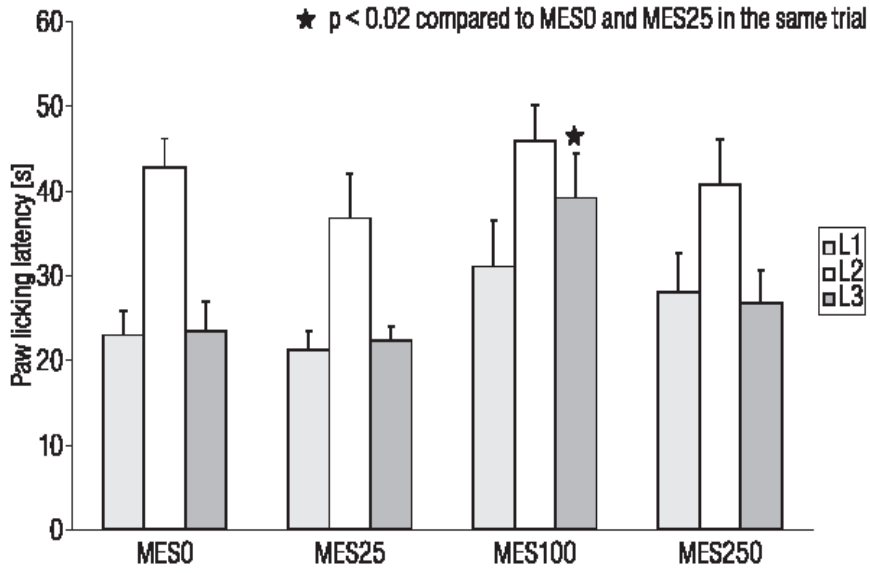


Figure 2. Hot plate. The comparison of latency of the reaction (paw-lick) to the thermal stimulus before (L1), immediately after (L2) and 24 hr after (L3) intermittent 2 min electric shock in rats exposed to 1,3,5-TMB.

The test was performed on days 50 and 51 after the exposure.
The bars represent group means and SE.
Source: Wiaderna et al. (2002)

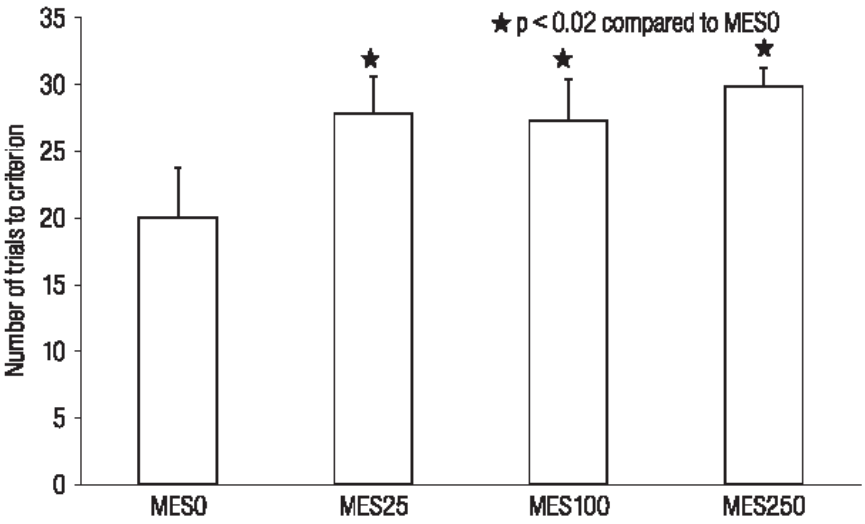


Figure 3. Active avoidance. The comparison of the rat groups exposed to 1,3,5-TMB for the number of trials (attempts) required to reach the avoidance criterion (4 shock avoidances) in 5 consecutive trials (attempts) during the training session.

The test was performed on day 54 (training) and day 60 (retraining) after the exposure.
The bars represent group means and SE. Source: Wiaderna et al. (2002)

Health Effect at LOAEL	NOAEL	LOAEL
Shorter retention of passive avoidance reaction	n/a	25 ppm (123 mg/m^3)

Comments: This study observed alterations in a number of behavioral tests. Values reported by authors can be used to determine LOAEL and NOAEL. CNS disturbances observed up to 2 months after termination of exposure, indicating the persistence of effects following metabolic clearance of 1,3,5-TMB from the test animals. Unable to quantify dose-response relationship from data because responses either equal at all exposure concentrations or elevated only at one exposure concentration. Duration of exposure only 4 weeks. Generally, short-term exposure studies have limited utility in quantitation of human health reference values.

Source: Wiaderna et al. (2002).

Table B-44. Characteristics and quantitative results for Wiglusz et al. (1975b)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	5–8 per dose	Inhalation	0, 1.5, 3.0, or 6.0 mg/L (0, 1,500, 3,000, or 6,000 mg/m^3) 1,3,5-TMB	Acute study: 6 hrs Short-term study: 6 hrs/day, 6 days/week for 5 weeks

Additional study details

- Male Wistar rats were exposed in a short-term study to 0, 1.5, 3.0, or 6.0 mg/L 1,3,5-TMB.
- In a separate chronic study, male Wistar rats were exposed to 3.0 mg/L 1,3,5-TMB for 6 hrs/day, 6 days/week, for 5 weeks.
- Rats weighed 240–280 g and were housed in stainless steel wire mesh cages, with food and water provided ad libitum.
- Blood samples were collected for 3 days before exposure then on days 1, 7, 14, and 28.

	1,3,5-TMB exposure concentration (mg/L)— hematological parameters following single 6 hour exposure			
Observation	**0**	**1.5**	**3.0**	**6.0**
Hemoglobin in g% (mean ± SD)				
Day 0	14.1 ± 1.3	15.2 ± 0.3	15.0 ± 0.8	14.2 ± 1.1
Day 1	--	--	14.8 ± 1.0	13.9 ± 2.1
Day 7	--	14.0 ± 0.5	13.5 ± 0.5	13.5 ± 0.8
Day 14	15.1 ± 0.8	14.6 ± 0.5	13.6 ± 0.6	13.1 ± 0.4
Day 28	14.8 ± 0.5	14.9 ± 0.7	13.6 ± 0.8	14.8 ± 0.4
Million erythrocytes per mm^3 serum (mean ± SD)				
Day 0	4.91 ± 0.19	5.35 ± 0.09	4.96 ± 0.15	5.51 ± 0.17
Day 1	--	--	5.32 ± 0.02	5.31 ± 0.11
Day 7	--	5.18 ± 0.18	4.93 ± 0.16	4.89 ± 0.17
Day 14	5.37 ± 0.90	4.99 ± 0.11	5.09 ± 0.10	4.77 ± 0.10
Day 28	5.17 ± 0.18	5.26 ± 0.07	5.12 ± 0.10	5.20 ± 0.27
Thousand leukocytes per mm^3 serum (mean ±SD)				
Day 0	11.08 ± 3.14	12.26 ± 3.50	13.01 ± 3.10	8.90 ± 3.88
Day 1	--	--	11.38 ± 1.37	8.24 ± 3.88
Day 7	--	11.70 ± 2.97	11.66 ± 1.50	12.32 ± 5.01
Day 14	8.0 ± 2.16	12.06 ± 3.33	11.70 ± 1.05	10.68 ± 1.21
Day 28	6.83 ± 1.27	11.50 ± 10.48	11.96 ± 1.16	9.92 ± 2.42

Table B-44 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975b)

Percent segmented neutrophilic granulocytes (mean ± SD)				
Day 0	8.5 ± 4.1	13.5 ± 3.6	18.5 ± 2.3	16.6 ± 2.8
Day 1	--	--	22.5 ± 5.4	53.6 ± 22.5
Day 7	--	20.2 ± 6.04	31.3 ± 10.3	26.7 ± 12.5
Day 14	10.6 ± 2.5	12.2 ± 5.9	30.1 ± 6.2	20.6 ± 23.7
Day 28	15.6 ± 6.3	12.5 ± 6.4	35.0 ± 6.7	15.8 ± 3.8
Percent bacciliform neutrophilic granulocytes (range)				
Day 0	0.6 (0–1)	0.0	0.0	0.0
Day 1	--	--	0.0	0.0
Day 7	--	0.0	0.0	0.0
Day 14	0.0	0.16 (0–1)	0.0	0.0
Day 28	0.0	1 (0–2)	0.0	0.0
Percent acidophilic granulocytes (mean ± SD)				
Day 0	1.1 ± 0.7	2.6 ± 1.9	0.5 ± 0.5	1.8 ± 1.7
Day 1	--	--	0.0	0.14 ± 0.3
Day 7	--	1.1 ± 1.1	3.1 ± 0.5	0.0
Day 14	2.8 ± 1.3	5.1 ± 3.2	4.8 ± 1.0	2.6 ± 2.6
Day 28	4.1 ± 2.9	3.1 ± 1.7	6.0 ± 4.1	2.2 ± 2.8
Percent lymphocyte (mean ± SD)				
Day 0	88.6 ± 4.4	82.8 ± 4.8	67.8 ± 2.3	79.4 ± 4.3
Day 1	--	--	73.3 ± 5.4	44.0 ± 21.3
Day 7	--	77.6 ± 4.8	65.0 ± 7.9	71.2 ± 12.5
Day 14	85.4 ± 1.5	82.0 ± 3.8	64.3 ± 5.8	75.0 ± 23.0
Day 28	78.6 ± 8.3	81.8 ± 7.6	57.1 ± 4.1	81.2 ± 5.8
Percent monocyte (mean ± SD)				
Day 0	1.6 ± 0.8	1.0 ± 0.6	1.1 ± 0.9	2.2 ± 1.0
Day 1	--	--	1.1 ± 0.4	2.3 ± 1.8
Day 7	--	0.8 ± 1.1	0.3 ± 0.5	1.7 ± 1.9
Day 14	0.8 ± 0.4	0.6 ± 0.5	0.3 ± 0.8	1.2 ± 0.4
Day 28	1.6 ± 1.0	1.6 ± 1.0	1.6 ± 1.2	1.0 ± 0.8

Table B-44 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975b)

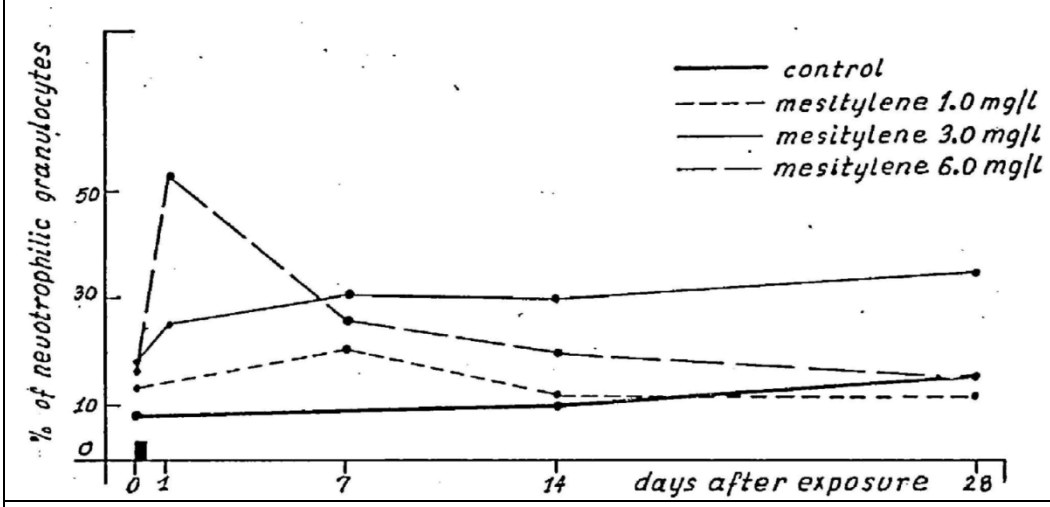


Figure 1. Percentage of segmented neutrophilic granulocytes after 6 hrs exposure to 1,3,5-TMB.

Source: Wiglusz et al. (1975b)

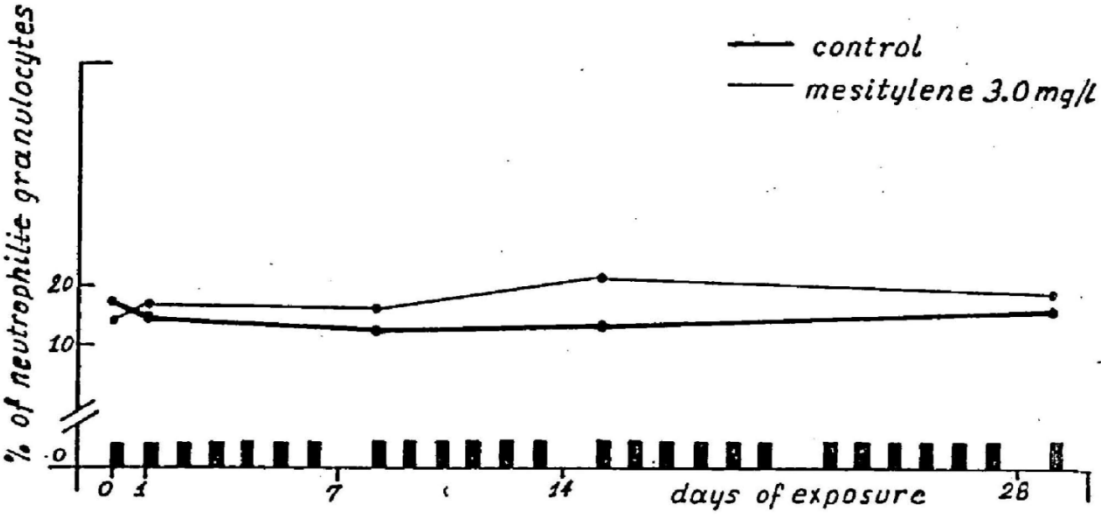


Figure 2. Percentage of segmented neutrophilic granulocytes during exposure to 1,3,5-TMB 3.0 mg/L for 6 hrs/day, 6 days/week, for 5 weeks.

Source: Wiglusz et al. (1975b)

Observation	Hematological parameters during 5 week exposure to 1,3,5-TMB (means ± SD)				
	Day 0	Day 1	Day 7	Day 14	Day 28
Hemoglobin in g%					
Control group	13.0 ± 4.7	14.6 ± 2.5	14.6 ± 2.5	15.6 ± 3.2	14.2 ± 5.0
1,3,5-TMB group	14.6 ± 0.7	15.5 ± 0.6	14.8 ± 1.1	14.5 ± 0.9	13.8 ± 0.5
Million erythrocytes per mm^3 Serum					
Control group	5.42 ± 0.78	6.12 ± 04	6.40 ± 0.25	6.46 ± 0.39	6.18 ± 0.61
1,3,5-TMB group	6.08 ± 1.18	6.35±0.38	6.11 ± 0.63	5.74 ± 1.1	5.05 ± 2.2
Thousand leukocytes per mm^3 Serum					
Control group	10.63 ± 4.27	13.66 ± 2.91	11.13 ± 2.52	14.53 ± 2.64	11.46 ± 2.74
1,3,5-TMB group	13.76 ± 3.70	11.43 ± 4.0	9.53 ± 2.55	12.23 ± 4.04	13.40 ± 5.18
% Segmented neutrophilic Granulocytes					
Control group	17.1 ± 11.9	14.5 ± 8.1	12.1 ± 2.5	13.6 ± 6.3	15.6 ± 3.2
1,3,5-TMB group	14.0 ± 5.0	17.0 ± 9.4	16.6 ± 5.0	21.5 ± 7.4	18.4 ± 8.6

Table B-44 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975b)

% Bacciliform neutrophilic granulocytes					
Control group	0.83 (1–2)	0.66 (1–2)	1.33 (1–3)	1.33 (1–2)	1.0 (0–1)
1,3,5-TMB group	0.6 (1–2)	0.4 (0–1)	1 (1–2)	1.8 (2–5)	1.4 (1–2)
% Acidophilic granulocytes					
Control group	1 (1–4)	2.1 (1–4)	3.3 (1–7)	1.8 (1–4)	1.6 (1–4)
1,3,5-TMB group	1.5 (1–3)	1.0 (1–3)	0.8 (1–2)	1.0 (1–2)	0.8 (0–1)
% Lymphocyte					
Control group	79.6 ± 11.7	81.6 ± 8.6	81.8 ± 4.7	81.1 ± 5.2	80.0 ± 2.4
1,3,5-TMB group	79.8 ± 5.5	81.0 ± 7.7	80.5 ± 6.5	74.0 ± 9.4	77.2 ± 8.4
% Monocyte					
Control group	1.1 (1–3)	1.0 (0–2)	1.5 (1–4)	1.0 (1–2)	1.5 (1–3)
1,3,5-TMB group	0.6 (1–3)	0.8 (1–2)	0.8 (1–2)	1.3 (1–3)	2.7 (2–4)

Health Effect at LOAEL	**NOAEL**	**LOAEL**
Increase in percent segmented neutrophilic granulocytes	1.5 mg/L	3.0 mg/L

Comments: This study slight increases in percent segmented neutrophilic granulocytes on day 14 of the short-term exposure study. Authors do not report statistical significance of results. Only one dose group used in chronic study.

Source: Wiglusz et al. (1975b)

Table B-45. Characteristics and quantitative results for Wiglusz et al. (1975a)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	6/dose	Inhalation	0, 0.3, 1.5, or 3.0 mg/L (0, 300, 1,500, or 3,000 mg/m^3) 1,3,5-TMB	Acute study: 6 hrs Short-term study: 6 hrs/day, 6 days/week for 5 weeks

Additional study details

- Male Wistar rats were exposed in a short-term study to 0, 0.3, 1.5, or 3.0 mg/L 1,3,5-TMB.
- In a separate chronic study, male Wistar rats were exposed to 3.0 mg/L 1,3,5-TMB for 6 hrs/day, 6 days/week, for 5 weeks.
- Rats weighed 240–280 g and were housed in stainless steel wire mesh cages, with food and water provided ad libitum.
- Blood samples were collected for 3 days before exposure then on days 1, 7, 14, and 28.

	1,3,5-TMB exposure concentration (mg/L)—hematological parameters following single 6 hour exposure (means ± SE)			
Observation	**0**	**0.3**	**1.5**	**3.0**
Aspartate amino transferase activity				
Day 0	79.0 ± 7.9	78.0 ± 7.7	75.3±7.3	81.6 ± 4.2
Day 2	81.8 ± 6.2	90.0 ± 5.7	71.8±3.3	74.6 ± 4.5
Day 7	82.2 ± 4.3	76.8 ± 4.2	71.2±2.2	84.1 ± 5.6
Day 14	82.6 ± 8.5	73.0 ± 4.2	76.3±6.7	76.1 ± 3.9
Day 28	79.6 ± 7.6	72.6 ± 7.2	84.2±7.9	79.5 ± 10.6
Alanine amino transferase activity				
Day 0	34.0 ± 4.5	35.6 ± 4.1	32.6 ± 4.5	29.1 ± 3.6
Day 2	34.0 ± 4.6	30.8 ± 2.7	30.6 ± 8.3	26.5 ± 1.2
Day 7	31.0 ± 3.1	37.5 ± 5.6	29.3 ± 4.5	39.5 ± 3.0
Day 14	32.0 ± 3.2	31.4 ± 2.5	34.6 ± 5.3	36.3 ± 1.7
Day 28	34.0 ± 3.8	31.3 ± 5.2	30.4 ± 9.4	39.3 ± 2.7
Alkaline phosphatase activity				
Day 0	28.6 ± 9.6	30.9 ± 3.3	27.4 ± 6.4	37.3 ± 5.6
Day 2	27.8 ± 5.1	26.0 ± 7.2	29.7 ± 2.6	30.5 ± 6.5
Day 7	31.8 ± 5.8	28.1 ± 5.9	32.8 ± 1.8	58.7 ± 8.9*
Day 14	27.0 ± 4.7	33.6 ± 2.4	28.9 ± 5.2	42.1 ± 2.9
Day 28	30.5 ± 3.2	28.0 ± 6.9	23.0 ± 4.7	--

Table B-45 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975a)

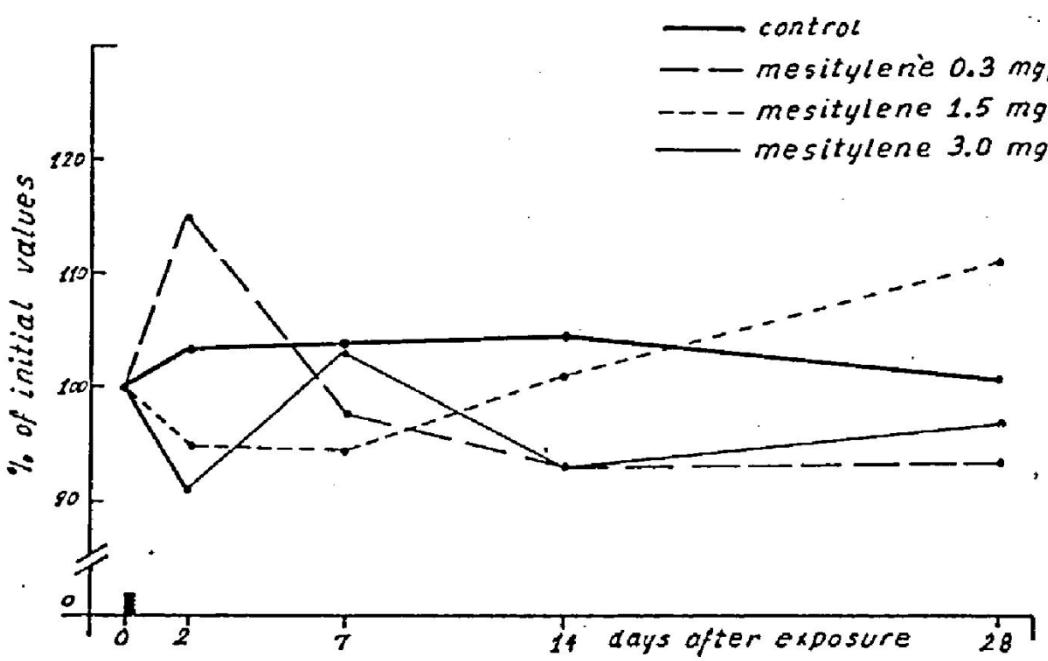


Figure 1. Serum activity of aspartate amino transferase after 6 hrs exposure to 1,3,5-TMB; values are expressed in % of initial values.

Source: Wiglusz et al. (1975a)

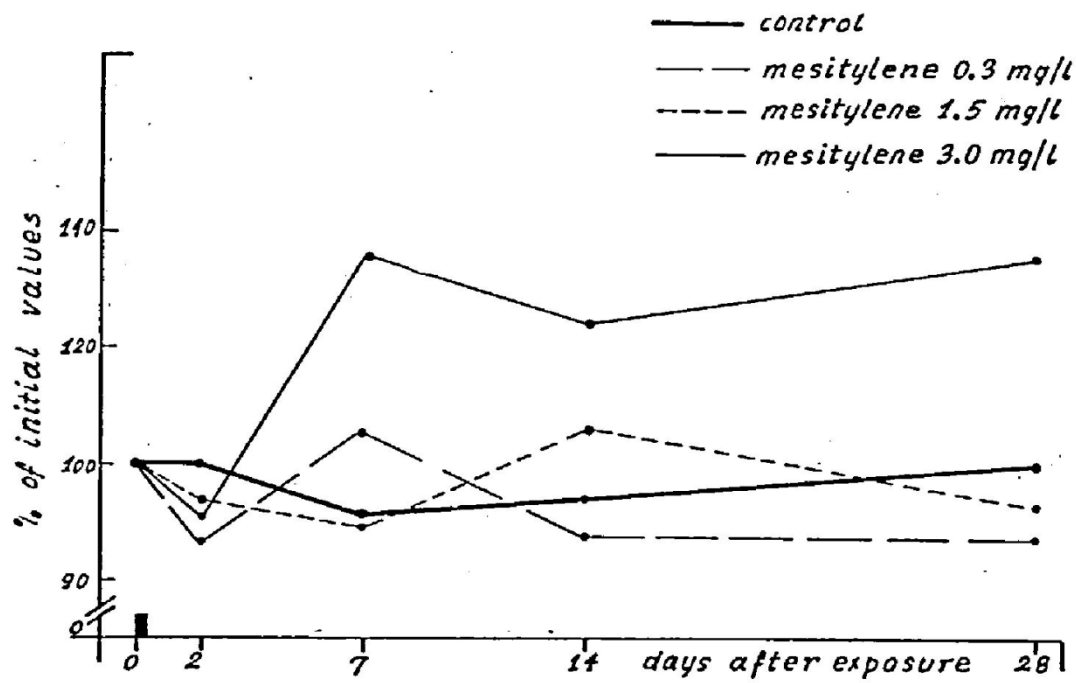


Figure 2. Serum activity of alanine amino transferase after 6 hrs exposure to 1,3,5-TMB; values are expressed in % of initial values.

Source: Wiglusz et al. (1975a)

Table B-45 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975a)

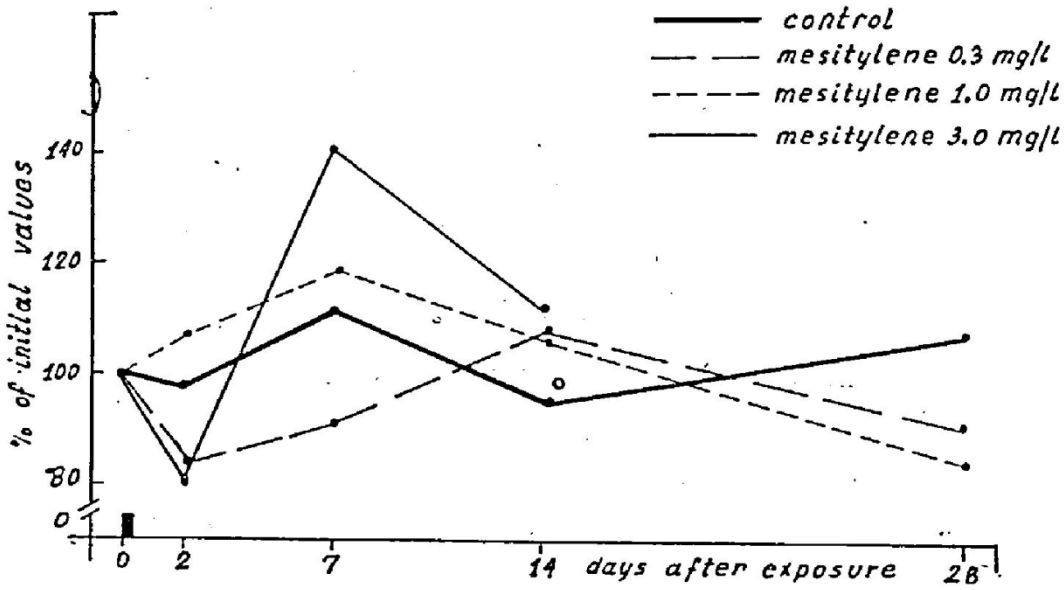


Figure 3. Serum activity of alkaline phosphatase after 6 hrs exposure to 1,3,5-TMB; values are expressed in % of initial values.

Source: Wiglusz et al. (1975a)

Observation	Hematological parameters during 5 week exposure to 1,3,5-TMB (means ± SD)					
	Day 0	Day 1	Day 3	Day 7	Day 14	Day 28
Aspartate amino transferase activity						
Control group	89.5 ± 2.3	74.5 ± 6.9	79.6 ± 10.5	83.2 ± 10.6	83.5 ± 7.3	82.2 ± 6.3
1,3,5-TMB group	72.0 ± 5.1	70.8 ± 5.2	81.3 ± 9.1	80.0 ± 6.3	93.4 ± 1.4*	79.6 ± 9.4
Alanine amino transferase activity						
Control group	34.0 ± 4.1	33.8 ± 5.0	35.6 ± 2.6	30.5 ± 4.9	30.0 ± 4.5	35.6 ± 4.6
1,3,5-TMB group	34.8 ± 3.6	28.0 ± 6.32	3.33 ± 3.8	35.1 ± 3.9	36.4 ± 4.0	36.5 ± 5.0
Ornithite carbamyl transferase activity						
Control group	2.7 ± 0.2	2.6 ± 0.2	3.1 ± 0.2	2.8 ± 0.1	2.6 ± 0.3	3.6 ± 0.3
1,3,5-TMB group	2.6 ± 0.4	2.5 ± 0.6	3.8 ± 0.4	3.5 ± 0.2	2.6 ± 0.2	3.7 ± 0.4
Alkaline phosphatase activity						
Control group	27.8 ± 4.0	28.8 ± 3.8	28.5 ± 6.8	26.5 ± 3.9	27.2 ± 8.8	25.8 ± 3.0
1,3,5-TMB group	32.4 ± 1.8	23.6 ± 3.6	22.2 ± 3.6	30.2 ± 6.9	25.6 ± 5.9	32.6 ± 4.8

Table B-45 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975a)

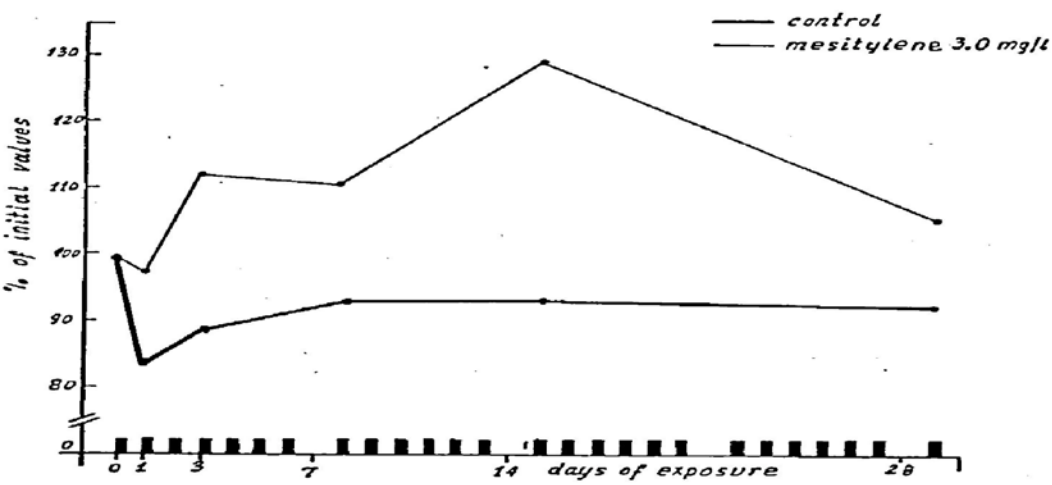


Figure 4. Serum activity of aspartate amino transferase during exposure to 1,3,5-TMB at 3.0 mg/L for 6 hrs/day, 6 days/week, for 5 weeks; values are expressed in % of initial values.

Source: Wiglusz t al. (1975a)

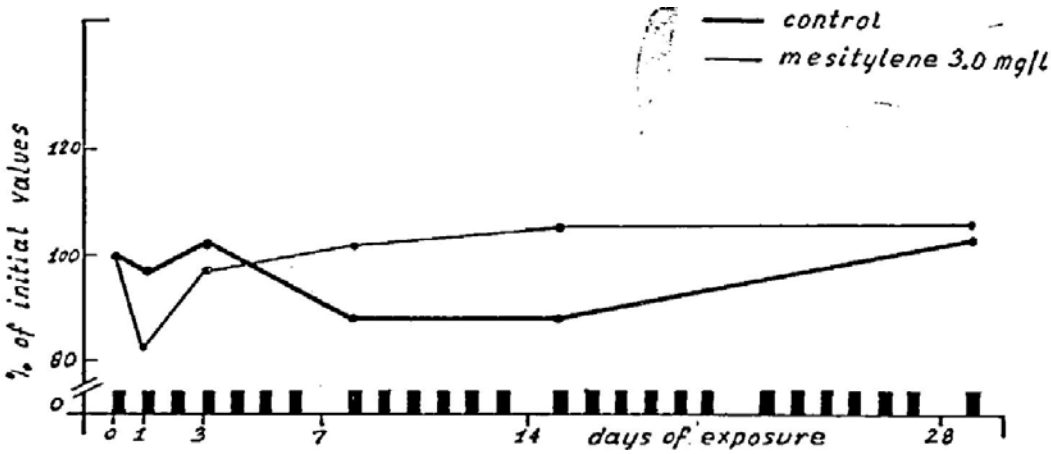


Figure 5. Serum activity of alanine amino transferase during exposure to 1,3,5-TMB at 3.0 mg/L for 6 hrs/day, 6 days per week, for 5 weeks; values are expressed in % of initial values.

Source: Wiglusz et al. (1975a)

Table B-45 (Continued): Characteristics and quantitative results for Wiglusz et al. (1975a)

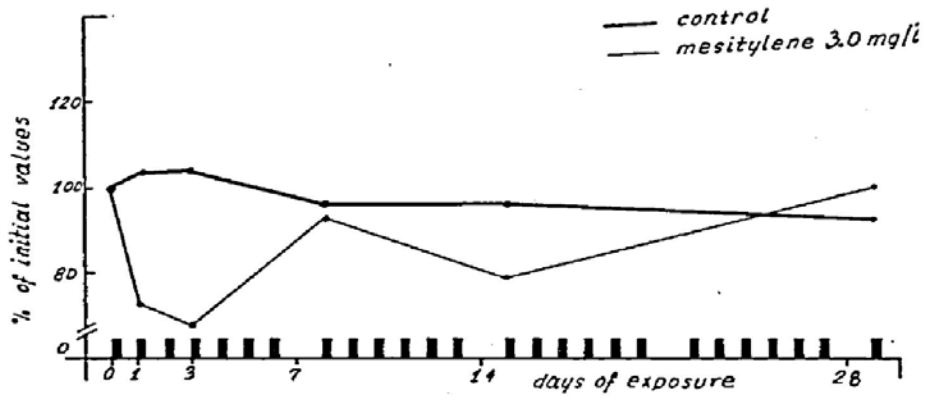


Figure 6. Serum activity of alkaline phosphatase during exposure to 1,3,5-TMB at 3.0 mg/L for 6 hrs/day, 6 days/week, for 5 weeks; values are expressed in % of initial values.

Source: Wiglusz et al. (1975a)

Health Effect at LOAEL	NOAEL	LOAEL
Increase in alkaline phosphatase activity	1.5 mg/L	3.0 mg/L

Comments: This study observed increases in alkaline phosphatase activity on day 7 of the short-term exposure study. Only one dose group used in chronic study. Data not recorded daily; significant gaps exist between sampling days.

*Statistically significant in relation to initial values ($p < 0.05$).

Source: Wiglusz et al. (1975a)

B.6. HUMAN TOXICOKINETIC STUDIES

Table B-46. Characteristics and quantitative results for Järnberg et al. (1996)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Caucasian humans	M	9 per dose	Inhalation	2 ppm and 25 ppm (~10 and 123 mg/m^3) 1,2,3-, 1,2,4-, or 1,3,5-TMB	2 hrs exposure, followed by 4 hrs observation

Additional study details

- Caucasian males were exposed to 2 ppm (~10 mg/m^3) 1,2,4-TMB and 25 ppm (123 mg/m^3) 1,2,3-, 1,2,4-, or 1,3,5-TMB in an inhalation chamber for 2 hrs.
- Study subjects were asked to perform light cycling to simulate a work environment, with participants generating 50 W power during 2 hr exposure.
- 1,2,3-, 1,2,4-, and 1,3,5-TMB concentrations in exhaled air, blood, and urine were determined via gas chromatography.
- No significant irritation or CNS effects were observed.
- Results imply extensive deposition in adipose tissue.
- Exhalation accounted for 20–37% of absorbed amount while urinary excretion of unchanged TMBs accounted for ≤0.002%.
- The study was approved by the Regional Ethical Committee at the Karolinska Institute

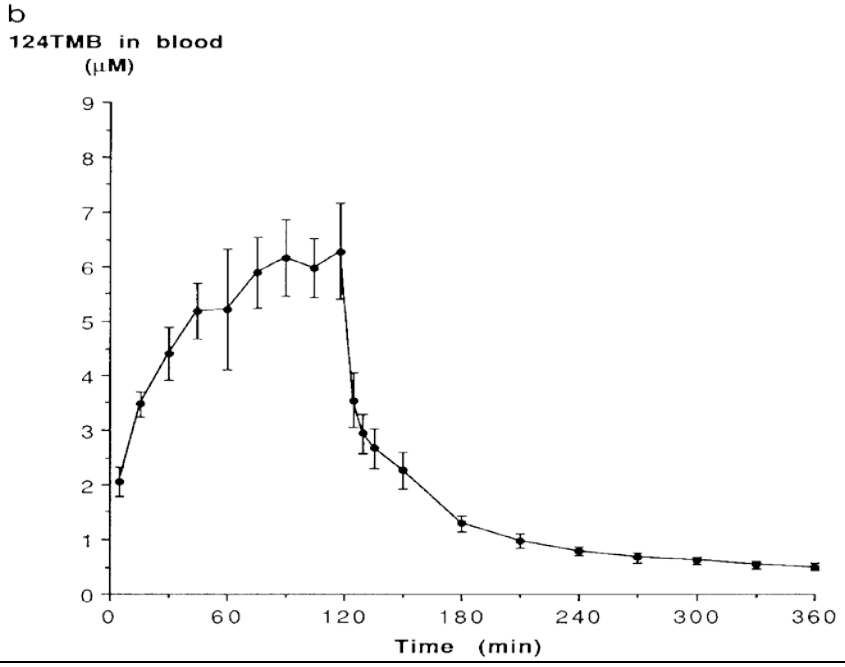
Respiratory uptake and urinary excretion of TMB isomers following 2 hour inhalation exposure (mean ± 95%CI)

Exposure	**25 ppm (**123 mg/m^3)**1,2,3-TMB**	**25 ppm (**123 mg/m^3)**1,3,5-TMB**	**25 ppm (**123 mg/m^3) **1,2,4-TMB**	**2 ppm** (~10 mg/m^3) **1,2,4-TMB**
Respiratory uptake (%)[a]	56 ± 4	62 ± 3	64 ± 3	63 ± 2
Net respiratory uptake (%)[b]	48 ± 3	55 ± 2	60 ± 3	61 ± 2
Respiratory uptake (mmol)[a]	1.4 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.16 ± 0.01
Net respiratory uptake (mmol)[b]	1.2 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	0.15 ± 0.01
Respiratory excretion (%)[c]	37 ± 9	25 ± 6	20 ± 3	15 ± 5
Net respiratory excretion (%)[d]	28 ± 8	16 ± 4	14 ± 2	9 ± 4
Urinary excretion (%)[e]	0.0023 ± 0.0008	0.0016 ± 0.0015	0.0010 ± 0.0004	0.0005 ± 0.0002

Table B-46 (Continued): Characteristics and quantitative results for Järnberg et al. (1996)

Kinetic values of TMB isomers following 2 hour inhalation exposure (mean ± 95%CI)				
Kinetic parameter	**25 ppm (**123 mg/m^3)**1,2,3-TMB**	**25 ppm (**123 mg/m^3)**1,3,5-TMB**	**25 ppm (**123 mg/m^3) **1,2,4-TMB**	**2 ppm** (~10 mg/m^3) **1,2,4-TMB**
Total calculated blood clearance (L/hr/kg)[f]	0.63 ± 0.13	0.97 ± 0.16	0.68 ± 0.13	0.87 ± 0.37
Total apparent calculated blood clearance (L/hr/kg)[g]	0.54 ± 0.11	0.86 ± 0.12	0.63 ± 0.11	0.82 ± 0.32
Exhalatory blood clearance (L/hr/kg)[f]	0.23 ± 0.07	0.24 ± 0.10	0.14 ± 0.04	0.14 ± 0.10
Metabolic blood clearance (L/hr/kg)[f]	0.39 ± 0.11	0.72±0.11	0.54 ± 0.10	0.74 ± 0.29
1st Phase half-life (min)	1.5 ± 0.9	1.7 ± 0.8	1.3 ± 0.8	1.4 ± 1.8
2nd Phase half-life (min)	24 ± 9	27 ± 5	21 ± 5	28 ± 14
3rd Phase half-life (min)	4.7 ± 1.6	4.9 ± 1.4	3.6 ± 1.1	5.9 ± 2.5
4th Phase half-ife (min)	78 ± 22	120 ± 41	87 ± 27	65 ± 20
AUC (µM x hrs)	32 ± 6	22 ± 4	35 ± 10	3.6 ± 2.0
Volume of distribution (L/kg)	30 ± 6	39 ± 8	38 ± 11	28 ± 3
Mean residence time (hrs)	57 ± 22	42 ± 11	69 ± 32	47 ± 22

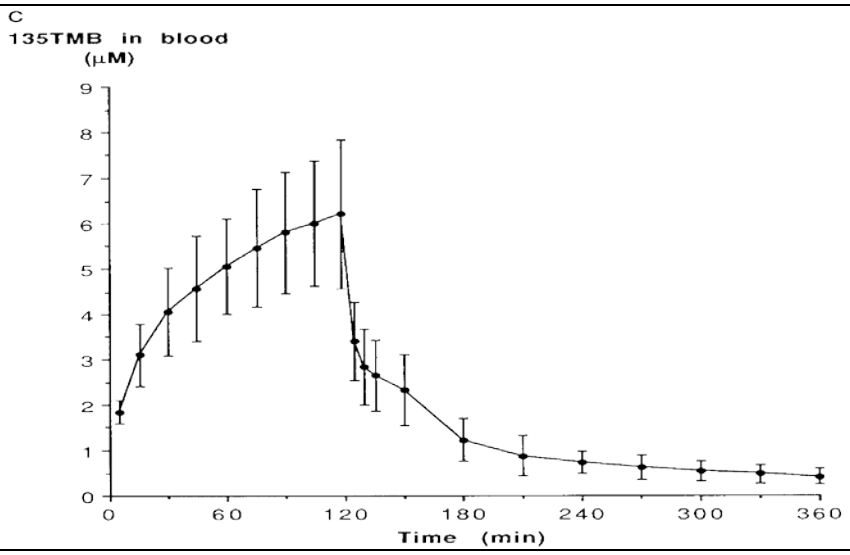
Figure 1. Concentration of 1,2,4-TMB in capillary blood during and after 2 hr exposure to 25 ppm (123 mg/m^3) 1,2,4-TMB (mean values ± 95% CI).



Source: Järnberg et al. (1996)

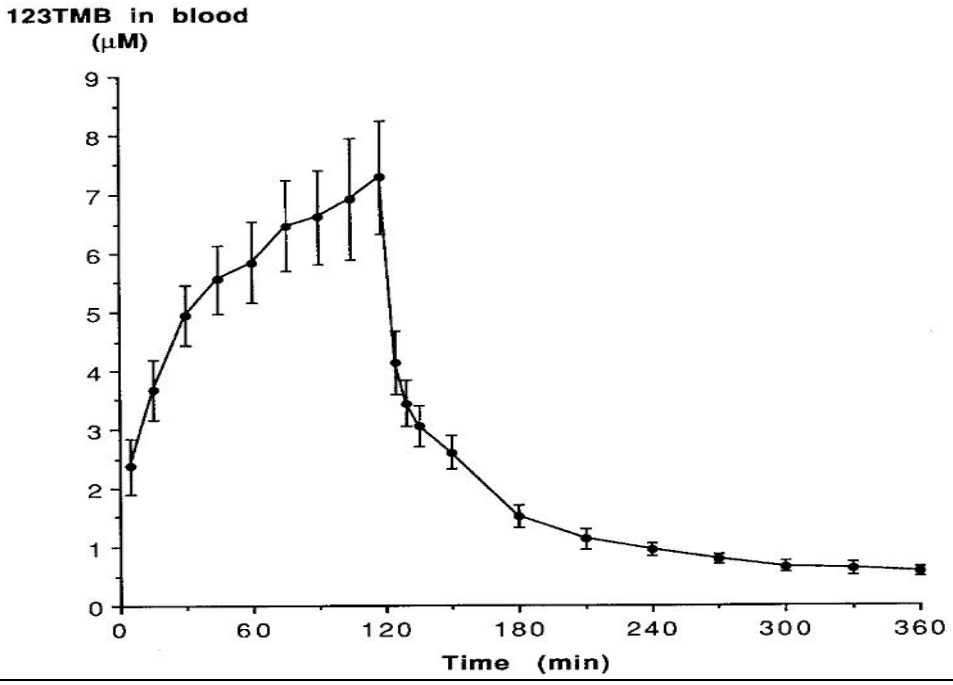
Table B-46 (Continued): Characteristics and quantitative results for Järnberg et al. (1996)

Figure 2. Concentration of 1,3,5-TMB in capillary blood during and after 2 hr exposure to 25 ppm (123 mg/m^3) 1,3,5-TMB (mean values ± 95% CI).



Source: Järnberg et al. (1996)

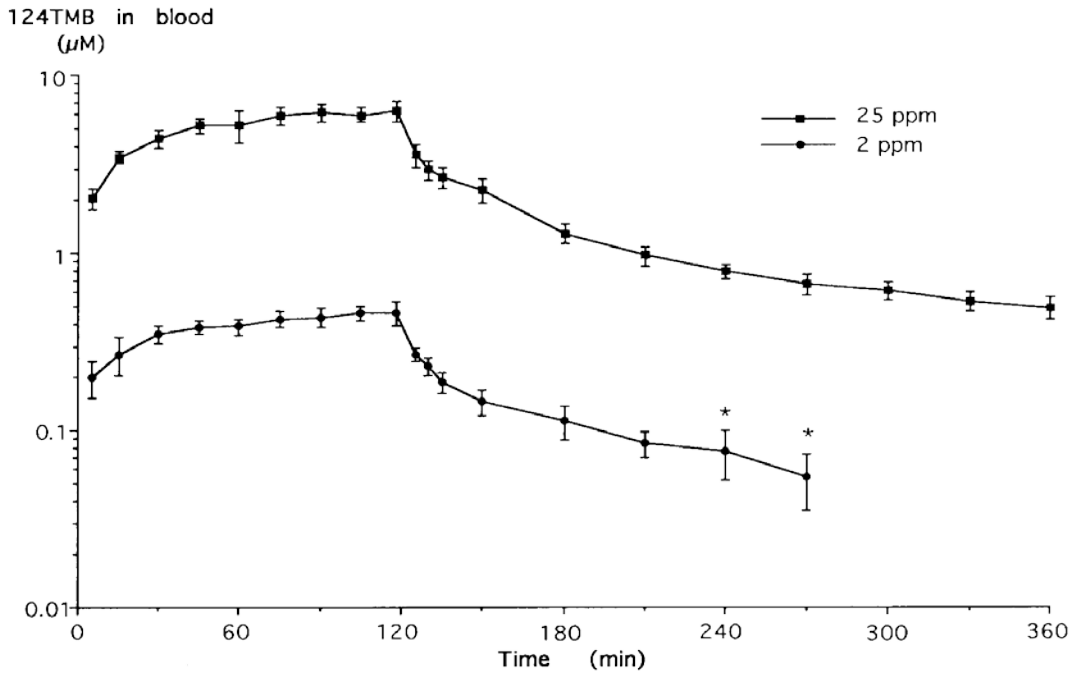
Figure 3. Concentration of 1,2,3-TMB in capillary blood during and after 2 hr exposure to 25 ppm (123 mg/m^3) 1,2,3-TMB (mean values ± 95% CI).



Source: Järnberg et al. (1996)

Table B-46 (Continued): Characteristics and quantitative results for Järnberg et al. (1996)

Figure 4. Concentration of 1,2,4-TMB in capillary blood from 10 subjects exposed to 2 and 25 ppm (~10 and 123 mg/m^3) of 1,2,4-TMB (mean values ± 95% CI)



Source: Järnberg et al. (1996)

Comments: Exposure duration possibly not sufficient to detect metabolic changes. Metabolites not measured.

[a]Percent of dose calculated as net uptake + amount cleared by exhalation during exposure .

[b]Percentage of dose calculated as net uptake.

[c]During and post-exposure, percentage of the respiratory uptake.

[d]Post-exposure, percentage of net respiratory uptake.

[e]Post-exposure, percentage of respiratory uptake.

[f]Calculated from respiratory uptake.

[g]Calculated from net respiratory uptake.

Source: Järnberg et al. (1996)

Table B-47. Characteristics and quantitative results for Järnberg et al. (1997a)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Caucasian Human	M	9	Inhalation	11 mg/m^3 1,2,4-TMB	2 hrs

Additional study details

- Nine Caucasian males were exposed to 11 mg/m^3 1,2,4-TMB alone or 11 mg/m^3 1,2,4-TMB as a component of 300 mg/m^3 WS.
- Exposure lasted 2 hrs, during which study subjects were required to cycle producing 50 W continuously to simulate a work environment.
- Gas chromatography was used to measure 1,2,4-TMB levels in air.
- HPLC was used to measure urinary metabolites.
- Irritation was not reported amongst subjects at these exposure levels.
- The study was approved by the Regional Ethical Committee at the Karolinska Institute and was only performed after informed consent.

Figure 1. Mean (± SD) capillary blood concentration of 1,2,4-TMB during and after exposure to 1,2,4-TMB alone and 1,2,4-TMB as a component of WS.

Source: Järnberg et al. (1997a)

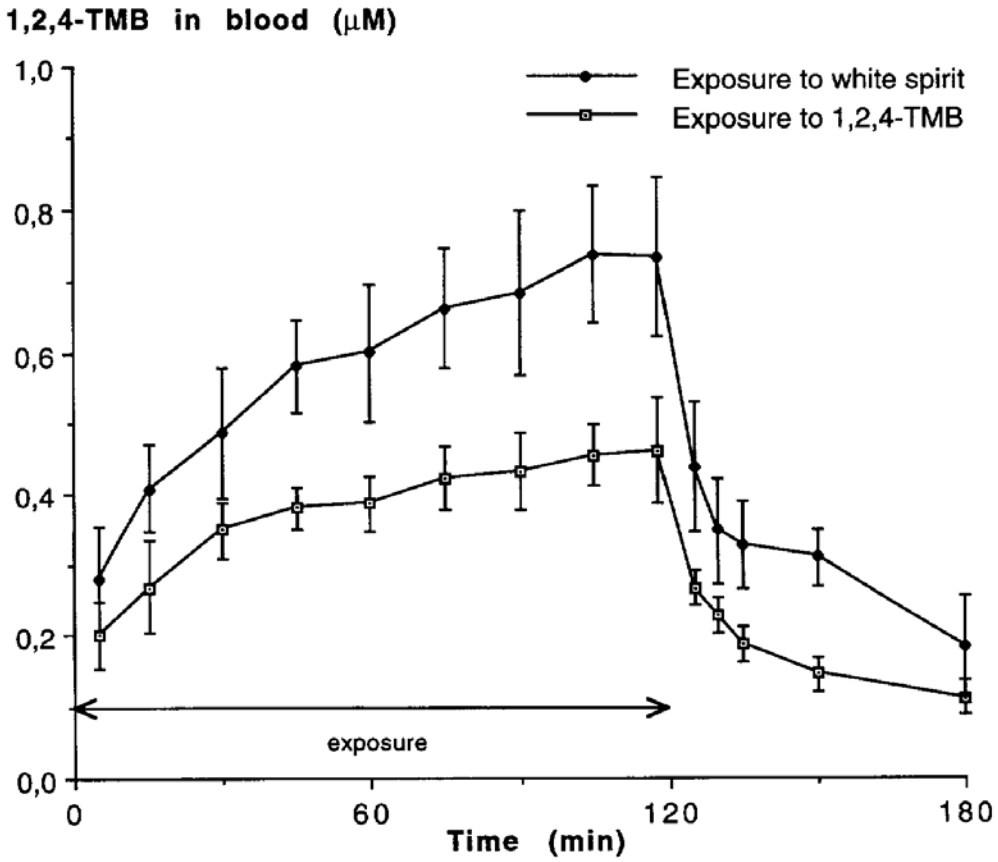
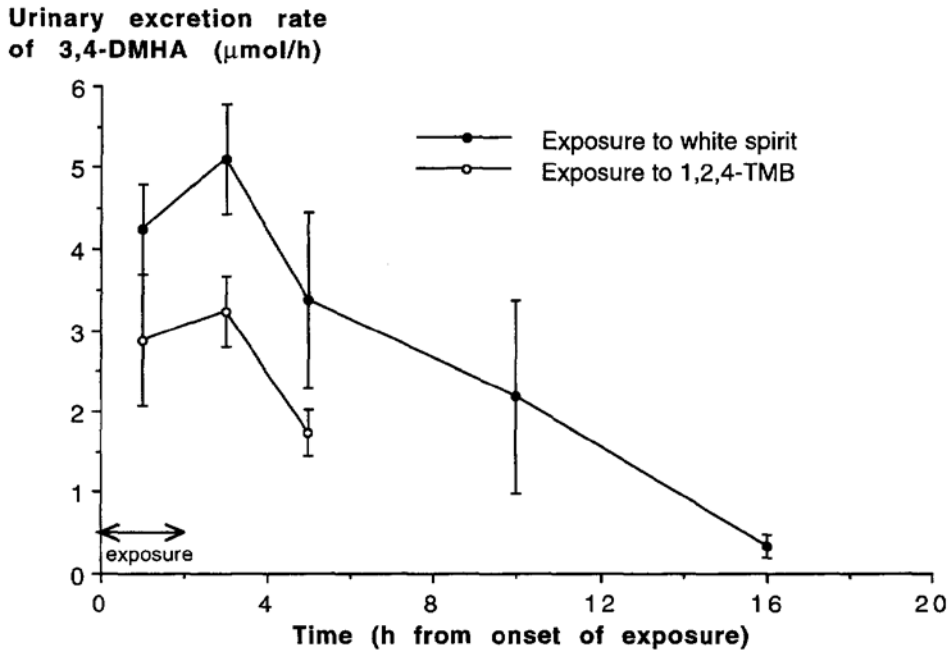


Table B-47 (Continued): Characteristics and quantitative results for Järnberg et al. (1997a)

Results from 2 hour exposure to 1,2,4-TMB alone or 1,2,4-TMB as a component of WS (mean ± SD)			
Exposure	**1,2,4-TMB alone**	**1,2,4-TMB in WS**	***p*-value**
Net respiratory uptake (mmol)	0.15 ± 0.01	0.14 ± 0.02	0.5[a]
AUC (µM × min), 0–3 hr	53 ± 4	86 ± 9	<0.0001[a]
Half-life of 3,4-DMHA (hr)	3.7 ± 0.4[b]	3.0 ± 0.7	0.2[c]
Excretion of 3,4-DMHA (%[d]), 0–6 hr	11 ± 2	18 ± 3	0.007[c]

Figure 2. Urinary excretion rate of 3,4-dimethylhippuric acid against the midpoint time of urine collection in 9 male volunteers exposed to 11 mg/m^3 of 1,2,4-TMB, either alone or as a component of WS (mean ± 95% CI).



Source: Järnberg et al. (1997a)

Comments: Metabolites (DMBAs) measured in urine. Exposure duration possibly not sufficient to detect other metabolic changes. Only one exposure group; multiple concentrations not tested.

[a] Student's t-test
[b] Recalculated for 9 subjects form a 120 mg/m^3 exposure to 1,2,4-TMB
[c] Analysis of variance
[d] 5 of net respiratory uptake

Source: Järnberg et al. (**1997a**)

Table B-48. Characteristics and quantitative results for Järnberg et al. (1997b)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Caucasian Humans	M	10	Inhalation	25 ppm (123 mg/m^3) 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB	2 hrs

Additional study details

- Ten males were exposed to 25 ppm (123 mg/m^3) 1,2,3-TMB, 1,2,4-TMB or 1,3,5-TMB for 2 hrs or 2 ppm (~10 mg/m^3) 1,2,4-TMB for 2 hrs.
- Study subjects were asked to perform light cycling to simulate a work environment, with participants generating 50 W power during 2 hr exposure.
- Isomers of all DMHA metabolites in urine were detected via HPLC.
- Approximately 22% of inhaled 1,2,4-TMB, 11% of inhaled 1,2,3-TMB, and 3% of inhaled 1,3,5-TMB was found to be excreted as DMHAs in urine within 24 hrs following exposure.
- The study was approved by the Regional Ethical Committee at the Karolinska Institute and only with the informed consent of the subjects and according to the 1964 Declaration of Helsinki

Half-times of urinary excretion rate, recoveries, and rates of urinary DMHA isomer excretion (mean ± 95% CI)

Exposure	**Isomer**	**Half-time (hr)**	**Urinary recovery % (24 hrs)**	**Excretion rate, µg/min, 0–24 hrs**
1,2,3-TMB	2,3-DMHA	4.8 ± 0.8	9 ± 3	19 ± 3
1,2,3-TMB	2,6-DMHA	8.1 ± 1.5	2 ± 2	4.2 ± 1.7
1,2,4-TMB	3,4-DMHA	3.80 ± 0.4	18 ± 3	44 ± 6
1,2,4-TMB	2,4-DMHA	5.8 ± 0.9	3 ± 0.8	8.2 ± 1.4
1,2,4-TMB	2,5-DMHA	5.3 ± 1.5	<1 ± 0.2	1.6 ± 0.5
1,3,5-TMB	3,5-DMHA	16 ± 6	3 ± 2	8.9 ± 2.1

Comments: Metabolites (DMBAs) measured in urine. Exposure duration possibly not sufficient to detect metabolic changes associated with longer time points. Toxicokinetics studied at only one concentration.

Source: Järnberg et al. (1997b)

Table B-49. Characteristics and quantitative results for Järnberg et al. (1998)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Caucasian humans	M	9 subjects	Inhalation	2 ppm (~10 mg/m^3) 1,2,4-TMB, 2 ppm (~10 mg/m^3) in WS, 25 ppm (123 mg/m^3) 1,2,4-TMB	2 hrs exposure, followed by 6 hrs observation

Additional study details

- Caucasian males were exposed to 2 ppm (~10 mg/m^3) 1,2,4-TMB, 2 ppm (~10 mg/m^3) in WS, 25 ppm (123 mg/m^3) 1,2,4-TMB in an inhalation chamber for 2 hrs.
- Study subjects were asked to perform light cycling to simulate a work environment.
- 1,2,4-TMB concentration was determined via gas chromatography.
- DMHA metabolites were measured with HPLC.
- Blood levels of 1,2,4 TMB and its urinary metabolites were found to be higher in the WS exposure group suggesting that components of WS could interfere with TMB metabolism.
- No significant irritation or CNS effects were observed.
- The study was approved by the Regional Ethics Committee of the Karolinska Institute and was only performed after informed consent.

Kinetic results following 2 hour inhalation exposure to 1,2,4-TMB and 1,2,4-TMB in WS—mean values (95% CI)

Kinetic parameter	**2 ppm** (~10 mg/m^3) **group**	**2 ppm** (~10 mg/m^3) **in WS**	**25 ppm** (123 mg/m^3) **alone**
Actual [TMB] (ppm)	2.22 (2.13–2.31)	2.26 (2.20–2.32)	23.9 (22.7–25.1)
Respiratory uptake (mmol)[a]	0.16 (0.14–0.18)	0.16 (0.14–0.18)	1.73 (1.61––1.85)
Net respiratory uptake	0.15 (0.14–0.16)	0.14 (0.12–0.16)	1.52 (1.37–1.67)
AUC_{blood} (µM × min)	95 (54–137)	157 (136–178)*	1286 (1131–1441)
Total blood clearance (L/min)	2.09 (1.52–2.66)	1.06 (0.89–1.23)**	1.38 (1.23–1.53)*
Metabolic blood clearance (L/min)	1.71 (1.15–2.26)	0.79 (0.62–0.96)*	1.06 (0.87–1.25)*
Exhalatory blood clearance (L/min)	0.39 (0.28–0.50)	0.28 (0.20–0.36)	0.32 (0.24–0.40)
Mean residence time (hr)	4.6 (-1.3–10.5)	4.8 (2.1–7.5)	3.8 (1.8–5.8)
Volume of distribution, steady state (L)	293 (69–517)	271 (139–403)	294 (165–423)

Table B-49 (Continued): Characteristics and quantitative results for Järnberg et al. (1998)

Half-life in blood, TMB, 1st phase (min)	3.9 (1.4–6.4)	5.9 (3.1–8.7)	6.1 (5.3–6.9)
Idem, TMB, 2nd phase (hr)	4.3 (-0.5–9.0)	4.8 (2.1–7.5)	4.0 (2.2-5.8)
Half-life in urine, 3,4-DMHA (hr)	ND[c]	3.0 (2.3–3.7)	3.8 (3.4–4.2)
Urinary recovery, 3,4-DMHA (%)[b], 0–6 hr	11 (9–13)	18(15–21) *	14 (12–16)
Idem (%)[b], 0–22 hR	ND	27 (23–31)	18 (15–21)
Comments: Multiple exposure concentrations were tested and multiple tissues were analyzed. Study of 1,2,4-TMB as a component of WS. Toxicokinetics of 1,2,3- and 1,3,5-TMB not studied.			

[a]Net respiratory uptake + amount cleared by exhalation during exposure.
[b]% of net respiratory uptake.
[c]Not determined.
*$p < 0.05$, **$p < 0.01$, compared to 2 ppm (~10 mg/m^3) alone by repeated measures ANOVA.

Source: Järnberg et al. (1998).

Table B-50. Characteristics and quantitative results for Jones et al. (2006)

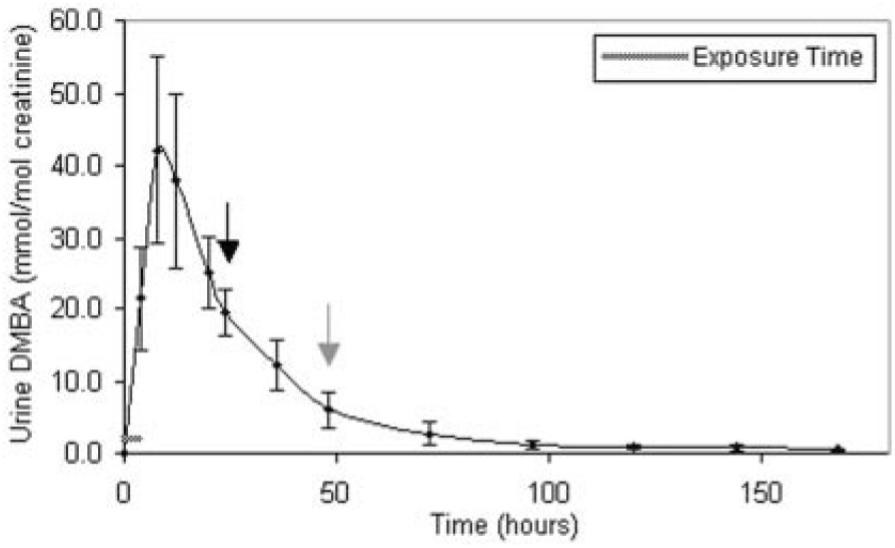
Study design

Species	Sex	N	Exposure route	Dose range	Exposure duration
Human	M/F	2 per sex	Inhalation	25 ppm (1,2,3-TMB mg/m^3) 1,3,5-TMB	4 hrs

Additional study details

- Two males and two females were exposed to 25 ppm (1,2,3-TMB mg/m^3) 1,3,5-TMB in an inhalation chamber for 4 hrs.
- 1,3,5-TMB concentration in exhaled air, venous blood, and urine was determined via gas chromatography.
- No significant irritation or CNS effects were observed during the inhalation study, although one volunteer was treated with a 2 cm^2 gauze patch soaked with liquid 1,3,5-TMB and reported mild itching, erythema, and oedema where gauze contacted skin.
- Authors conclude that urinary DMBA and breath TMB are suitable markers of TMB exposure, and that repeated exposures during work week can result in significant accumulation in tissues.
- The study was approved by the Health and Safety Executive's Research Ethics Committee

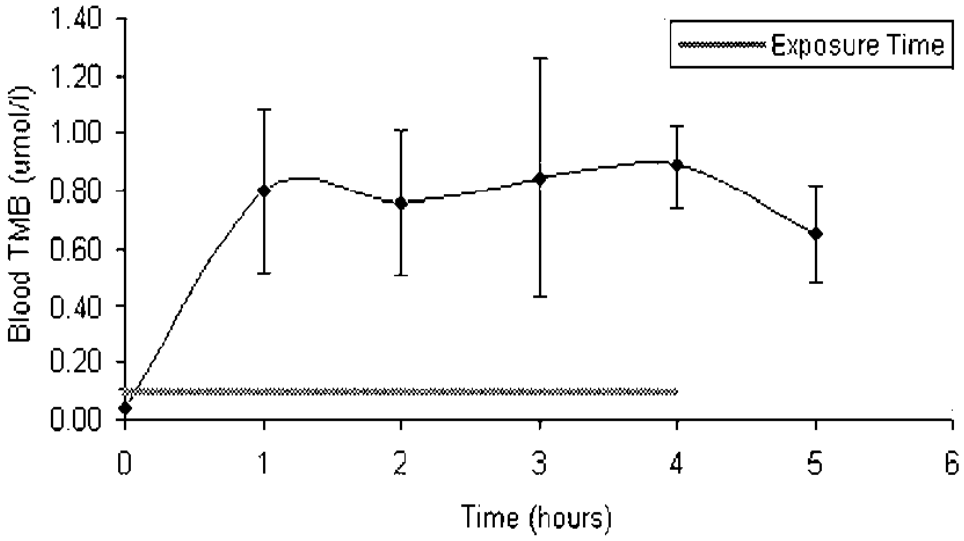
Figure 1. Mean ± SD urinary total DMBAs. Black and grey arrows represent 24 and 48 hrs respectively, following a single 4 hr exposure to 25 ppm (1,2,3-TMB mg/m^3) 1,3,5-TMB.



Source: Jones et al. (2006)

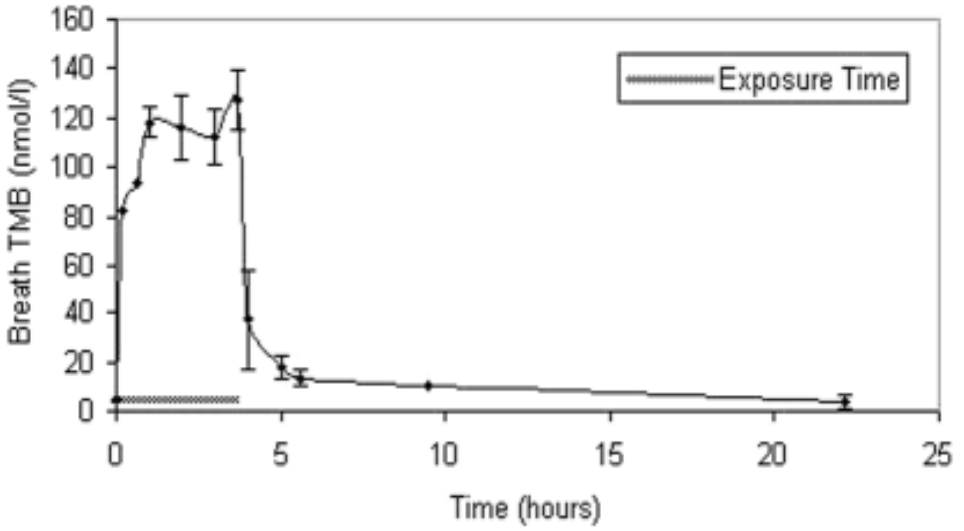
Table B-50 (Continued): Characteristics and quantitative results for Jones et al. (2006)

Figure 2. Mean ± SD blood levels of 1,3,5-TMB during and after 4 hr exposure to 25 ppm (1,2,3-TMB mg/m^3) 1,3,5-TMB.



Source: Jones et al. (2006)

Figure 3. Mean ± SD breath levels of 1,3,5-TMB during and after 4 hr exposure to 1,3,5-TMB.



Source: Jones et al. (2006)

Comments: Metabolite (DMBA) concentration measured in urine. Subjects tested included males and females. Small number of study subjects (n = 4). Exposure duration possibly not sufficient to detect metabolic changes. Other metabolites not measured.

Source: Jones et al. (2006)

Table B-51. Characteristics and quantitative results for Kostrzewski et al. (1997)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Human	M/F	5	Inhalation	Between 5 and 150 mg/m^3 1,2,4-TMB, 1,3,5-TMB, and 1,2,3-TMB	4 or 8 hrs

Additional study details

- Five humans were exposed to 1,2,4-TMB, 1,3,5-TMB, and 1,2,3-TMB at concentrations between 5 and 150 mg/m^3.
- Exposure durations were either 4 or 8 hrs.
- TMBs were measured in blood and urine, via gas chromatography.
- DMBA excretion was found to follow an open, two-compartment model.

1,2,3-, 1,2,4-, and 1,3,5-TMB concentration in blood before, during, and after exposure

	1,2,3-TMB		**1,2,4-TMB**		**1,3,5-TMB**	
Sampling time (hrs)	**Blood concen-tration ($\mu g/dm^3$ [µg/L])**	**SD**	**Blood concen-tration ($\mu g/dm^3$ [µg/L])**	**SD**	**Blood concen-tration ($\mu g/dm^3$ [µg/L])**	**SD**
0	0	0	0	0.00	0	0.00
0.25	259	94.5	194	19.80	181	25.01
0.50	290	91.54	460	57.36	308	5.29
1	295	57.11	533	46.61	355	44.80
2	380	93.17	730	128.89	482	201.57
4	341	186.94	810	112.40	603	184.13
8	520	129.42	979	171.12	751	122.87
0.05	261	50.36	580	36.2	434	36.40
0.10	277	57.89	496	85.03	388	64.16
0.15	287	38.18	447	106.69	309	38.78
0.25	277	35.47	387	65.83	298	65.48
0.50	--	--	246	128.54	247	34.00
1	204	17.78	131	19.87	190	41.13
2	133	38.55	101	14.17	121	24.60
4	85	8.96	85	13.65	94	16.52
6	65	23.69	63	11.03	76	25.81
8	64	11.59	69	7.09	74	20.16
25	54	14.57	54	3.74	45	13.93
32	29	3.51	48	10.24	44	20.19
49	19	13.01	46	9.98	42	7.93
56	21	11.31	31	9.32	42	9.81
73	14	3.50	26	9.49	--	--

Table B-51 (Continued): Characteristics and quantitative results for Kostrzewski et al. (1997)

Excretion rate (*V* [mg/hr]) of dimethylbenzoic acid (DMBA) in urine during and after exposure to 1,2,3-TMB, 1,2,4-TMB, or 1,3,5-TMB				
	1,2,3-TMB exposure			
	2,3-DMBA		2,6-DMBA	
Sampling time (hr)	*V* (mg/hr)	SD	*V* (mg/hr)	SD
0	0.000	0.000	0.000	0.000
0–2	3.518	0.852	0.099	0.097
2–4	10.745	1.856	0.097	0.084
4–6	16.594	5.028	0.146	0.039
6–8	23.468	5.291	0.202	0.070
8–10	16.874	2.353	0.160	0.004
10–12	14.769	1.964	0.150	0.035
12–14	11.929	2.070	0.161	0.048
14–16	7.715	2.236	0.129	0.038
16–23	3.976	0.782	0.110	0.042
23–27	1.876	0.213	0.067	0.021
27–31	1.822	0.893	0.079	0.052
31–35	1.471	0.551	0.081	0.055
35–39	2.292	0.998	0.143	0.032
39–47	1.388	0.660	0.102	0.037
47–51	1.125	0.414	0.109	0.041
51–55	1.543	0.468	0.172	0.058
55–59	1.505	0.683	0.139	0.050
59–63	1.154	0.481	0.055	0.063
63–71	0.535	0.119	0.031	0.030
71–75	0.802	0.383	0.053	0.001
75–79	0.999	0.712	0.059	0.030
79–83	0.886	0.343	0.086	0.078
83–87	0.349	0.165	0.046	0.050
87–95	0.365	0.163	0.000	0.000

Table B-51 (Continued): Characteristics and quantitative results for Kostrzewski et al. (1997)

Sampling time (hr)	1,2,4-TMB exposure			
	2,4- and 2,5-DMBA		3,4-DMBA	
	V (mg/hr)	SD	*V* (mg/hr)	SD
0	0.000	0.000	0.000	0.000
0–2	6.632	3.069	19.949	5.489
2–4	12.931	4.315	22.731	4.536
4–6	21.148	7.067	26.906	6.525
6–8	29.263	9.240	35.346	11.017
8–10	16.616	11.451	12.082	10.205
10–12	15.619	2.935	6.198	2.325
12–14	17.328	2.218	6.029	2.135
14–16	13.832	2.176	4.415	1.372
16–23	7.023	2.565	2.520	1.043
23–27	4.052	0.674	1.870	0.525
27–31	2.570	0.760	2.005	0.460
31–35	2.209	0.666	1.523	0.610
35–39	1.211	1.075	1.247	0.895
39–47	1.262	0.256	0.957	0.099
47–51	1.174	0.459	0.953	0.623
51–55	0.370	0.228	0.659	0.231
55–59	0.928	0.327	0.936	0.515
59–63	1.591	1.162	1.286	0.391
63–71	0.948	0.276	0.869	0.141
71–75	1.122	0.049	0.851	0.246
75–79	0.748	0.441	0.422	0.231
79–83	1.082	0.733	0.744	0.328
83–87	--	--	--	--
87–95	--	--	--	--

Table B-51 (Continued): Characteristics and quantitative results for Kostrzewski et al. (1997)

	1,3,5-TMB exposure	
	3,5-DMBA	
Sampling time (hr)	*V* (mg/hr)	SD
0	0.000	0.000
0–2	3.538	0.833
2–4	8.854	2.955
4–6	12.334	3.905
6–8	19.204	6.092
8–10	19.413	6.329
10–12	23.535	7.606
12–14	22.460	3.254
14–16	16.941	4.350
16–23	10.790	3.116
23–27	6.908	2.691
27–31	6.558	3.657
31–35	3.983	2.367
35–39	3.946	2.073
39–47	3.110	0.838
47–51	3.244	1.140
51–55	2.343	1.355
55–59	3.669	1.882
59–63	2.436	1.303
63–71	1.600	1.305
71–75	1.025	0.639
75–79	1.044	0.825
79–83	0.750	0.645
83–87	--	--
87–95	--	--

Comments: Metabolites (DMBAs) measured in urine. Toxicokinetics studied over a range of exposures. Exposure duration possibly not sufficient to detect other metabolic changes. Only one study subject per exposure group.

Source: Kostrzewski et al. (1997)

B.7. ANIMAL TOXICOKINETIC STUDIES

Table B-52. Characteristics and quantitative results for Dahl et al. (1988)

Study Design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
F344 Rats	M	2 rats	Inhalation	1-5,000 ppm 1,2,4-TMB	80 minutes per day for 5 consecutive days

Additional study details

- Male F344 rats weighing between 264 and 339 g were housed in polycarbonate cages for the duration of the experiment.
- Vapors were pumped into exposure chamber at flow rate of 400ml/min past the nose of each rat in the nose-only exposure tube.
- The amount of absorbed hydrocarbon vapor was calculated from the flow rate and the output from the nose-only tube as measured by gas chromatography every minute during each 80 minute exposure.
- Concentrations were increased each day. Days 1-5 concentrations were 1ppm, 10ppm, 100ppm, 1000ppm, and 5000ppm respectively.
- 1,2,4-TMB uptake in one rat was observed to be 11.5±2 nmol/kg/min/ppm. For the second rat, uptake was observed to be 15.7±2.4 nmol/kg/min/ppm.

Comments: Study duration was short term (5 days). Reported values for uptake represent averages of uptake throughout experiment, despite the widely differing doses administered. This makes it difficult to quantify dose-specific uptake. Statistical power is limited because only two rats were used.

Source: Dahl et al. (1988)

Table B-53. Characteristics and quantitative results for Eide and Zahlsen et al. (1996)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Sprague-Dawley rats	M	4 per dose	Inhalation	0, 75, 150, 300, 450 ppm (0, 369, 738, 1,476, or 2,214 mg/m^3) 1,2,4-TMB	12 hr exposures in inhalation chamber

Additional study details

- Male Sprague-Dawley rats were exposed to 75, 150, 300, or 450 ppm (0, 369, 738, 1,476, or 2,214 mg/m^3) 1,2,4-TMB in an inhalation chamber for 12 hrs.
- Food and water was give ad libitum except during exposure, and animal weight ranged between 200 g and 250 g prior to exposure.
- Hydrocarbon concentration tissue concentrations were determined via head space gas chromatography. Daily mean concentrations did not vary by more than ±5.3% from nominal concentrations.
- 1,2,4-TMB was found in higher concentrations in blood than *n*-nonane and trimethylcyclohexane.

Tissue 1,2,4-TMB concentrations following 12 hour 1,2,4-TMB inhalation exposure

Exposure	**Blood (µmol/kg)**	**Brain (µmol/kg)**	**Liver (µmol/kg)**	**Kidneys (µmol/kg)**	**Fat (µmol/kg)**
75ppm (369 mg/m^3)	14.1	23.6	53.4	53.4	516
150 ppm (738 mg/m^3)	57.5	97.5	123.1	168.5	3,806
300 ppm (1,476 mg/m^3)	115.5	220.9	256.3	282.4	12,930
450 ppm (2,214 mg/m^3)	221.3	400.2	468.6	492.5	19,270

Comments: Fat was analyzed and shown to retain higher concentrations of 1,2,4-TMB than all other tissues. Multiple exposure concentrations were tested and multiple tissues were analyzed. No data on urinary elimination. No data on metabolites of 1,2,4-TMB.

Source: Eide and Zahlsen et al. (1996).

Table B-54. Characteristics and quantitative results for Huo et al. (1989)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	3 rats per dose	Oral, in olive oil	0.08 mmol/kg, 0.8 mmol/kg, 0.49 µCi/kg 1,2,4-TMB	3, 6, 12, and 24 hrs

Additional study details

- Single doses of ^{14}C labeled 1,2,4-TMB administered orally to rats.
- Tissues were analyzed at 3, 6, 12, and 24 hr time points for the tissue distribution study and continuously for 24 hrs in the metabolism study.
- Percent 1,2,4-TMB distributed to individual tissues determined via liquid scintillation counter, concentration of metabolites analyzed via gas chromatography.
- 1,2,4-TMB was distributed widely throughout the body, though particularly high levels were found in adipose tissue.
- Over 99% of radio-labeled material was recovered from urine within 24 hrs.
- Three most common metabolites were 3,4-DMHA (30.2%), 2,4-DMBA (12.7%), and 2,5-DMBA (11.7%).

Tissue distribution and urinary excretion following single oral dose of ^{14}C-1,2,4-TMB

% Dose of radioactivity in tissue and urine (mean ± SD for three rats)

Tissue/Urine	**3 hrs**	**6 hrs**	**12 hrs**	**24 hrs**
Liver	2.76 ± 0.39	2.69 ± 0.60	1.54 ± 0.38	0.13 ± 0.04
Kidney	0.56 ± 0.11	0.52 ± 0.12	0.14 ± 0.10	0.06 ± 0.05
Lung	0.10 ± 0.03	0.06 ± 0.03	0.03 ± 0.03	0.01 ± 0.01
Heart	0.03 ± 0.01	0.01	--	--
Testis	0.09 ± 0.04	0.12 ± 0.03	0.04 ± 0.04	--
Spleen	0.03 ± 0.02	0.03 ± 0.01	0.01 ± 0.01	--
Brain	0.08 ± 0.04	0.03 ± 0.02	0.03 ± 0.03	--
Stomach	2.39 ± 1.47	1.33 ± 0.98	0.09 ± 0.06	0.04 ± 0.03
Intestine	2.96 ± 1.82	3.33 ± 1.31	1.39 ± 1.03	0.25 ± 0.35
Serum	0.67 ± 0.14	0.57 ± 0.09	0.26 ± 0.15	0.12 ± 0.21
Muscle	2.38 ± 0.23	1.88 ± 1.63	0.64 ± 0.10	--
Skin	3.99 ± 1.51	2.29 ± 0.98	0.16 ± 0.25	--
Adipose Tissue	28.05 ± 9.28	26.31 ± 18.18	4.97 ± 0.97	0.67 ± 0.15
Urine	15.0 ± 1.1	32.6 ± 7.9	50.7 ± 7.9	99.8 ± 4.1

Table B-54 (Continued): Characteristics and quantitative results for Huo et al. (1989)

Concentration (μg/g) radioactive material in tissue (mean ± SD)				
Tissue	**3 hrs**	**6 hrs**	**12 hrs**	**24 hrs**
Liver	72 ± 9	81 ± 20	45 ± 12	5 ± 2
Kidney	68 ± 16	60 ± 13	17 ± 12	7 ± 6
Lung	17 ± 9	12 ± 6	4 ± 4	2 ± 4
Heart	8 ± 2	2 ± 1	--	--
Testis	8 ± 4	11 ± 2	3 ± 4	--
Spleen	11 ± 5	13 ± 5	5 ± 5	--
Brain	11 ± 5	6 ± 2	4 ± 4	--
Stomach	509 ± 313	263 ± 218	18 ± 11	10 ± 7
Intestine	35 ± 22	47 ± 17	21 ± 15	4 ± 6
Serum	17 ± 3	15 ± 1	6 ± 3	3 ± 6
Muscle	6 ± 1	5 ± 4	1 ± 0	--
Skin	20 ± 7	12 ± 4	1 ± 1	--
Adipose Tissue	200 ± 64	193 ± 125	33 ± 8	5 ± 1

Urinary metabolites of 1,2,4-TMB 24 hours after single oral dose in rats (values ± SD)									
	%Dose (0.08 mmol/kg) in urine			**%Dose (0.8 mmol/kg) in urine**					
	Free	**Conjugated**	**Total**	**Free**		**Conjugated**		**Total**	
Metabolite	**All rats**	**All rats**	**All rats**	**Rat 1**	**Rat 2**	**Rat 1**	**Rat 2**	**Rat 1**	**Rat 2**
2,3,5-AND 2,4,5-TMP[a]	2.6 ± 1.2	5.1 ± 1.4	7.7 ± 2.2	2.5	1.5	4.3	2.0	6.7	3.5
2,3,6-TMP	--	3.9 ± 0.7	4.0 ± 0.6	0.1	0.4	2.1	1.5	2.1	1.8
Total phenols	2.7 ± 1.1	9.0 ± 2.0	11.8 ± 2.9	2.6	1.9	6.3	3.5	8.8	5.3
2,4-DMBOH[b]	0.1 ± 0.1	12.5 ± 2.6	12.7 ± 2.6	0.1	0.4	11.5	7.2	11.6	7.6
2,5-DMBOH	0.1 ± 0.0	11.6 ± 2.7	11.7 ± 2.7	0.1	0.2	8.7	8.7	8.8	8.9
3,4-DMBOH	--	1.9 ± 0.9	1.9 ± 0.8	--	0.1	0.9	0.8	0.9	0.9
Total alcohols	0.2 ± 0.1	26.0 ± 5.5	26.3 ± 5.4	0.1	0.7	21.1	16.8	21.2	17.5
2,4-DMBA[c]	0.8 ± 0.1	5.2 ± 2.0	6.0 ± 2.0	0.8	2.5	6.8	1.5	7.6	4.0
2,5-DMBA	0.5 ± 0.0	3.1 ± 1.3	3.6 ± 1.3	0.3	1.2	3.5	2.1	3.9	2.3
3,4-DMBA	0.2 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	0.1	0.2	0.5	0.2	0.5	0.4
Total benzoic acids	1.5 ± 0.1	8.9 ± 3.4	10.4 ± 3.3	1.2	3.9	10.8	3.8	11.9	6.7
2,4-DMHA[d]	5.0 ± 1.9	2.0 ± 1.0	7.0 ± 2.6	3.3	2.7	4.8	1.2	8.1	3.7
2,5-DMAH	0.5 ± 0.2	0.3 ± 0.3	0.8 ± 0.3	0.2	0.1	0.5	0.1	0.7	0.2
3,4-DMHA	27.3 ± 8.4	3.3 ± 1.2	30.2 ± 9.4	23.1	17.9	15.6	7.1	38.7	25.0
Total hippuric acids	32.7 ± 10.5	5.6 ± 2.3	37.9 ± 12.1	26.6	20.8	20.9	8.4	47.5	28.9
Total metabolies	37.1 ± 11.4	49.5 ± 13.0	86.4 ± 23.0	30.4	27.2	59.1	32.4	89.5	58.4

Comments: Many tissues examined for radioactive and metabolite content. Multiple metabolites measured. Small numbers of rats per dose group, particularly for the 0.8 mmol/kg group (n = 2). Time points only extend to 24 hours.

[a]trimethylphenol, [b]dimethylbenzoic alcohol, [c]dimethylbenzoic acid, [d]dimethylyhippuric acid.

Source: Huo et al. (1989)

Table B-55. Characteristics and quantitative results for Mikulski and Wiglusz (1975)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	9 rats/dose	Unspecified	1.2 g/kg BW 1,2,3-, 1,2,4-, and 1,3,5-TMB	48 hrs

Additional study details

- Rats weighing between 210 and 350 g were with treated with 1,2,3-, 1,2,4-, or 1,3,5-TMB at 1.2 g/kg body weight.
- In one experiment, urine was collected every 4 hrs over a period of 3 days.
- In a second experiment, metabolites were collected from rats were treated with mesitylene (1,3,5-TMB), pseudocumene (1,2,4-TMB), or hemimellitene (1,2,3-TMB).
- Phenobarbital was found to inhibits the metabolism of TMBs to dimethylhippuric acids

Urinary excretion of glycine, glucuronic, and sulphuric acid conjugates of TMBs

	% of dose (mean ± SD)			
Not treated	**Glycine conjugates**	**Glucuronides**	**Organic sulphates**	**Total**
1,3,5-TMB	59.1 ± 5.2	4.9 ± 1.0	9.2 ± 0.8	73.2
1,2,4-TMB	23.9 ± 2.3	4.0 ± 0.5	9.0 ± 2.1	36.9
1,2,3-TMB	10.1 ± 1.2	7.9 ± 1.3	15.0 ± 3.5	33.0
Treated with Phenobarbital				
1,3,5-TMB	35.1 ± 3.4	9.8 ± 1.3	8.1 ± 1.4	53.0
1,2,4-TMB	30.6 ± 2.5	12.2 ± 2.8	17.4 ± 3.6	60.2
1,2,3-TMB	5.7 ± 1.1	11.3 ± 2.0	22.3 ± 3.0	39.3

Comments; Kinetic data for all three TMB isomers and their metabolites were included in study. However, the authors did not report method for dosing.

Source: Mikulski and Wiglusz (1975)

Table B-56. Characteristics and quantitative results for Swiercz et al. (2002)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Imp:DAK Wistar rats	M	4/dose	Inhalation	25, 100, or 250 ppm (123, 492, 1,230 mg/m^3) 1,2,4-TMB	6 hrs

Additional study details

- Two males and two females were exposed to 25, 100, or 250 ppm (123, 492, 1,230 mg/m^3) 1,2,4-TMB in an inhalation chamber for 6 hrs.
- 1,2,4-TMB concentration was determined via gas chromatography.
- Blood samples were taken from the tail vein at various time points up to 6 hrs after start of exposure.
- The half-life of 1,2,4-TMB elimination was found to increase with increasing exposure.

Air concentrations of 1,2,4-TMB and body mass of rats (means ± SD)

Biological material	**1,2,4-TMB nominal concentration**	**1,2,4-TMB actual concentration (ppm)**	**Rat body weight (g)**
Blood during 6 hr exposure	25 ppm (123 mg/m^3)	25 ± 2	200 ± 10
	100 ppm (492 mg/m^3)	109 ± 10	228 ± 10
	250 ppm (1,230 mg/m^3)	262 ± 21	190 ± 12
Blood after 6 hr exposure	25 ppm (123 mg/m^3)	26 ± 3	349 ± 6
	100 ppm (492 mg/m^3)	101 ± 3	333 ± 18
	250 ppm (1,230 mg/m^3)	238 ± 9	336 ± 5
Urine after 6 hr exposure	25 ppm (123 mg/m^3)	27 ± 3	355 ± 10
	100 ppm (492 mg/m^3)	98 ± 3	338 ± 10
	250 ppm (1,230 mg/m^3)	240 ± 7	330 ± 12

Blood 1,2,4-TMB concentration: During 6 hour inhalation exposure (mean ± SD)

	1,2,4-TMB concentration		
Time	**25 ppm (123 mg/mg^3)**	**100 ppm (492 mg/mg^3)**	**250 ppm 1,230 mg/mg^3)**
15 (min)	0.22 ± 0.07	1.12 ± 0.80	4.02 ± 0.85
30	0.33 ± 0.08	1.99 ± 1.09	4.87 ± 1.61
45	0.49 ± 0.16	3.56 ± 0.49	6.97 ± 1.22
1 (hrs)	0.53 ± 0.14	4.29 ± 0.60	8.67 ± 0.54
2	0.73 ± 0.16	5.10 ± 0.34	14.5 ± 2.6
3	0.80 ± 0.17	6.22 ± 0.70	17.8 ± 1.6
4	0.72 ± 0.15	7.40 ± 1.05	20.0 ± 0.5
5	0.79 ± 0.22	7.72 ± 1.48	23.3 ± 2.6
6	0.94 ± 0.16	8.32 ± 1.34	23.6 ± 1.8

Table B-56 (Continued): Characteristics and quantitative results for Swiercz et al. (2002)

Blood concentrations of 1,2,4-TMB: Following 6 hour exposure (mean ± SD)			
	1,2,4-TMB concentration		
Time	**25 ppm (123 mg/mg^3)**	**100 ppm (492 mg/mg^3)**	**250 ppm 1,230 mg/mg^3)**
3 (min)	0.68 ± 0.09	4.44 ± 1.54	20.9 ± 4.03
15	0.47 ± 0.04	3.72 ± 0.96	20.7 ± 5.13
30	0.40 ± 0.05	2.98 ± 0.88	17.1 ± 4.71
45	0.36 ± 0.04	2.89 ± 0.86	15.9 ± 5.74
1 (hrs)	0.34 ± 0.03	1.79 ± 0.49	14.9 ± 3.77
2	0.23 ± 0.04	1.25 ± 0.33	10.2 ± 3.04
3	0.17 ± 0.04	0.88 ± 0.29	8.05 ± 2.25
4	0.12 ± 0.02	0.61 ± 0.20	6.13 ± 1.64
5	0.10 ± 0.02	0.41 ± 0.14	3.98 ± 0.43
6	0.08 ± 0.02	0.33 ± 0.06	3.20 ± 0.52
Dimethylbenzoic acid (DMBA) urine concentrations: After 6 hour exposure to 1,2,4-TMB (mean ± SD)			
1,2,4-TMB	**2,5-DMBA (mg/L)**	**2,4-DMBA (mg/L)**	**3,4-DMBA (mg/L)**
25 ppm (123 mg/m^3)	23.6 ± 8.6	37.6 ± 12.9	79.9 ± 33.3
100 ppm (492 mg/m^3)	54.0 ± 5.4	130.9 ± 22.1	200.8 ± 25.8
250 ppm (1,230 mg/m^3)	109.4 ± 71.1	308.8 ± 220.1	571.8 ± 381.6

Comment: Metabolites (DMBAs) measured in urine. Appropriate number of animals per dose group (n = 4). Exposure duration possibly not sufficient to detect other metabolic changes.

Source: Swiercz et al. (2002)

Table B-57. Characteristics and quantitative results for Swiercz et al. (2003)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	4/dose	Inhalation	25, 100, or 250 ppm (123, 492, 1,230 mg/m^3) 1,2,4-TMB	6 hrs or 4 weeks

Additional study details

- Male Wistar rats were exposed to either 25, 100, or 250 ppm (123, 492, 1,230 mg/m^3) pseudocumene (1,2,4-TMB) in an inhalation chamber for either 6 hrs or 4 weeks.
- Rats were sacrificed following exposure period and tissues were analyzed 1,2,4-TMB content via gas chromatography.
- Venous elimination was found to follow an open two-compartment model.
- Within brain structures, the brainstem was found to contain the highest levels of 1,2,4-TMB.

Air concentrations of 1,2,4-TMB in inhalation chamber and body weight (mean ± SD)

Biological material	**1,2,4-TMB nominal concentration in inhaled air**	**1,2,4-TMB actual concentration in inhaled air (ppm)**	**Rat body weight (g)**
Arterial blood and brain structure from rats after 6 hrs	25 ppm (123 mg/m^3)	21 ± 2	219 ± 13
	100 ppm (492 mg/m^3)	116 ± 5	180 ± 28
	250 ppm (1,230 mg/m^3)	215 ± 15	220 ± 24
Arterial blood and brain structure from rats after 4 weeks	25 ppm (123 mg/m^3)	24 ± 3	327 ± 21
	100 ppm (492 mg/m^3)	99 ± 7	295 ± 31
	250 ppm (1,230 mg/m^3)	249 ± 19	268 ± 21
Liver, lung, and brain homogenate after 6 hrs	25 ppm (123 mg/m^3)	28 ± 1	227 ± 15
	100 ppm (492 mg/m^3)	123 ± 9	246 ± 11
	250 ppm (1,230 mg/m^3)	256 ± 7	228 ± 12
Liver, lung, and brain homogenate after 4 weeks	25 ppm (123 mg/m^3)	25 ± 2	310 ± 10
	100 ppm (492 mg/m^3)	103 ± 8	328 ± 23
	250 ppm (1,230 mg/m^3)	249 ± 13	320 ± 20
Venous blood collected following 4 week exposure	25 ppm (123 mg/m^3)	24 ± 3	321 ± 6
	100 ppm (492 mg/m^3)	99 ± 7	300 ± 22
	250 ppm (1,230 mg/m^3)	249 ± 19	373 ± 48

Table B-57 (Continued): Characteristics and quantitative results for Swiercz et al. (2003)

Venous blood 1,2,4-TMB concentrations after 4 week inhalation exposure			
	1,2,4-TMB concentration mean ± SD		
Time	**25 ppm (123 mg/mg^3)**	**100 ppm (492 mg/mg^3)**	**250 ppm 1,230 mg/mg^3)**
3 (min)	0.56 ± 0.18	4.06 ± 0.46	13.77 ± 3.34
15	0.43 ± 0.10	3.73 ± 1.21	11.82 ± 3.05
30	0.33 ± 0.03	3.02 ± 1.43	8.28 ± 2.07
45	0.28 ± 0.05	2.86 ± 0.89	7.21 ± 1.84
1 (hr)	0.22 ± 0.02	2.62 ± 0.82	6.27 ± 1.72
2	0.17 ± 0.06	1.83 ± 0.17	4.50 ± 1.04
3	0.11 ± 0.04	0.88 ± 0.24	3.17 ± 0.76
4	0.07 ± 0.04	0.64 ± 0.21	1.73 ± 0.37
5	0.07 ± 0.01	0.39 ± 0.11	1.30 ± 0.22
6	0.06 ± 0.02	0.37 ± 0.14	1.25 ± 0.22
Liver, lung, and brain homogenates and arterial blood 1,2,4-TMB concentrations following inhalation exposure (mean ± SD)			
Exposure	**25 ppm (123 mg/mg^3)**	**100 ppm (492 mg/mg^3)**	**250 ppm 1,230 mg/mg^3)**
Blood 6 hrs (mg/L)	0.31 ± 0.12	1.24 ± 0.41	7.76 ± 1.64
Blood 4 weeks (mg/L)	0.33 ± 0.11	1.54 ± 0.32	7.52 ± 2.11
Brain 6 hrs (mg/kg)	0.49 ± 0.06	2.92 ± 0.73	18.34 ± 1.92
Brain 4 weeks (mg/kg)	0.45 ± 0.05	2.82 ± 0.40	18.63 ± 4.27
Liver 6 hrs (mg/kg)	0.44 ± 0.01	7.13 ± 1.31	28.18 ± 5.34
Liver 4 weeks (mg/kg)	0.45 ± 0.15	3.00 ± 0.49*	22.47 ± 4.10
Lung 6 hrs (mg/kg)	0.43 ± 0.11	4.14 ± 0.54	18.90 ± 3.72
Lung 4 weeks (mg/kg)	0.47 ± 0.20	3.74 ± 0.82	22.47 ± 4.10
1,2,4-TMB in various brain structures following 1,2,4-TMB inhalation exposure			
	1,2,4-TMB concentration (mg/kg), mean ± SD		
Brain structure (time)	**25 ppm (123 mg/mg^3)**	**100 ppm (492 mg/mg^3)**	**250 ppm 1,230 mg/mg^3)**
Brain stem (6 hrs)	0.54 ± 0.11	3.38 ± 0.84	26.91 ± 5.33
Temporal cortex (6 hrs)	0.31 ± 0.06*	2.30 ± 0.71	13.54 ± 2.33*
Hippocampus (6 hrs)	0.28 ± 0.09*	1.89 ± 0.29*	12.99 ± 2.18*
Cerebellum (6 hrs)	0.32 ± 0.09*	1.99 ± 0.40*	12.91 ± 2.05*
Brain stem (4 weeks)	0.38 ± 0.23	2.33 ± 1.24	21.95 ± 3.81
Temporal cortex (4 weeks)	0.25 ± 0.07	2.03 ± 0.66	15.71 ± 3.54
Hippocampus (4 weeks)	0.41 ± 0.27	3.03 ± 0.48	12.44 ± 2.63*
Cerebellum (4 weeks)	0.33 ± 0.05	3.20 ± 0.40	10.85 ± 2.47*
Comments: Adipose tissue was not examined for 1,2,4-TMB content. Metabolite concentration was not measured. No control group.			

P < 0.05 in comparison to brainstem

Source: Swiercz et al. (2003).

Table B-58. Characteristics and quantitative results for Swiercz et al. (2006)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
IMP:WIST Wistar rats	M	5/dose	Inhalation	25, 100, or 250 ppm (123, 492, 1,230 mg/m^3) 1,3,5-TMB	6 hrs or 4 weeks

Additional study details

- Male Wistar rats were exposed to either 0, 25, 100, or 250 ppm (123, 492, 1.230 mg/m^3) mesitylene (1,3,5-TMB) in an inhalation chamber for either 6 hrs or 4 weeks.
- Rats were sacrificed following exposure period and tissues were analyzed for 1,3,5-TMB content via gas chromatography.
- 1,3,5-TMB was found in the lungs in greater quantities following repeated exposures at 100 ppm (492 mg/m^3) and 250 ppm (1.230 mg/m^3).

Air concentrations of 1,3,5-TMB in inhalation chamber and body weight (mean ± SD)

Biological material	**1,3,5-TMB nominal concentration in inhaled air**	**1,3,5-TMB actual concentration in inhaled air (ppm)**	**Rat body weight (g)**
Liver, lung, and kidney homogenates after 6 hr exposure	Control	0	246 ± 9
	25 ppm (123 mg/m^3)	25 ± 2	254 ± 11
	100 ppm (492 mg/m^3)	97 ± 14	242 ± 14
	250 ppm (1,230 mg/m^3)	254 ± 20	249 ± 7
Liver, lung, and kidney homogenates after 4 week exposure	Control	0	331 ± 17
	25 ppm (123 mg/m^3)	23 ± 2	311 ± 26
	100 ppm (492 mg/m^3)	101 ± 8	320 ± 38
	250 ppm (1,230 mg/m^3)	233 ± 16	328 ± 21
Blood collected after 6 hr exposure	Control	0	251 ± 7
	25 ppm (123 mg/m^3)	24 ± 2	250 ± 5
	100 ppm (492 mg/m^3)	101 ± 7	239 ± 7
	250 ppm (1,230 mg/m^3)	240 ± 22	249 ± 10
Blood collected after 4 week exposure	Control	0	310 ± 9
	25 ppm (123 mg/m^3)	23 ± 2	307 ± 15
	100 ppm (492 mg/m^3)	101 ± 8	310 ± 33
	250 ppm (1,230 mg/m^3)	233 ± 16	309 ± 19
Urine collected after 6 hr exposure	Control	0	280 ± 9
	25 ppm (123 mg/m^3)	25 ± 2	278 ± 10
	100 ppm (492 mg/m^3)	102 ± 10	335 ± 15
	250 ppm (1,230 mg/m^3)	238 ± 27	273 ± 18
Urine collected after 4 week exposure	Control	0	310 ± 10
	25 ppm (123 mg/m^3)	25 ± 2	295 ± 15
	100 ppm (492 mg/m^3)	102 ± 10	331 ± 19
	250 ppm (1,230 mg/m^3)	238 ± 27	320 ± 28

Table B-58 (Continued): Characteristics and quantitative results for Swiercz et al. (2006)

Concentrations of 1,3,5-TMB in various tissues after exposure to 1,3,5-TMB (mean ± SD)				
1,3,5-TMB exposure duration and target concentration	**Liver (µg/g tissue)**	**Lung (µg/g tissue)**	**Kidney (µg/g tissue)**	**Blood (µg/g tissue)**
6 Hrs—25 ppm (123 mg/m^3)	0.30 ± 0.07	0.31 ± 0.12	4.49 ± 1.93	0.31 ± 0.12
6 Hrs—100 ppm (492 mg/m^3)	3.09 ± 0.50	2.87 ± 0.57	13.32 ± 2.58	3.06 ± 0.65
6 Hrs—250 ppm (1,230 mg/m^3)	17.00 ± 6.08	17.36 ± 5.56	31.80 ± 9.44	13.36 ± 1.54
4 Wks—25 ppm (123 mg/m^3)	0.22 ± 0.01	0.42 ± 0.12	1.73 ± 0.30*	0.31 ± 0.08
4 Wks—100 ppm (492 mg/m^3)	3.01 ± 0.58	1.99 ± 0.75	15.61 ± 2.14	2.30 ± 0.52
4 Wks—250 ppm (1,230 mg/m^3)	12.98 ± 4.16	11.20 ± 3.61	35.97 ± 8.53	7.55 ± 1.43**
Concentrations of 3,5-DMBA in various tissues after exposure to 1,3,5-TMB (mean ± SD)				
1,3,5-TMB exposure duration and target concentration (ppm)	**Liver (µg/g tissue)**	**Lung (µg/g tissue)**	**Kidney (µg/g tissue)**	**Urine (mg/18 hrs)**
6 Hrs—25 ppm (123 mg/m^3)	12.62 ± 1.62	2.87 ± 0.55	8.77 ± 0.99	0.52 ± 0.03
6 Hrs—100 ppm (492 mg/m^3)	26.05 ± 2.77	5.50 ± 0.55	27.01 ± 9.86	3.66 ± 0.57
6 Hrs—250 ppm (1,230 mg/m^3)	36.92 ± 1.61	13.39 ± 1.90	60.91 ± 19.78	10.99 ± 3.90
4 Wks—25 ppm (123 mg/m^3)	6.52 ± 0.67**	3.69 ± 1.21	11.06 ± 4.33	0.83 ± 0.15*
4 Wks—100 ppm (492 mg/m^3)	21.67 ± 3.14**	8.90 ± 0.98**	31.03 ± 18.56	4.36 ± 0.86
4 Wks—250 ppm (1,230 mg/m^3)	53.07 ± 5.41**	19.79 ± 2.70**	82.10 ± 14.48	11.92 ± 3.05

Venous blood 1,3,5-TMB concentration following 6 hr 1,3,5-TMB inhalation exposure			
	1,3,5-TMB (µg/mL)		
Time	**25 ppm (123 mg/mg^3)**	**100 ppm (492 mg/mg^3)**	**250 ppm 1,230 mg/mg^3)**
3 (min)	0.31 ± 0.12	3.06 ± 0.65	13.36 ± 1.54
15	0.26 ± 0.13	2.51 ± 0.17	13.05 ± 1.61
30	0.15 ± 0.04	2.35 ± 0.57	12.06 ± 1.23
45	0.10 ± 0.03	1.41 ± 0.27	10.53 ± 1.71
1 (hrs)	0.06 ± 0.02	1.35 ± 0.30	8.85 ± 0.90
2	0.04 ± 0.02	1.34 ± 0.39	6.14 ± 0.53
3	ND	0.79 ± 0.30	4.54 ± 0.67
4	ND	0.57 ± 0.14	3.49 ± 1.16
5	ND	0.38 ± 0.14	2.31 ± 0.67
6	ND	0.20 ± 0.04	0.76 ± 0.06

Table B-58 (Continued): Characteristics and quantitative results for Swiercz et al. (2006)

Venous blood 1,3,5-TMB concentration following 4 week 1,3,5-TMB inhalation exposure			
	1,3,5-TMB (µg/mL)		
Time	25 ppm (123 mg/mg^3)	100 ppm (492 mg/mg^3)	250 ppm 1,230 mg/mg^3)
3 (min)	0.31 ± 0.08	2.30 ± 0.52	7.55 ± 1.43
15	0.26 ± 0.03	1.83 ± 0.47	6.51 ± 1.50
30	0.19 ± 0.02	1.57 ± 0.39	4.56 ± 0.98
45	0.17 ± 0.03	1.41 ± 0.13	3.65 ± 0.62
1 (hrs)	0.12 ± 0.03	1.33 ± 0.15	3.69 ± 1.25
2	0.05 ± 0.01	0.95 ± 0.22	3.14 ± 0.64
3	ND	0.72 ± 0.17	2.28 ± 0.19
4	ND	0.41 ± 0.11	1.74 ± 0.17
5	ND	0.39 ± 0.05	1.23 ± 0.34
6	ND	0.29 ± 0.13	1.14 ± 0.20
Comments: Kinetics of 1,3,5-TMB elimination are reported and discussed in detail. Extensive analysis of 3,5-DMBA. Adipose tissue was not examined for 1,3,5-TMB content.			

*p < 0.05; ** p < 0.01 (respectively: significantly differect from the signal exposure (Student's t-test).

Source: Swiercz et al. (2006)

Table B-59. Characteristics and quantitative results for Tsujimoto et al. (2000)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Slc Wistar rats	M	4 per dose	i.p. in corn oil	0, 0.3, 1, and 3 mmol/kg BW 1,2,4-TMB	2 days

Additional study details

- Groups of four male Wistar rats dosed with 0, 0.3, 1, or 3 mmol/kg BW 1,2,4-TMB.
- Urine samples collected for 2 days.
- HPLC used to quantify amount of dimethylbenzyl mercapturic acid in urine.

Urinary excretion of dimethylbenzyl mercapturic acid in 1,2,4-TMB treated rats

	% of dose ± SD		
Dose (mmol/kg)	**0–24 hr**	**24–48 hr**	**Total**
0.3	14.0 ± 1.2	ND	14.0 ± 1.2
1.0	19.4 ± 1.8	ND	19.4 ± 1.8
3.0	16.7 ± 6.2	2.5 ± 1.6	19.2 ± 4.8

Comments: This study observed a marked decrease in dimethylbenzyl mercapturic acid excretion between 24 and 48 hours following exposure. Authors do not report specific speciation data for 2,4-, 2,5-, or 3,4-dimethylbenzyl mercapturic acid.

Source: Tsujimoto et al. (2000)

Table B-60. Characteristics and quantitative results for Tsujimoto et al. (2005)

Study Design

Species	Sex	N	Exposure route	Dose range	Exposure duration
Wistar rats	M	4 per dose	i.p. in corn oil	0, 0.3, 1, and 3 mmol/kg BW given 1,2,3- or 1,3,5-TMB	2 days

Additional study details

- Groups of four male Wistar rats were given 1,2,3- or 1,3,5-TMB intraperitoneally in doses of 0, 0.3, 1, or 3 mmol/kg BW.
- Urine samples collected for 2 days, then analyzed for trimethylphenols (TMP) via GC-MS

Urinary excretion (% of dose ± SD) of phenolic metabolites in 1,2,3-TMB treated rats

Dose (mmol/kg)	2,3,4-Trimethylphenol			3,4,5-Trimethylphenol		
	0-24 hr	24-48 hr	Total	0-24 hr	24-48 hr	Total
0.3	5.90 ± 2.62	0.46 ± 0.34	6.36 ± 2.92	ND	ND	ND
1.0	7.93 ± 5.00	0.35 ± 0.16	8.28 ± 4.85	≤0.24	ND	≤0.24
3.0	6.20 ± 3.45	0.57 ± 0.34	6.77 ± 3.60	≤0.19	≤0.04	≤0.19

Urinary excretion (% of dose ± SD) of phenolic metabolites in 1,3,5-TMB treated rats

Dose (mmol/kg)	2,4,6-Trimethylphenol		
	0-24 hr	24-48 hr	Total
0.3	7.04 ± 1.24	0.53 ± 0.29	7.57 ± 0.99
1.0	4.39 ± 0.61	0.51 ± 0.12	4.90 ± 0.64
3.0	3.32 ± 0.58	0.82 ± 0.34	4.14 ± 0.67

Comments: This study observed a marked decrease in TMP excretion between 24 and 48 hours following exposure. This study does not include data for 1,2,4 TMB and phenolic metabolites. Variation between rats (high standard deviation) within exposure groups.

ND – not detected

Source: Tsujimoto et al. (2005)

Table B-61. Characteristics and quantitative results for Tsujino et al. (2002)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Wistar rats	M	3 for Experiment 1, 36 for Experiment 3 (shown below in Figure 3)	Dermal (via saturated cotton)	1 mL kerosene	0, 1, 3, or 6 hrs

Additional study details

- In first experiment, rats were dermally exposed to kerosene on a saturated, sealed piece of cotton for 1 hr to analyze TMB and aliphatic hydrocarbon (AHC) dermal absorption.
- In second experiment, 44 rats were divided into four groups which varied by exposure duration, post-exposure time, and/or exposure either before or after death.
- TMBs were detected at greater levels than AHCs, and were only detected in traces following post-mortem exposure.
- Trace concentrations of TMBs following post-mortem exposure suggest TMB must circulate in blood before being distributed to organs.

1 hr exposure and ratio of TMBs to internal standard (o-xylene d_{10}) (mean ± SD)

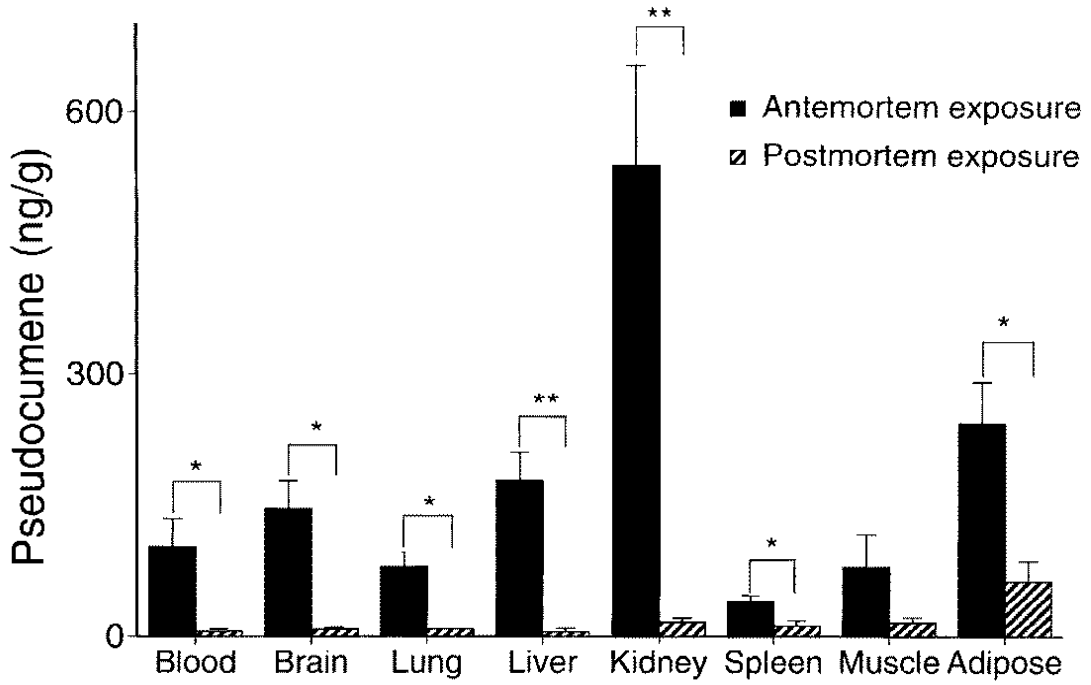
Tissue source	**Post-mortem samples spiked with kerosene (positive control)**	**Post-mortem samples following dermal exposure**
Blood	3.6 ± 1.6	0.4 ± 0.4
Brain	1.2 ± 0.5	0.14 ± 0.05*
Lung	1.2 ± 0.5*	0.09 ± 0.03
Liver	1.1 ± 0.5	0.3 ± 0.09**
Spleen	0.7 ± 0.3	0.1 ± 0.04
Kidney	1.0 ± 0.4	0.5 ± 0.1**
Muscle	1.2 ± 0.5*	0.09 ± 0.02
Adipose	0.9 ± 0.3*	0.15 ± 0.07
OVERALL	1.4 ± 0.3***	0.21 ± 0.05*

Table B-61 (Continued): Characteristics and quantitative results for Tsujino et al. (2002)

1,2,4-TMB in Various Tissues following 1 hr Exposure and Ante vs. Post-Mortem Exposure

Figure 3. 1,2,4-TMB levels in rats immediately after 1 hour of dermal exposure to kerosene are compared between ante-mortem (group I) and post-mortem (group IV) groups.

Data represent mean ± SE. The data were analyzed using two-way ANOVA (* $p < 0.05$, ** $p < 0.01$)



Source: Tsujino et al. (2002)

Comments: Number of tissues were tested and number of animals used in the ante- and post-mortem 1 hr exposure groups (20 and 16 respectively). The authors conclude that their data shows that TMBs are dispersed throughout the body by circulation in blood following dermal exposure. Small number of animals used to determine dermal absorption at 1 hour (n = 3). No data provided for effects of exposure (if any).

*, **, *** $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$

Source: Tsujimoto et al. (2005)

Table B-62. Characteristics and quantitative results for Zahlsen et al. (1990)

Study design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Sprague-Dawley rats	M	24	Inhalation	1,000 ppm (4,920 mg/m^3) 1,2,4-TMB	12 hr exposures on days 1, 3, 7, 10, and 14

Additional Study details

- Male Sprague-Dawley rats were exposed to 1,000 ppm (4,920 mg/m^3) 1,2,4-TMB in an inhalation for 12 hrs on days 1, 3, 7, 10, and 14.
- Food and water was given ad libitum except during exposure, and animal weight ranged between 150 g and 200 g prior to exposure on day 1.
- Hydrocarbon concentration in blood was determined via head space gas chromatography. Daily mean concentrations did not vary by more than ±10% from nominal concentrations.
- Multiple exposures to 1,2,4-TMB resulted in decreases in blood concentrations following subsequent exposures, possibly due to the induction of metabolic enzymes that play a role in the metabolism of 1,2,4-TMB.

Figure 1. Blood concentrations (+SD) of n-nonane, 1,2,4-TMB, and 1,2,4-trimethylcyclohexane following 12 hr exposures on days 1, 3, 7, 10, and 14.

Source: Zahlsen et al. (1990)

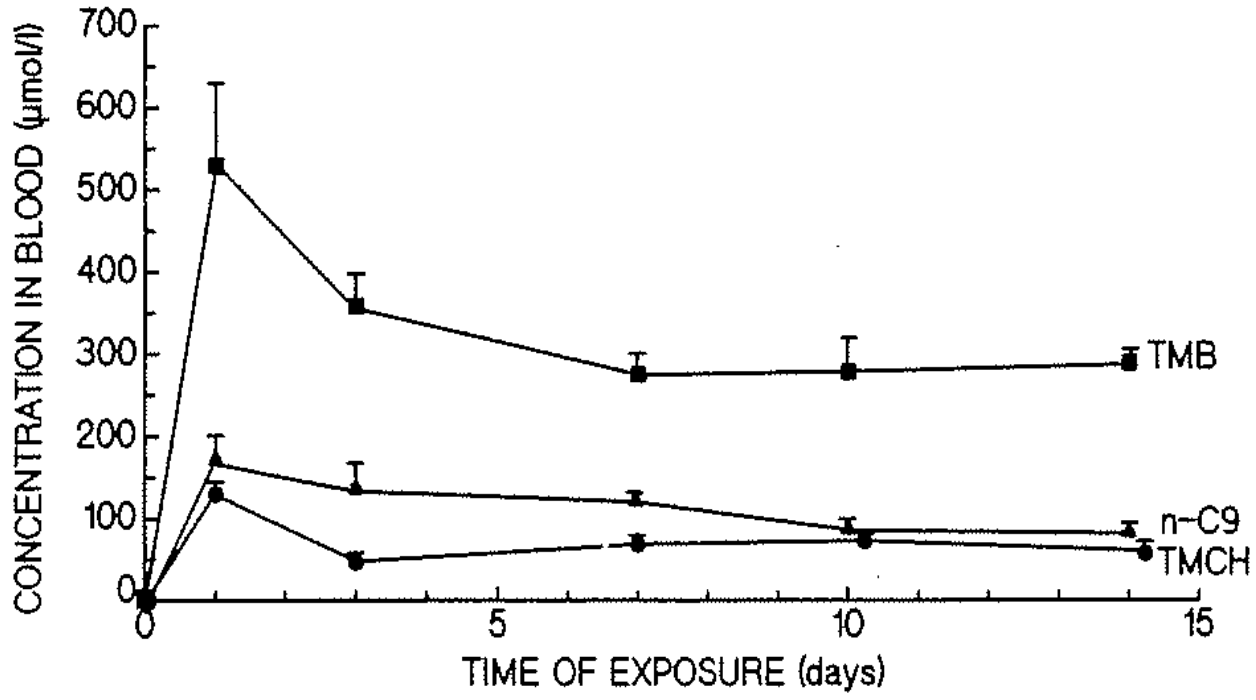


Table B-62 (Continued): Characteristics and quantitative results for Zahlsen et al. (1990)

Figure 2. Brain concentrations (+SD) of n-nonane, 1,2,4-TMB, and 1,2,4-trimethylcyclohexane following 12 hr exposures on days 1, 3, 7, 10, and 14.

Source: Zahlsen et al. (**1990**)

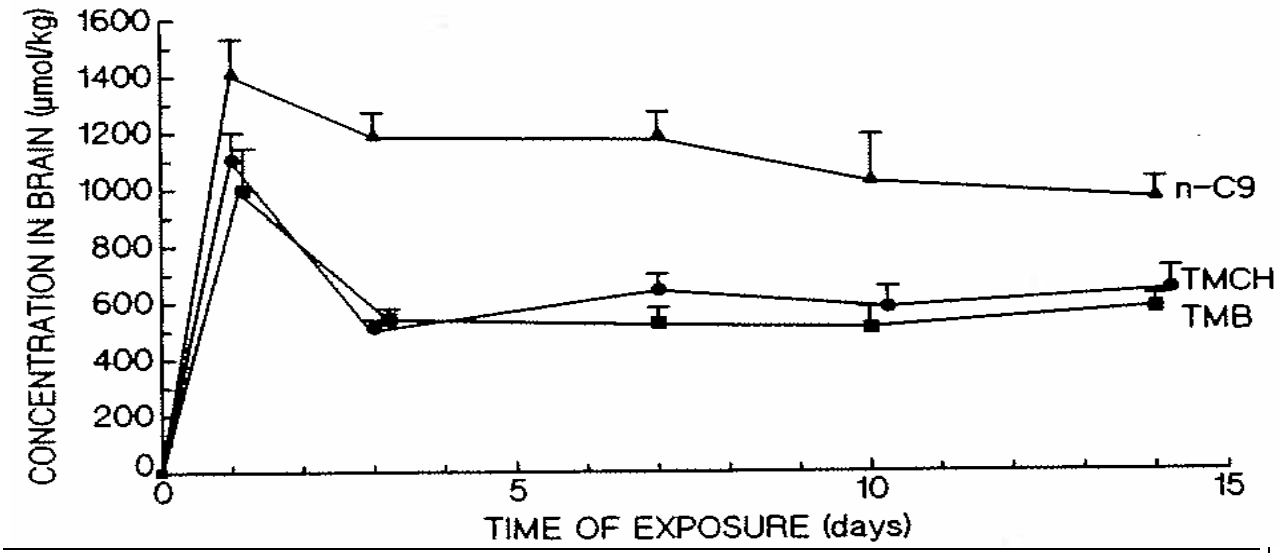


Figure 3. Perirenal fat concentrations (+SD) of n-nonane, 1,2,4-TMB, and 1,2,4-trimethylcyclohexane following 12 hr exposures on days 1, 3, 7, 10, and 14.

Source: Zahlsen et al. (1990)

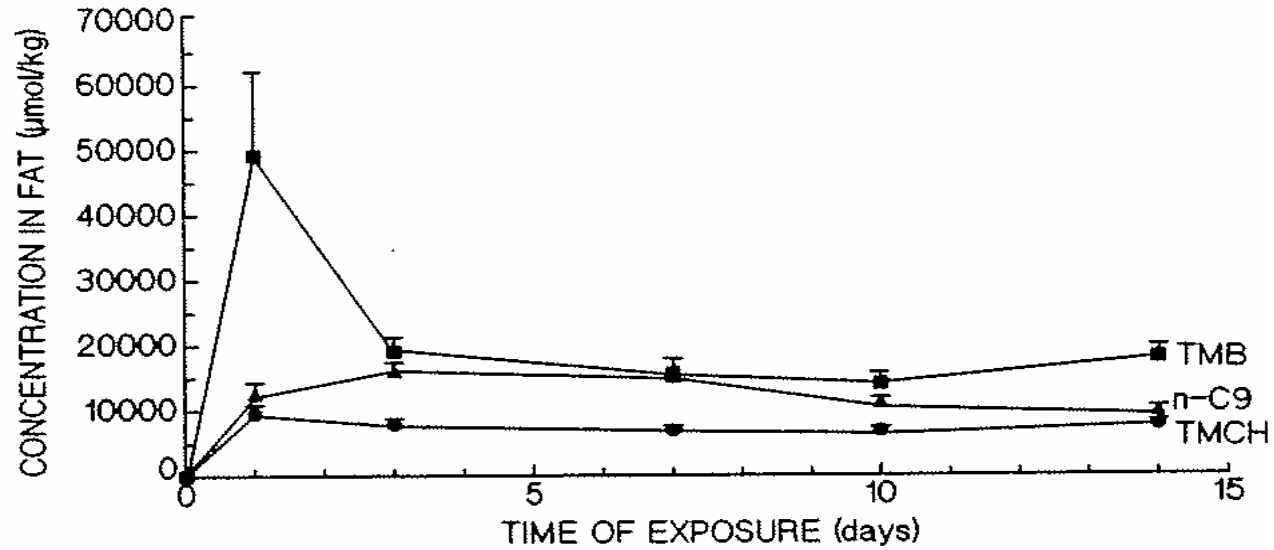


Table B-62 (Continued): Characteristics and quantitative results for Zahlsen et al. (1990)

Brain:blood and fat:blood TMB distribution after 12 hr exposure at end of day 14	
Compound	**Concentration ratio**
Brain:blood TMB ratio	2.0
Fat:blood TMB ratio	63

Comments: Perirenal fat was analyzed and shown to retain higher concentrations of 1,2,4-TMB than blood. Exposure was not continuous (only occurred on days 1, 3, 7, 10, and 15). Only one exposure concentration (1,000 ppm [4,920 mg/m^3]) was tested, and there were no control groups.

Source: Zahlsen et al. (1990).

Table B-63. Characteristics and quantitative results for Zahlsen et al. (1992)

Study Design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Sprague-Dawley rats	M	4/ time point	Inhalation	100 ppm C9-aromate	12 hours/day for 3 days

Additional study details

- Food and water was given ad libitum, except during exposure.
- Rats weighed between 150-200 g and were between 40 and 50 days of age.
- 4 rats were housed in each cage, and each exposure chamber contained 4 cages; 16 rats were present at the beginning of exposure.
- At each time point, 4 rats were sacrificed and their tissues analyzed for C9-aromate presence.

	C9-Aromate Concentration in Rat Tissues at Various Time Points (Mean ± S.D)
Observation	**100 ppm C9 Exposure Group**
Blood Day 1	14.2±0.7
Blood Day 2	12.6±0.9
Blood Day 3	17.1±2.2
Blood Rec[a]	0.2±0.1
Brain Day 1	38.1±1.5
Brain Day 2	34.9±3.9
Brain Day 3	36.5±2.2
Brain Rec	nd
Liver Day 1	41.0±4.5
Liver Day 2	30.5±3.4
Liver Day 3	35.4±2.4
Liver Rec[a]	0.6±0.1
Kidney Day 1	113.8±26.5
Kidney Day 2	142.0±35.2
Kidney Day 3	103.6±18.8
Kidney Rec[a]	2.0±0.3
Fat Day 1	1741±329
Fat Day 2	1375±88
Fat Day 3	1070±93
Fat Rec[a]	120±52

Comments: Data was collected immediately following exposure and 12 hours following exposure, providing insight into metabolic clearance and excretion. Study duration was short term (5 days), making it difficult to determine if tissue concentration changes following chronic exposure.

[a]Rec=After 12 hour recovery

Source: Zahlsen et al. (**1992**)

B.8. ANIMAL AND HUMAN TOXICOKINETIC STUDIES

Table B-64. Characteristics and quantitative results for Meulenberg and Vijverberg (2000)

Study Design					
Species	**Sex**	**N**	**Exposure route**	**Dose range**	**Exposure duration**
Rat and Human	F & M	Varies	n/a	Not given	Not given
Additional study details • Authors examined partition coefficients for many volatile organic compounds from multiple studies. • 1,2,3-, 1,2,4-, and 1,3,5-TMB were among the volatile organic compounds considered for review. • Partition coefficients for blood, fat, brain, liver, muscle, and kidney were reported for both rats and humans.					

Observation	**Partition Coefficients for 1,2,3-, 1,2,4- and 1,3,5-TMB**		
	1,2,3-TMB	**1,2,4-TMB**	**1,3,5-TMB**
Reported and predicted partition coefficients For oil, saline, and air			
$P_{oil:air}$	10,900[a]	10,200[a]	9,880[a]
$P_{saline:air}$	2.73[a]	1.61[a]	1.23[a]
Reported and predicted $P_{tissue:air}$ values for various human tissues			
Blood	66.5[a]	59.1[a]	43[a]
Fat	4879[b]	4566	4423
Brain	220	206	199
Liver	306	286	277
Muscle	155	144	140
Kidney	122	114	110
Reported and predicted $P_{tissue:air}$ values for various rat tissues			
Blood	62.6	55.7	55.7
Fat	6484	6068	5878
Brain	591	552	535
Liver	288	269	260
Muscle	111	104	100
Kidney	1064	995	963
Comment: This study evaluated a number of parameters, presenting predicted partition coefficients for blood, fat, brain, liver, muscle, and kidney tissue in both humans and rats. Reported values based on single trial.			
[a]Averaged values as reported by Järnberg and Johanson (1995). [b]All other values predicted by Meulenberg and Vijverberg (2000).			

APPENDIX C. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

C.1. BENCHMARK DOSE MODELING SUMMARY

This appendix provides technical detail on dose-response evaluation and determination of points of departure (POD) for relevant neurological, hematological, and developmental toxicity endpoints in the TMB database. The endpoints were modeled using the U.S. EPA's Benchmark Dose Software (BMDS, version 2.2). Sections C.1.1.1 and C.1.1.2 (non-cancer) describe the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). In some cases it may be appropriate to use alternative methods, based on statistical judgement; exceptions are noted as necessary in the summary of the modeling results.

C.1.1. Non-Cancer Endpoints

C.1.1.1. Evaluation of Model Fit

For each continuous endpoint, BMDS continuous models were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

C.1.1.2. Model Selection

For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most threefold, the model selected was the one that yielded the lowest Akaike Information Criterion (AIC) value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

Table C-1. Non-cancer endpoints selected for dose-response modeling for 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB

Study, Species (generation) / Sex, and Endpoint	Internal Doses, External Exposure Concentrations, and Effect Data				
Korsak and Rydzyński (1996)					
1,2,4-TMB					
Rat (Wistar) / Male	**Internal Dose (mg/L)**	**0**	**0.1272**	**0.8666**	**5.4424**
CNS: Pawlick (seconds)	No. of animals Mean ± SD	9 15.4 ± 5.8	10 18.2 ± 5.7	9 27.6 ± 4.6	10 30.1 ± 6.1
1,2,3-TMB					
Rat (Wistar) / Male	**Concentration (mg/m^3)**	**0**	**123**	**492**	**1230**
CNS: Pawlick (seconds)	No. of animals Mean ± SD	30 9.7 ± 2.1	20 11.8 ± 3.8	10 16.3 ± 6.3	10 17.3 ± 3.4
Korsak et al. (2000a) – 1,2,4-TMB					
Rat (Wistar) / Male	**Internal Dose (mg/L)**	**0**	**0.1339**	**0.8671**	**5.2481**
Decreased RBC (10^6/cm^3 [10^6 cells/mL])	No. of animals Mean ± SD	10 9.98 ± 1.6	10 9.84 ± 1.82	10 8.50 ± 1.11	10 7.70 + 1.38
Rat (Wistar) / Female	**Internal Dose (mg/L)**	**0**	**0.1335**	**0.8899**	**5.5189**
Clotting time (seconds)	No. of animals Mean ± SD	10 30 ± 10	10 23 ± 4	10 19 ± 5	10 22 ± 7
Korsak et al. (2000b) – 1,2,3-TMB					
Rat (Wistar) / Male	**Concentration (mg/m^3)**	**0**	**128**	**523**	**1269**
Decreased segmented neutrophils (%)	No. of animals Mean ± SD	10 24.8 ± 4.5	10 25.4 ± 5.8	10 20.7 ± 5.8	10 17.7 ± 8.3
Increased reticulocytes (%)	No. of animals Mean ± SD	10 2.8 ± 1.3	10 2.1 ± 1.7	10 3.8 ± 2.1	10 4.5 ± 1.8
Rat (Wistar) / Female	**Concentration (mg/m^3)**	**0**	**128**	**523**	**1269**
Decreased segmented neutrophils (%)	No. of animals Mean ± SD	10 23.1 ± 6.1	10 19.7 ± 3.4	10 16.4 ± 4.2	10 11.9 ± 7.1

Table C-1 (Continued): Non-cancer endpoints selected for dose-response modeling for 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB

Study, Species (generation) / Sex, and Endpoint	**Internal Doses, External Exposure Concentrations, and Effect Data**					
Saillenfait et al. (2005)						
1,2,4-TMB						
F1 rat pups and Dams (SD)	**Concentration (mg/m^3)**	**0**	**492**	**1471**	**2913**	**4408**
Male fetal weight (g)	Number of liters Mean ± SD	23 5.86 ± 0.34	22 5.79 ± 0.30	22 5.72 ± 0.49	22 5.55 ± 0.48	24 5.20 ± 0.42
Female fetal weight (g)	Number of liters Mean ± SD	23 5.57 ± 0.33	22 5.51 ± 0.31	22 5.40 ± 0.45	22 5.28 ± 0.40	24 4.92 ± 0.40
Maternal weight gain (g)	Number of dams Mean ± SD	24 29 ± 12	22 31 ± 14	22 27 ± 12	22 15 ± 17	24 0 ± 14
1,3,5-TMB						
F1 rat pups and Dams (SD)	**Concentration (mg/m^3)**	**0**	**497**	**1471**	**2974**	**5874**
Male fetal weight (g)	Number of liters Mean ± SD	21 5.80 ± 0.41	22 5.76 ± 0.27	21 5.50 ± 0.31	17 5.39 ± 0.55	18 5.10 ± 0.57
Female fetal weight (g)	Number of liters Mean ± SD	21 5.50 ± 0.32	22 5.47 ± 0.21	21 5.27 ± 0.47	17 5.18 ± 0.68	18 4.81 ± 0.45
Maternal weight gain (g)	Number of dams Mean ± SD	21 29 ± 14	22 30 ± 9	21 20 ± 12	17 7 ± 20	18 -12 ± 19

For all endpoints from Korsak et al. (2000a; 1997) and Korsak and Rydzyński (1996), external exposure concentrations were first converted into the internal dose metric of weekly average venous blood concentration (mg/L), and these dose metrics were used as the dose inputs for BMD modeling. Due to PBPK model insufficiency at the high dose (i.e., estimating higher internal blood metrics compared to observed blood data), all high doses were dropped prior to modeling (see Dose-Response Analysis section in Volume 1 for more detail). Section C.2 is included for comparison at the end of this appendix that includes BMD modeling results when the high doses were not dropped. All modeling results (i.e., BMDs and BMDLs) for the Korsak studies are provided in mg/L. As a PBPK model was not applied to the endpoints from Saillenfait et al. (2005), modeling results for these endpoints are provided in mg/m^3. Additionally, as no PBPK model was available for 1,2,3-TMB, all endpoints from Korsak et al. (2000b) are provided in mg/m^3.

Comprehensive modeling results for all endpoints are provided on EPA's Health Effects Research Online (HERO) database (U.S. EPA, 2011b).

C.1.1.3. Model Selection

Below are tables summarizing the modeling results for the non-cancer endpoints modeled. The following parameter restrictions were applied, unless otherwise noted.

- Continuous models: For the polynomial models, restrict beta's in the appropriate direction (i.e., ≥ 0 for responses that increase with dose, and ≤ 0 for responses that decrease with dose); for the Hill, power, and exponential models, restrict power ≥ 1.

Table C-2. Summary of BMD modeling results for increased latency to paw-lick in male Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(constant variance, high dose dropped), (Korsak and Rydzyński, 1996)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b] Exponential (M3)	0.5045	122.2153	0.42102	0.328286	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential model 4 was selected based on lowest BMDL (BMDLs differed by at least 3-fold)
Exponential (M4)	**n/a[c]**	**123.7699**	**0.233402**	**0.0864608**	
Linear[d] Polynomial 2° Polynomial 3° Power	0.6236	122.010727	0.354545	0.259068	

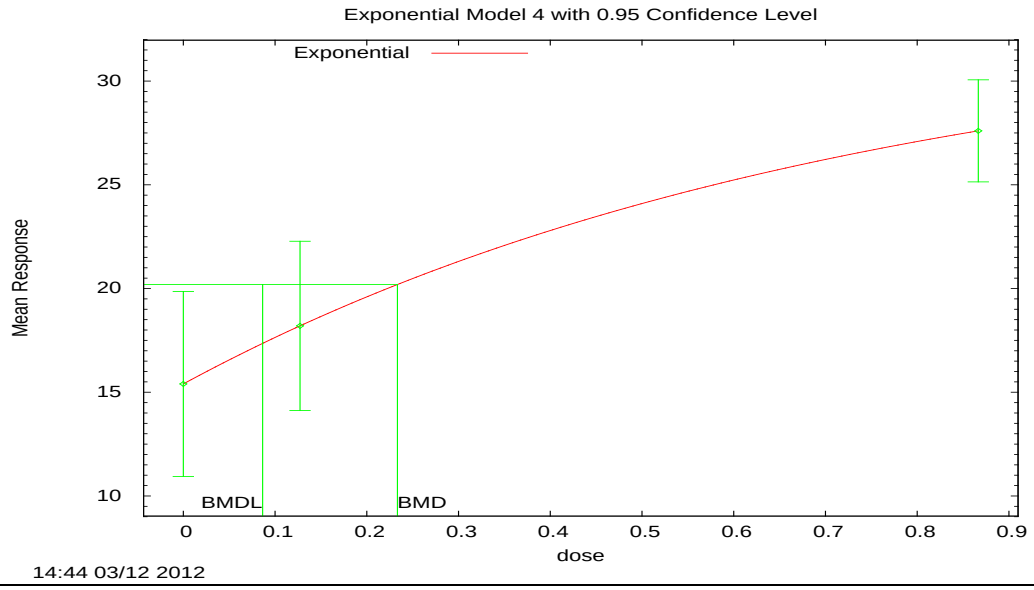
[a] Constant variance case presented (Test 2 *p*-value = 0.169). Selected model in bold; scaled residuals for selected model for concentrations 0, 0.1272, and 0.8666 mg/L were 6.09×10^{-08}, -1.09×10^{-08}, and -3.65×10^{-08} respectively.

[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.

[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). However, inspection of scaled residuals and visual fit indicated appropriate model fit.

[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

Data Source: (Korsak and Rydzyński, 1996)



Note: BMR = 1 SD change from control mean; dose shown in mg/L 1,2,4-TMB (high dose dropped). (Korsak and Rydzyński, 1996)

Figure C-1. Plot of mean response by dose for increased latency to paw-lick in male Wistar rats, with the fitted curve for Exponential model 4 with constant variance.

Exponential Model.
(Version: 1.7; Date: 12/10/2009)
The form of the response function is: Model 2: Y[dose] = a * exp{sign * b * dose}
A constant variance model is fit.

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 0.233402
BMDL at the 95% confidence level = 0.0864608

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
lnalpha	3.13464	3.13464
rho	0	0
a	15.4	14.63
b	13.6063	2.69257
c	2.14406	1.98086
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	9	15.4	15.4	5.8	4.794	6.09×10^{-08}
0.1272	10	18.2	18.2	5.7	4.794	-1.09×10^{-08}
0.8666	9	27.6	27.6	3.2	4.794	-3.65×10^{-08}

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-57.88496	4	123.7699
A2	-56.10689	6	124.2138
A3	-57.88496	4	123.7699
R	-68.59968	2	141.1994
4	-57.88496	4	123.7699

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	24.99	4	< 0.0001
Test 2 (Are Variances Homogeneous, A2 vs. A1)	3.556	2	0.169
Test 3 (Are variances adequately modeled, A2 vs. A3)	3.556	1	0.169
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	0	0	n/a

Table C-3. Summary of BMD modeling results for decreased red blood cells in male Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean (constant variance, high dose dropped), (Korsak et al., 2000a)

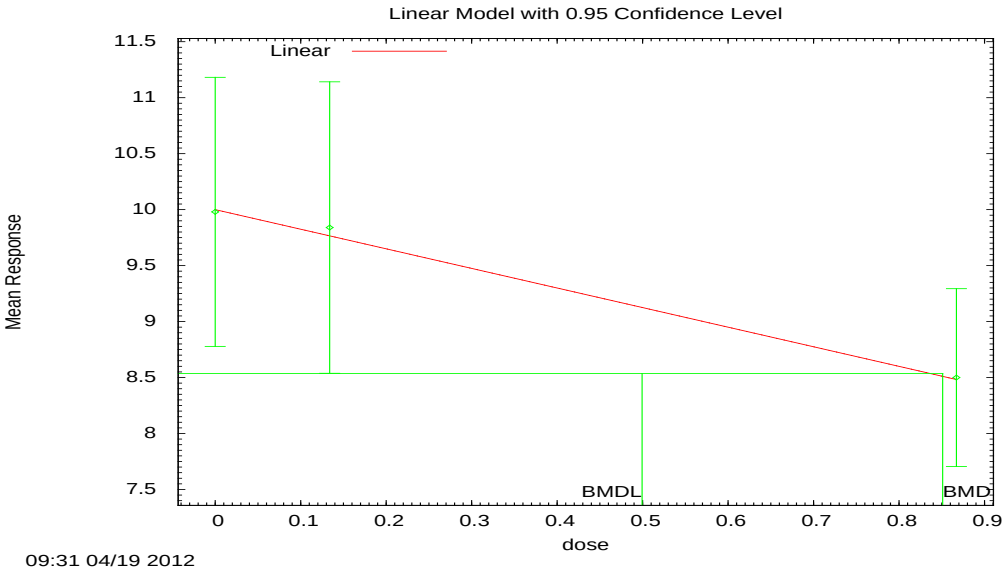
Model[a]	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)	0.8653	59.81949	0.847227	0.467889	Of the models that provided an adequate fit and a valid BMDL estimate, the Linear model was selected based on lowest AIC
Exponential (M3)	n/a[b]	61.79073	0.870338	0.469066	
Exponential (M4)	0.8653	59.81949	0.847227	0.184658	
Linear	**0.8864**	**59.811121**	**0.851043**	**0.499419**	
Polynomial 2°[c] Polynomial 3°	n/a[b]	61.790726	0.869761	0.5002	
Power	n/a[b]	61.790726	0.870176	0.5002	

[a]Constant variance case presented (Test 2 *p*-value = 0.2848). Although Test 1 *p*-value (0.091) was greater than 0.05, visual inspection of the dose-response curve indicates that responses do differ between dose groups. Selected model in bold; scaled residuals for selected model for concentrations 0, 0.1339, and 0.8671 mg/L were -0.0916, 0.108, and -0.0167 respectively.

[b] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). However, inspection of scaled residuals and visual fit indicated appropriate model fit.

[c] For the polynomial 3° model, the b3 coefficient estimate was 0 (boundary). The models in this row reduced to the polynomial 2° model.

Data Source: (Korsak et al., 2000a)



Note: BMR = 1 SD change from control mean; dose shown in mg/L 1,2,4-TMB (high dose dropped) (Korsak et al., 2000a)

Figure C-2. Plot of mean response by dose for decreased red blood cells in male Wistar rats, with the fitted curve for Linear model with constant variance.

Polynomial Model.
(Version: 2.16; Date: 05/26/2010)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + … beta_n*dose^n
A constant variance model is fit.

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 0.851043
BMDL at the 95% confidence level = 0.499419

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	2.21157	2.45563
rho	0	0
beta_0	10.0231	10.0231
beta_1	-1.74743	-1.74743

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	10	9.98	10	1.68	1.49	-0.0916
0.1339	10	9.84	9.79	1.82	1.49	0.108
0.8671	10	8.5	8.51	1.11	1.49	-0.0167

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-26.895363	4	61.790726
A2	-25.639495	6	63.278991
A3	-26.895363	4	61.790726
fitted	-26.905560	3	59.811121
R	-29.647442	2	63.294884

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	8.01589	4	0.091
Test 2 (Are Variances Homogeneous, A2 vs. A1)	2.51173	2	0.2848
Test 3 (Are variances adequately modeled, A2 vs. A3)	2.51173	2	0.2848
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	0.0203948	1	0.8864

Table C-4. Summary of BMD modeling results for decreased clotting time in female Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean (constant and modeled variance, high dose dropped) (Korsak et al., 2000a)

Constant Variance					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/L)**	**$BMDL_{1SD}$ (mg/L)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.0676	151.6841	0.624689	0.35101	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (*U.S. EPA, 2012a*), the data were remodeled using a non-homogenous variance model
Exponential (M4)	n/a[c]	150.3436	0.118085	0.0006662	
Linear[d] Polynomial 2° Polynomial 3° Power	0.05648	151.99019	0.69465	0.441274	
Modeled Variance					
Model[e]	**Goodness-of-fit**		**BMD_{1SD} (mg/L)**	**$BMDL_{1SD}$ (mg/L)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.00949	150.0056	0.829105	0.456483	No model selected as the only appropriate fitting model (Exponential model 4) returned an implausibly low BMDL estimate.
Exponential (M4)[f]	n/a[c]	145.2775	0.154524	0.000850437	
Linear[d] Polynomial 2° Polynomial 3° Power	0.007771	150.362869	0.866447	0.533906	

[a] Constant variance case presented (Test 2 *p*-value = 0.008489).
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups).
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.
[e] Modeled variance case presented (Test 3 *p*-value = 0.1159).
[f] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). However, inspection of scaled residuals and visual fit indicated appropriate model fit. However, this model returned an unreasonably low BMDL value. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

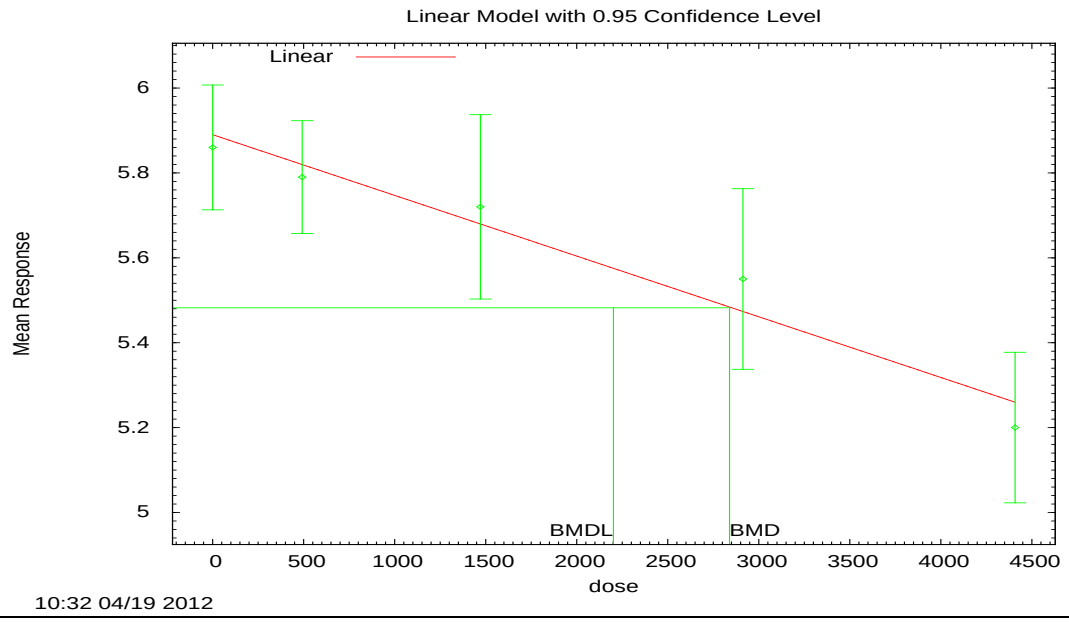
Data Source: (Korsak et al., 2000a).

Table C-5. Summary of BMD modeling results for decreased fetal weight in male Sprague-Dawley rats exposed to 1,2,4-TMB by maternal inhalation on GD6-GD20; BMR = 1 SD or 5% change from control mean (constant variance)(Saillenfait et al., 2005)

BMR = 1 SD change from control mean					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)	0.5714	-84.27301	2,803.48	2,139.69	Of the models that provided an adequate fit and valid BMDL estimate, the Linear model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M3)	0.8333	-83.91341	3,440.45	2,348.58	
Exponential (M4)	0.5714	-84.27301	2,803.48	2,052.08	
Exponential (M5)	0.5459	-81.91341	3,440.45	2,348.58	
Hill	0.5588	-81.936294	3,440.86	2,367.37	
Linear	**0.6217**	**-84.509084**	**2,839.22**	**2,201.74**	
Polynomial 2°	0.8828	-84.028802	3,398.61	2,382.65	
Polynomial 3°	0.9521	-84.179982	3,444.47	2,408.2	
Power	0.8432	-83.937043	3,440.84	2,368.19	
BMR = 5% change from control mean					
Model[a]	**Goodness-of-fit**		**$BMD_{5\%}$ (mg/m^3)**	**$BMDL_{5\%}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)	0.5714	-84.27301	2,009.49	1,577.44	Of the models that provided an adequate fit and valid BMDL estimate, the Linear model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M3)	0.8333	-83.91341	2,861.09	1,716	
Exponential (M4)	0.5714	-84.27301	2,009.49	1,427.9	
Exponential (M5)	0.5459	-81.91341	2,861.09	1,716	
Hill	0.5588	-81.936294	2,857.59	1,749.71	
Linear	**0.6217**	**-84.509084**	**2,057.05**	**1,640.07**	
Polynomial 2°	0.8828	-84.028802	2,798.98	1,760.54	
Polynomial 3°	0.9521	-84.179982	2,841.49	1,777.39	
Power	0.8432	-83.937043	2,857.43	1,750.98	

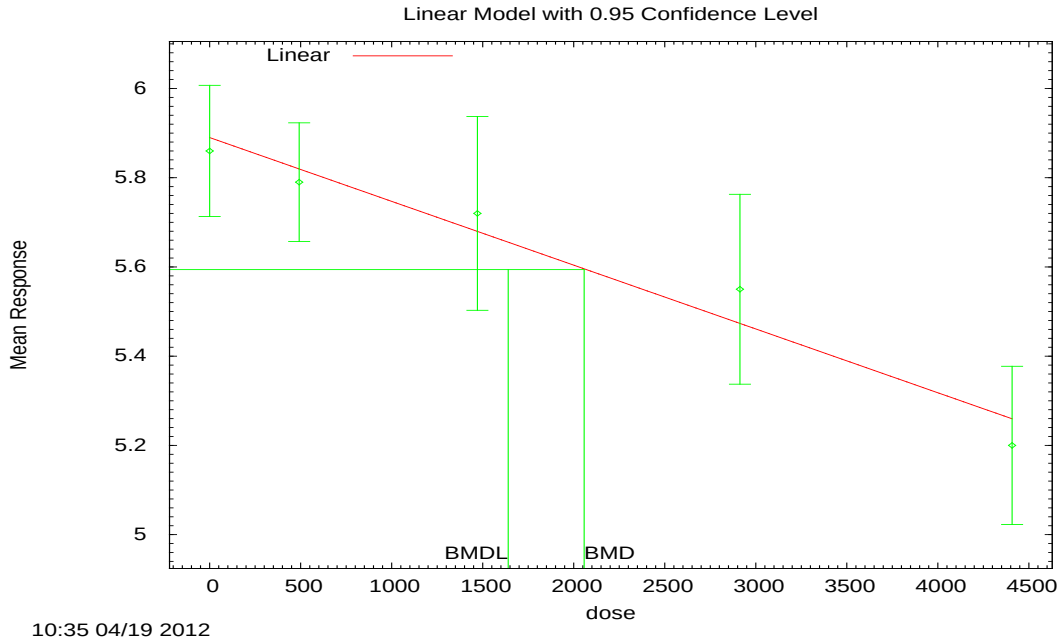
[a]Constant variance case presented (Test 2 *p*-value = 0.1008), selected model in bold; scaled residuals for selected model for concentrations 0, 492, 1,471, 2,913, and 4,408 mg/m^3 were -0.336, -0.324, 0.486, 0.906, -0.694, respectively.

Data source: (Saillenfait et al., 2005)



Note: BMR = 1 SD change from control mean, dose shown in mg/m^3 1,2,4-TMB (Saillenfait et al., 2005)

Figure C-3. Plot of mean response by dose for decreased fetal weight in male Sprague-Dawley rats, with the fitted curve for Linear model with constant variance.



Note: BMR = 5% change from control mean, dose shown in mg/m^3 1,2,4-TMB (Saillenfait et al., 2005).

Figure C-4. Plot of mean response by dose for decreased fetal weight in male Sprague-Dawley rats, with the fitted curve for Linear model with constant variance.

Polynomial Model.
(Version: 2.16; Date: 05/26/2010)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + … beta_n*dose^n
A constant variance model is fit.

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 2839.22
BMDL at the 95% confidence level = 2201.74

BMR = 5% Relative risk
BMD = 2057.05
BMDL at the 95% confidence level = 1640.07

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	0.16139	0.170101
rho	0	0
beta_0	5.88846	5.88821
beta_1	-0.000143129	-0.000142292

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	23	5.86	5.89	0.34	0.406	-0.336
492	22	5.79	5.82	0.3	0.406	-0.324
1471	22	5.72	5.63	0.49	0.406	0.486
2913	22	5.55	5.47	0.48	0.406	0.906
4408	24	5.2	5.26	0.42	0.406	-0.694

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	46.139026	6	-80.278052
A2	50.018128	10	-80.036256
A3	46.139026	6	-80.278052
fitted	45.254542	3	-84.509084
R	28.974008	2	-53.948016

Tests of Interest

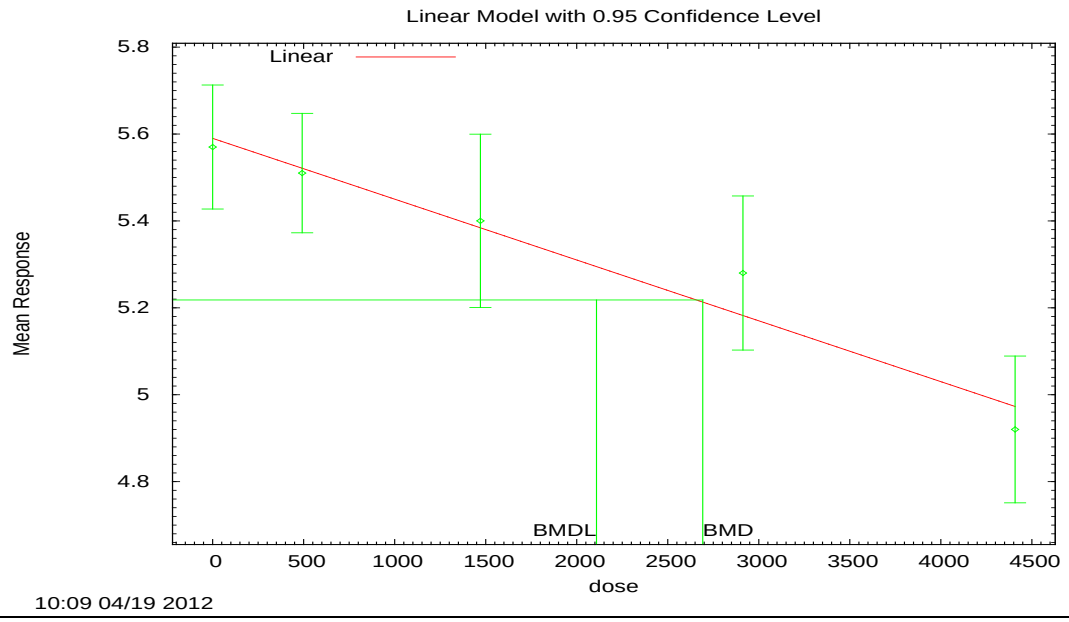
Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	42.0882	8	< 0.0001
Test 2 (Are Variances Homogeneous, A2 vs. A1)	7.7582	4	0.1008
Test 3 (Are variances adequately modeled, A2 vs. A3)	7.7582	4	0.1008
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	1.76897	3	0.6217

Table C-6. Summary of BMD modeling results for decreased fetal weight in female Sprague-Dawley rats exposed to 1,2,4-TMB by maternal inhalation on GD6-GD20; BMR = 1 SD or 5% change from control mean (constant variance; Saillenfait et al., 2005)

BMR = 1 SD change from control mean					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)	0.5056	-101.6488	2,650.97	2,044.51	Of the models that provided an adequate fit and valid BMDL estimate, the linear model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M3)	0.654	-101.1358	3,312.88	2,212.4	
Exponential (M4)	0.5056	-101.6488	2,650.97	1,947.94	
Exponential (M5)	0.3568	-99.13583	3,312.88	2,212.4	
Hill	0.3698	-99.180649	3,311.58	2,241.33	
Linear	**0.5547**	**-101.899075**	**2,692.29**	**2,108.65**	
Polynomial 2°	0.7252	-101.342513	3,258.79	2,264.38	
Polynomial 3°	0.832	-101.617243	3,322.13	2,306.76	
Power	0.6693	-101.182018	3,311.53	2,242.38	
BMR = 5% change from control mean					
Model[a]	**Goodness-of-fit**		**$BMD_{5\%}$ (mg/m^3)**	**$BMDL_{5\%}$ (mg/m^3)**	**Basis for Model Selection *p*-value**
	***p*-value**	**AIC**			
Exponential (M2)	0.5056	-101.6488	1,951.39	1,549	Of the models that provided an adequate fit and valid BMDL estimate, the linear model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M3)	0.654	-101.1358	2,778.64	1,662.76	
Exponential (M4)	0.5056	-101.6488	1,951.39	1,398.32	
Exponential (M5)	0.3568	-99.13583	2,778.64	1,662.76	
Hill	0.3698	-99.180649	2,773.5	1,702.36	
Linear	**0.5547**	**-101.899075**	**2,001.36**	**1,612.89**	
Polynomial 2°	0.7252	-101.342513	2,703.42	1,718.54	
Polynomial 3°	0.832	-101.617243	2,764.88	1,746.99	
Power	0.6693	-101.182018	2,773.32	1,703.72	

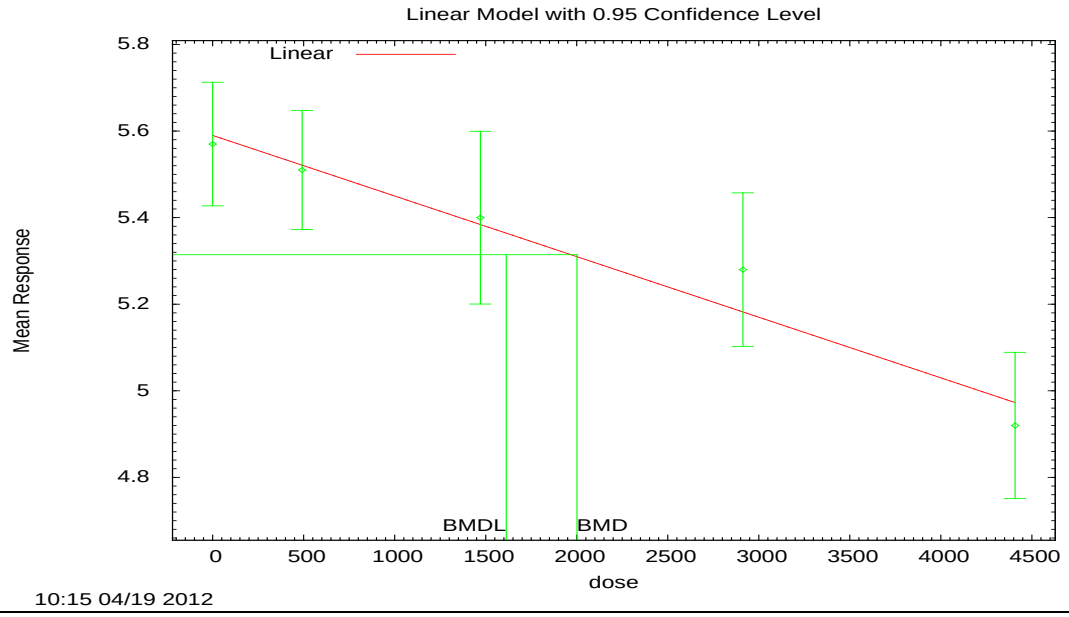
[a]Constant variance case presented (Test 2 *p*-value = 0.3936), selected model in bold; scaled residuals for selected model for concentrations 0, 492, ,1471, 2,913, and 4,408 mg/m^3 were 0.39, -0.187, -0.566, 0.519, -0.158, respectively.

Data source: (Saillenfait et al., 2005)



Note: BMR = 1 SD change from control mean, dose shown in mg/m^3 1,2,4-TMB (Saillenfait et al., 2005)

Figure C-5. Plot of mean response by dose for decreased fetal weight in female Sprague-Dawley rats, with the fitted curve for Linear model with constant variance.



Note: BMR = 5% change from control mean, dose shown in mg/m^3 1,2,4-TMB (Saillenfait et al., 2005)

Figure C-6. Plot of mean response by dose for decreased fetal weight in female Sprague-Dawley rats, with the fitted curve for Linear model with constant variance.

Polynomial Model.
(Version: 2.16; Date: 05/26/2010)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ... beta_n*dose^n
A constant variance model is fit.

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 2692.29
BMDL at the 95% confidence level = 2108.65

BMR = 5% Relative risk
BMD = 2001.36
BMDL at the 95% confidence level = 1612.89

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	0.141584	0.14543
rho	0	0
beta_0	5.59423	5.59388
beta_1	-0.000139761	-0.000138886

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	23	5.57	5.59	0.33	0.376	-0.309
492	22	5.51	5.53	0.31	0.376	-0.193
1471	22	5.4	5.39	0.45	0.376	0.142
2913	22	5.28	5.19	0.4	0.376	1.16
4408	24	4.92	4.98	0.4	0.376	-0.757

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	54.992554	6	-97.985109
A2	57.038880	10	-94.077760
A3	54.992554	6	-97.985109
fitted	53.949538	3	-101.899075
R	36.104870	2	-68.209740

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	41.868	8	< 0.001
Test 2 (Are Variances Homogeneous, A2 vs. A1)	4.09265	4	0.3936
Test 3 (Are variances adequately modeled, A2 vs. A3)	4.09265	4	0.3936
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	2.08603	3	0.5547

Table C-7. Summary of BMD modeling results for decreased maternal body weight gain in female Sprague-Dawley rats exposed to 1,2,4-TMB by inhalation on GD6-GD20; BMR = 1 SD change from control mean (constant variance) (Saillenfait et al., 2005)

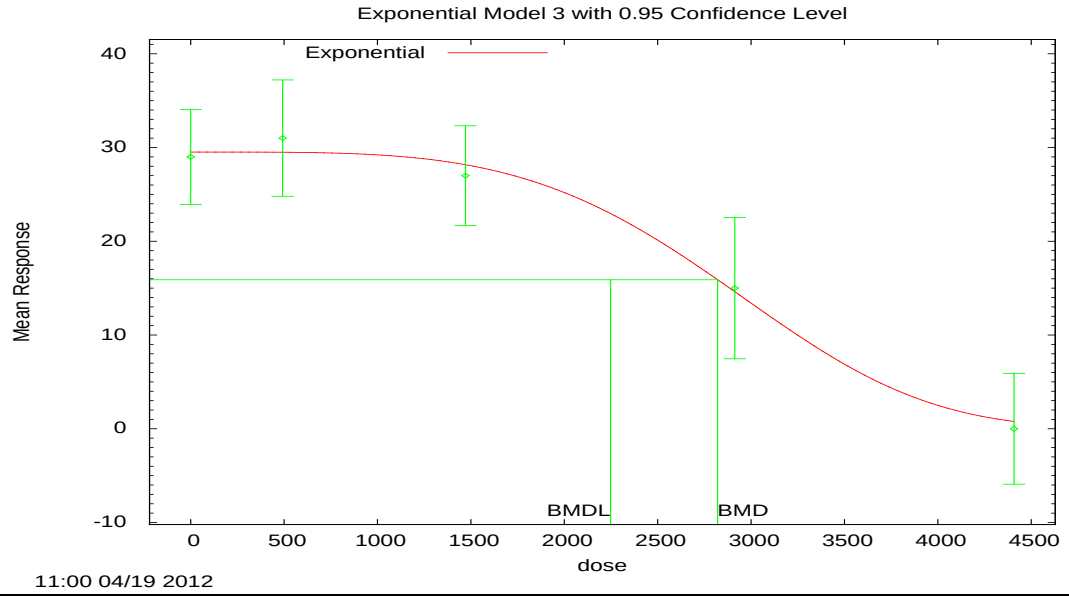
Model[a]	Goodness-of-fit		BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b]	< 0.0001	1,025.385	3.67497	Bad Completion	Of the models that provided an adequate fit and valid BMDL estimate, the Exponential 3 model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M3)	**0.7552**	**717.3518**	**2,821.1**	**2,247.99**	
Exponential (M4)[b]	< 0.0001	773.2296	Not Computed	0	
Exponential (M5)	0.4537	719.3518	2,821.1	2,247.99	
Hill	0.593	719.075964	2,781.23	2,161.92	
Linear	0.1319	720.406291	2,009.47	1,649.63	
Polynomial 2°[c] Polynomial 3°	0.7004	717.502596	2,888.45	2,132.32	
Power	0.7393	717.394507	2,821.04	2,129.53	

[a]Constant variance case presented (Test 2 *p*-value = 0.4284). Selected model in bold; scaled residuals for selected model for concentrations 0, 492, 1,471, 2,913, and 4,408 mg/m^3 were -0.1845, 0.5186, -0.4013, 0.1315, -0.2808, respectively.

[b]The Exponential models 2 and 4 models did not return BMD and/or BMDL values and were excluded from further consideration.

[c] For the polynomial 3° model, the b3 coefficient estimate was 0 (boundary). The models in this row reduced to the polynomial 2° model.

Data source: (Saillenfait et al., 2005).



Note: BMR = 1 SD change from control mean; dose shown in mg/m^3 1,2,4-TMB (Saillenfait et al., 2005).

Figure C-7. Plot of mean response by dose for decreased maternal body weight gain in female Sprague-Dawley rats, with fitted curve for Exponential model 3 with constant variance.

Exponential Model.
(Version: 1.7; Date: 12/10/2009)
The form of the response function is: Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
A constant variance model is fit.

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 2821.1
BMDL at the 95% confidence level = 2247.99

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
lnalpha	5.22238	5.21746
rho	0	0
a	29.5127	0
b	0.000314053	0.000203897
c	0	0
d	3.96638	18

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	24	29	29.51	12	13.62	-0.1845
492	22	31	29.49	14	13.62	0.5186
1471	22	27	28.16	12	13.62	-0.4013
2913	22	15	14.62	17	13.62	0.1315
4408	24	0	0.7804	14	13.62	-0.2808

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-354.3952	6	720.7904
A2	-352.4764	10	724.9529
A3	-354.3952	6	720.7904
R	-386.383	2	776.7661
4	-354.6759	4	717.3518

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	67.81	8	< 0.0001
Test 2 (Are Variances Homogeneous, A2 vs. A1)	3.837	4	0.4284
Test 3 (Are variances adequately modeled, A2 vs. A3)	3.837	4	0.4284
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	0.5615	2	0.7552

Table C-8. Summary of BMD modeling results for increased latency to paw-lick in male Wistar rats exposed to 1,2,3-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(constant variance and modeled variance), (Korsak and Rydzyński, 1996)

Constant Variance					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/L)**	**$BMDL_{1SD}$ (mg/L)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.005704	262.2082	700.938	566.333	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the data were remodeled using a non-homogenous variance model
Exponential (M4)	0.5461	254.2393	192.288	107.132	
Exponential (M5)	n/a[c]	255.8749	201.187	111.315	
Hill	n/a[c]	255.874906	185.863	110.398	
Linear[d] Polynomial 2° Polynomial 3° Power	0.01728	259.991214	577.555	442.59	
Modeled Variance					
Model[e]	**Goodness-of-fit**		**BMD_{1SD} (mg/L)**	**$BMDL_{1SD}$ (mg/L)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	<0.0001	259.5324	496.844	329.318	No model selected as Test 3 *p*-value was < 0.1. This was due to the variance in high dose group. Therefore, the data were remodeled using a non-homogenous variance model and with the high dose dropped (see Table C-9)
Exponential (M4)	0.301	241.4193	86.2091	46.7265	
Exponential (M5)	n/a[c]	242.5858	113.028	51.9836	
Hill	n/a[c]	265.438765	334.7333	Not calculated	
Linear[f] Polynomial 2° Power	0.0003247	254.414778	319.651	195.989	

[a] Constant variance case presented (Test 2 *p*-value = 0.0.0001146).
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups).
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.
[e] Modeled variance case presented (Test 3 *p*-value = 0.07076). This *p*-value indicates that a modeled variance model does not adequately describe the observed variances.
[f] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° model, the b2 coefficient estimate was 0 (boundary). The polynomial 3° did not converge. The models in this row reduced to the Linear model.

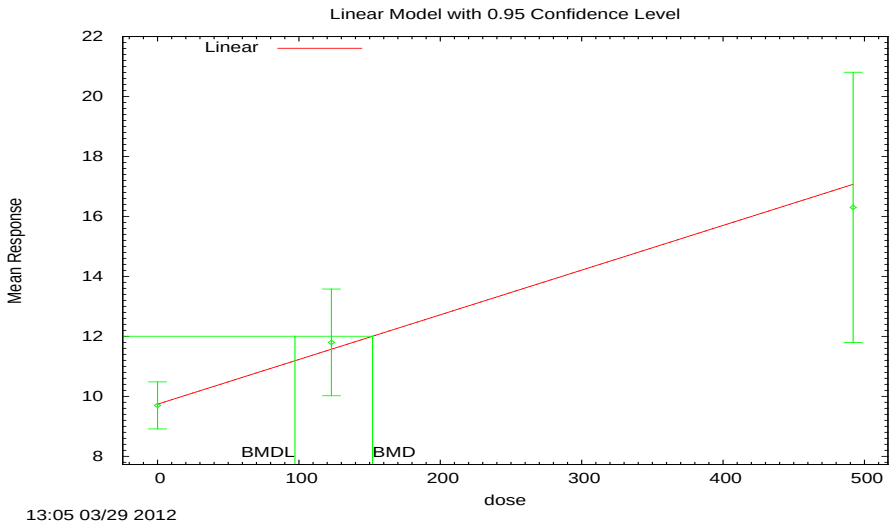
Data Source: (Korsak and Rydzyński, 1996)

Table C-9. Summary of BMD modeling results for increased latency to paw-lick in male Wistar rats exposed to 1,2,3-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(modeled variance, high dose dropped), (Korsak and Rydzyński, 1996)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) [b] Exponential (M3)	0.07449	203.2651	192.144	131.627	Of the models that provided an adequate fit and valid BMDL estimate, the linear model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M4)	n/a[c]	202.0839	104.546	52.5736	
Linear[d] Polynomial 2° Polynomial 3° Power	**0.2016**	**201.714812**	**152.065**	**97.1911**	

[a]Modeled variance case presented (Test 3 *p*-value = 0.5008). Selected model in bold; scaled residuals for selected model for concentrations 0, 123, and 492 mg/m^3 were -0.102, 0.319, and -0.354, respectively.
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). However, inspection of scaled residuals and visual fit indicated appropriate model fit.
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

Data Source: (Korsak and Rydzyński, 1996)



Note: BMR = 1 SD change from control mean; dose shown in mg/m^3 1,2,3-TMB (high dose dropped) (Korsak and Rydzyński, 1996)

Figure C-8. Plot of mean response by dose for increased latency to paw-lick in male Wistar rats, with fitted curve for Linear model with modeled variance.

Polynomial Model.
(Version: 2.16; Date: 05/26/2010)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + … beta_n*dose^n
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 152.065
BMDL at the 95% confidence level = 97.1911

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	-7.3421	2.58956
rho	3.94293	0
beta_0	9.74214	9.90769
beta_1	0.0148851	0.0131332

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	30	9.7	9.74	2.1	2.26	-0.102
123	20	11.8	11.6	3.8	3.18	0.319
492	10	16.3	17.1	6.3	6.84	-0.354

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-106.147893	4	220.295786
A2	-95.815379	6	203.630758
A3	-96.041973	5	202.083946
fitted	-96.857406	4	201.714812
R	-116.956260	2	237.912520

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	42.2818	4	<0.0001
Test 2 (Are Variances Homogeneous, A2 vs. A1)	20.665	2	<0.0001
Test 3 (Are variances adequately modeled, A2 vs. A3)	0.453187	1	0.5008
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	1.63087	1	0.2016

Table C-10. Summary of BMD modeling results for decreased segmented neutrophils in male Wistar rats exposed to 1,2,3-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(constant variance), (Korsak et al., 2000b)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b] Exponential (M3)	**0.7155**	**189.1052**	**915.77**	**534.809**	Of the models that provided an adequate fit and valid BMDL estimate, the Exponential 2 model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M4)	0.4482	191.0108	814.879	261.734	
Exponential (M5)	n/a[c]	192.4867	547.805	137.551	
Hill	n/a[c]	192.486705	564.348	Not calculated	
Linear[d] Polynomial 2° Polynomial 3° Power	0.6711	189.233222	979.089	632.777	

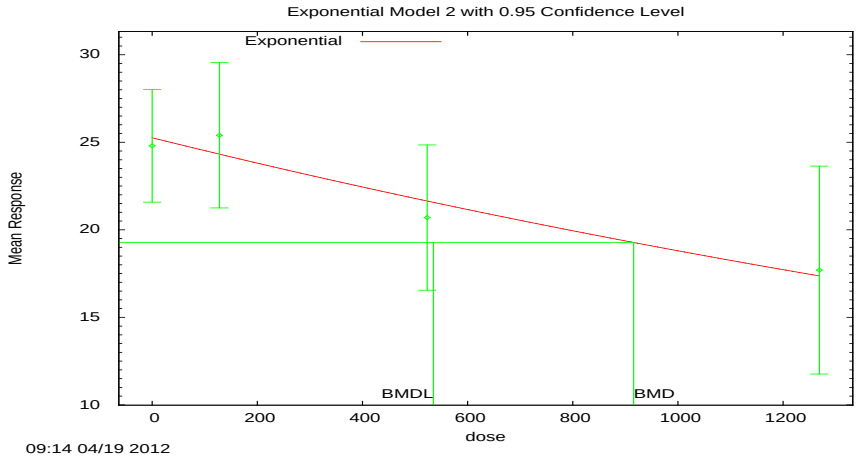
[a]Constant variance case presented (Test 2 *p*-value = 0.2692). Selected model in bold; scaled residuals for selected model for concentrations 0, 123, 492 and 1,230 mg/m^3 were -0.242, 0.5701, -0.4994, and 0.176, respectively.

[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.

[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). Inspection of scaled residuals indicated appropriate model fit. However, inspection of visual fit indicated uncertain dose-response characteristics, and therefore, these models were excluded from consideration.

[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

Data Source: (Korsak et al., 2000b)



Note: BMR = 1 SD change from control mean; dose shown in mg/m^3 1,2,3-TMB (Korsak et al., 2000b)

Figure C-9. Plot of mean response by dose for decreased segmented neutrophils in male Wistar rats, with fitted curve for Exponential model 2 with constant variance.

Exponential Model
(Version: 1.7; Date: 12/10/2009)
The form of the response function is: Model 2: Y[dose] = a * exp{sign * b * dose}
A constant variance model is fit.

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 915.77
BMDL at the 95% confidence level = 534.809

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
lnalpha	3.57763	3.56089
rho	0	0
a	25.2579	19.0843
b	0.000295164	0.00028845
c	0	0
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	10	24.8	25.26	4.5	5.982	-0.242
128	10	25.4	24.32	5.8	5.982	0.5701
523	10	20.7	21.64	5.8	5.982	-0.4994
1269	10	17.7	17.37	8.3	5.982	0.176

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-91.2178	5	192.4356
A2	-89.25328	8	194.5066
A3	-91.2178	5	192.4356
R	-96.16301	2	196.326
4	-91.55261	3	189.1052

Tests of Interest

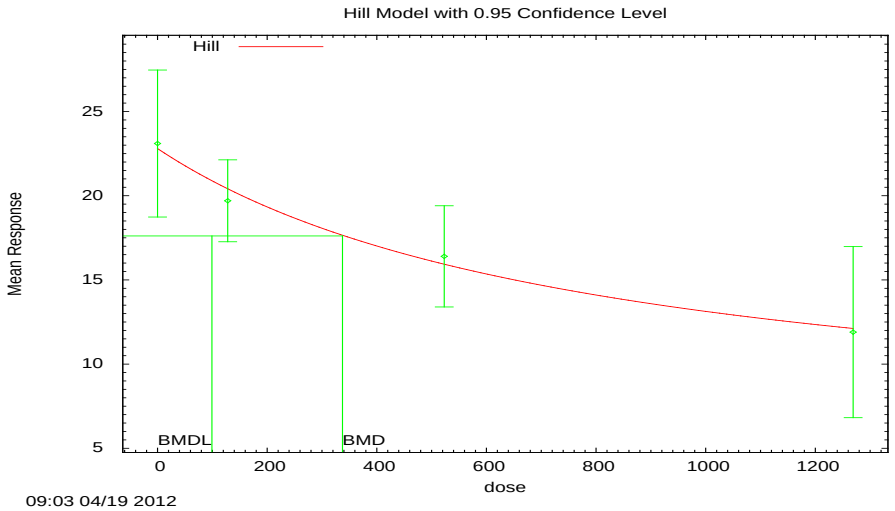
Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	13.82	6	0.03172
Test 2 (Are Variances Homogeneous, A2 vs. A1)	3.929	3	0.2692
Test 3 (Are variances adequately modeled, A2 vs. A3)	3.929	3	0.2692
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	0.6696	2	0.7155

Table C-11. Summary of BMD modeling results for decreased segmented neutrophils in female Wistar rats exposed to 1,2,3-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(constant variance), (Korsak et al., 2000b)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b] Exponential (M3)	0.6401	177.6514	517.048	334.805	Of the models that provided an adequate fit and valid BMDL estimate, the Hill model was selected based on the lowest BMDL (BMDLs differed by more than 3-fold).
Exponential (M4)[b] Exponential (M5)	0.5208	179.1714	365.397	134.354	
Hill	**0.5692**	**179.083138**	**337.442**	**99.2111**	
Linear[c] Polynomial 2° Polynomial 3° Power	0.4533	178.341743	645.521	465.309	

[a] Constant variance case presented (Test 2 *p*-value = 0.09252). Although this *p*-value is less than 0.10, it indicates a marginal fit at the 95% confidence level, and therefore a constant variance is determined to adequately fit the observed variance data. Selected model in bold; scaled residuals for selected model for concentrations 0, 128, 523, and 1,269 mg/m^3 were 0.209, -0.412, 0.312, and -0.108, respectively.
[b] For Exponential models 3 and 5, the estimate of d was 1 (boundary). Therefore Exponential model 3 reduced to Exponential model 2, and Exponential model 5 reduced to Exponential model 4.
[c] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

Data Source: (Korsak et al., 2000b)



Note: BMR = 1 SD change from control mean; dose shown in mg/m^3 1,2,3-TMB (Korsak et al., 2000b).

Figure C-10. Plot of mean response by dose for decreased segmented neutrophils in female Wistar rats, with fitted curve for Hill model with constant variance.

Hill Model.
(Version: 2.16; Date: 04/06/2011)
The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)
A constant variance model is fit

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 337.442
BMDL at the 95% confidence level = 99.2111

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	26.4982	29.205
rho	0	0
intercept	22.76	23.1
v	-17.5024	-11.2
N	1	1.05772
k	809.89	391.333

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	10	23.1	22.8	6.1	5.15	0.209
128	10	19.7	20.4	3.4	5.15	-0.412
523	10	16.4	15.9	4.2	5.15	0.312
1269	10	11.9	12.1	7.1	5.15	-0.108

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-85.379588	5	180.759176
A2	-82.165225	8	180.330450
A3	-85.379588	5	180.759176
fitted	-85.541569	4	179.083138
R	-95.409822	2	194.819645

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	26.4892	6	0.0001804
Test 2 (Are Variances Homogeneous, A2 vs. A1)	6.42873	3	0.09252
Test 3 (Are variances adequately modeled, A2 vs. A3)	6.42873	3	0.09252
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	0.323962	1	0.5692

Table C-12. Summary of BMD modeling results for increased reticulocytes in male Wistar rats exposed to 1,2,3-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(constant variance), (Korsak et al., 2000b)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b] Exponential (M3)	0.2733	89.08418	1112.25	806.744	Of the models that provided an adequate fit and valid BMDL estimate, the Linear model was selected based on the lowest AIC (BMDLs differed by less than 3-fold).
Exponential (M4)	0.1397	90.67033	900.404	308.017	
Exponential (M5)	n/a[c]	91.37006	540.186	140.925	
Hill	n/a[c]	91.370061	554.848	Not calculated	
Linear[d] Polynomial 2° Polynomial 3° Power	**0.3105**	**88.828645**	**1025.1**	**652.898**	

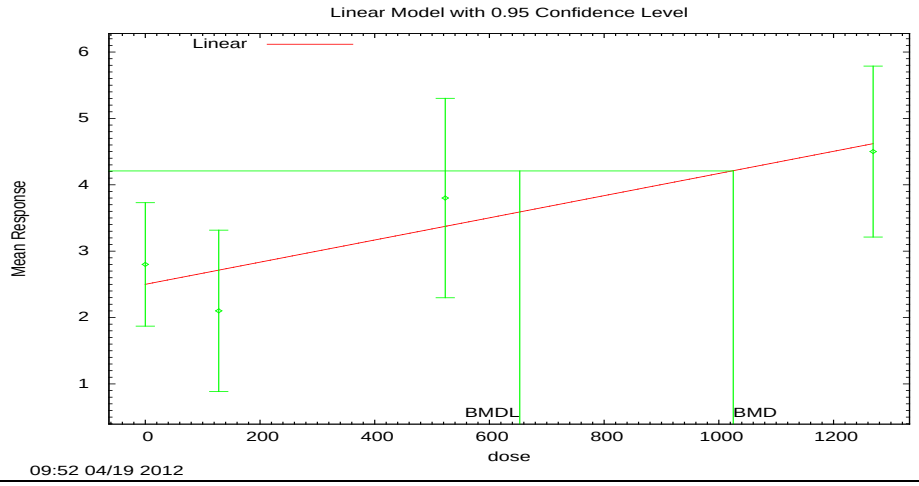
[a] Constant variance case presented (Test 2 *p*-value = 0.5223). Selected model in bold; scaled residuals for selected model for concentrations 0, 128, 523 and 1,269 mg/m^3 were 0.555, -1.14, 0.793, and -0.212, respectively.

[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.

[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). Inspection of scaled residuals indicated appropriate model fit. However, inspection of visual fit indicated uncertain dose-response characteristics, and therefore, these models were excluded from consideration.

[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

Data Source: (Korsak et al., 2000b).



Note: BMR = 1 SD change from control mean; dose shown in mg/m^3 1,2,3-TMB (Korsak et al., 2000b).

Figure C-11. Plot of mean response by dose for increased reticulocytes in male Wistar rats, with fitted curve for Linear model with constant variance.

Polynomial Model.
(Version: 2.16; Date: 05/26/2010)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ... beta_n*dose^n
A constant variance model is fit

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 1025.1
BMDL at the 95% confidence level = 652.989

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	2.91747	3.0575
rho	0	0
beta_0	2.50021	2.50021
beta_1	0.0016623	0.00166623

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	10	2.8	2.5	1.3	1.71	0.555
128	10	2.1	2.71	1.7	1.71	-1.14
523	10	3.8	3.37	2.1	1.71	0.793
1269	10	4.5	4.61	1.8	1.71	-0.212

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-40.244741	5	90.489483
A2	-39.119955	8	94.239910
A3	-40.244741	5	90.489483
fitted	-41.414322	3	88.828645
R	-45.600613	2	95.201226

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	12.9613	6	0.04365
Test 2 (Are Variances Homogeneous, A2 vs. A1)	2.24957	3	0.5223
Test 3 (Are variances adequately modeled, A2 vs. A3)	2.24957	3	0.5223
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	2.33916	2	0.3105

Table C-13. Summary of BMD modeling results for decreased fetal weight in male Sprague-Dawley rats exposed to 1,3,5-TMB by maternal inhalation on GD6-GD20; BMR = 1 SD change from control mean (constant and modeled variance)(Saillenfait et al., 2005)

Constant Variance					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.6927	-66.94125	3,396.62	2,560.01	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the data were remodeled using a non-homogenous variance model
Exponential (M4)	0.6981	-65.6776	2,604.81	1,341.07	
Exponential (M5)	0.397	-63.67902	2,603.37	1,341.3	
Hill	0.4094	-63.715888	2,572.4	1,274.69	
Linear[c] Polynomial 2° Polynomial 3° Power	0.6496	-66.753074	3,513.03	2,694.51	
Modeled Variance					
Model[d]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.5214	-73.29149	2,523.27	1,779.29	No model selected as Test 3 *p*-value was < 0.1. This was due to high variance in control group. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.
Exponential (M4)	0.4304	-71.85947	2,041.7	1,125.34	
Exponential (M5)	0.3877	-70.79949	2,044.66	1,237.6	
Hill	0.4276	-65.644335	2,407.38	1,295.43	
Linear[c] Polynomial 2° Polynomial 3° Power	0.4791	-73.066751	2,636.36	1,890.46	

[a] Constant variance case presented (Test 2 *p*-value = 0.002368)
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.
[d] Modeled variance case presented (Test 3 *p*-value = 0.06027, except the Hill model, for which Test 3 *p*-value = 0.00544).

Data source: (Saillenfait et al., 2005).

Table C-14. Summary of BMD modeling results for decreased fetal weight in female Sprague-Dawley rats exposed to 1,3,5-TMB by maternal inhalation on GD6-GD20; BMR = 1 SD change from control mean (constant and modeled variance)(Saillenfait et al., 2005)

Constant Variance					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.9112	-61.96218	3,581.71	2,669	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the data were remodeled using a non-homogenous variance model
Exponential (M4)[b] Exponential (M5)	0.7655	-59.96227	3,573.06	1,915.99	
Hill	0.7656	-59.962704	3,569.61	1,865.62	
Linear[c] Polynomial 2° Polynomial 3° Power	0.9085	-61.950195	3,676.95	2,794.36	
Modeled Variance					
Model[d]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[b] Exponential (M3)	0.01931	-67.53742	2692.79	1827.72	No model selected as Test 3 *p*-value was < 0.1. This was due to high variance in control group and low variance in the high dose group. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.
Exponential (M4)	0.05097	-69.49883	1481.66	798.275	
Exponential (M5)	0.5334	-73.06401	1469.46	1069.57	
Hill	0.4769	-59.505126	3161.1	1614.44	
Linear[e] Polynomial 2° Polynomial 3° Power	0.0148 0.01552	-67.061071	2841.13	1969.76	

[a] Constant variance case presented (Test 2 *p*-value < 0.0001)

[b] For Exponential models 3 and 5, the estimate of d was 1 (boundary). Therefore Exponential model 3 reduced to Exponential model 2, and Exponential model 5 reduced to Exponential model 4.

[c] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

[d] Modeled variance case presented (Test 3 *p*-value = 0.01301)

[e] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model. The Test 4 *p*-value for the power model (0.01552) was different from the Test 4 *p*-value for the linear or polynomial models (0.0148)

Data source: (Saillenfait et al., 2005).

Table C-15. Summary of BMD modeling results for decreased maternal body weight gain in female Sprague-Dawley rats exposed to 1,3,5-TMB by inhalation on GD6-GD20; BMR = 1 SD change from control mean (constant and modeled variance), (Saillenfait et al., 2005)

Constant Variance					
Model[a]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)	< 0.0001	805.8321	3.36×10^{-51}	Bad Completion	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the data were remodeled using a non-homogenous variance model
Exponential (M3)	< 0.0001	807.8353	6.29281	Bad Completion	
Exponential (M4)	< 0.0001	701.8275	Not Computed	0	
Exponential (M5)	0.00262	649.4267	2,057.15	1,396.23	
Hill	0.5141	639.963339	2,035.36	1,353.4	
Linear[b] Polynomial 2° Polynomial 3°	0.6919	636.99599	1,982.21	1,655.52	
Power	0.4835	638.991033	2,014.88	1,655.77	
Modeled Variance					
Model[c]	**Goodness-of-fit**		**BMD_{1SD} (mg/m^3)**	**$BMDL_{1SD}$ (mg/m^3)**	**Basis for Model Selection**
	***p*-value**	**AIC**			
Exponential (M2)[d]	< 0.0001	921.089	Not Computed	0	Only the power model provided an adequate fit and calculated a BMD and BMDL, and therefore was selected.
Exponential (M3)[d]	< 0.0001	923.089	Not Computed	0	
Exponential (M4)	< 0.0001	698.0766	3.76×10^{-46}	3.76×10^{-46}	
Exponential (M5)	< 0.0001	650.9354	1,476.12	601.777	
Hill	<.0001	728.727708	29.7037	11.8372	
Linear	0.0003338	645.262934	2,749.72	2,330.78	
Polynomial 2°	<.0001	710.199993	-9,999	2,491.63	
Polynomial 3°[,d]	0.2014	631.886974	1,797.1	Not calculated	
Power	**0.1981**	**631.236865**	**1,826.86**	**1,302.02**	

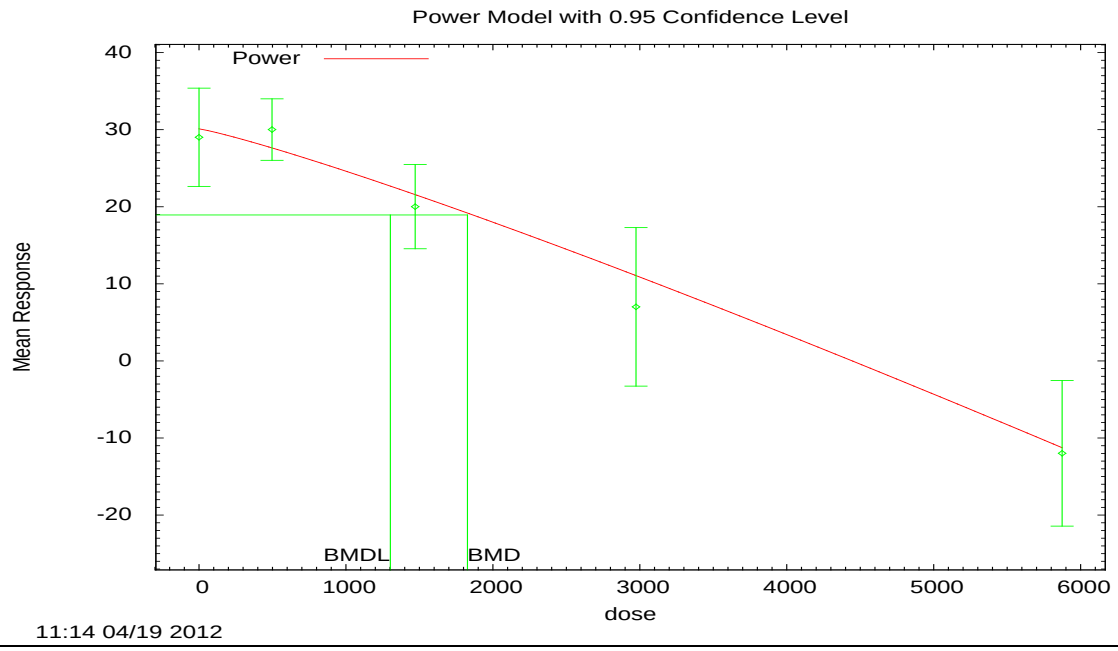
[a] Constant variance case presented (Test 2 *p*-value = 0.003114)

[b] For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

[c] Modeled variance case presented (Test 3 *p*-value = 0.2221). Selected model in bold; scaled residuals for selected model for concentrations 0, 497, 1,471, 2,974, 5,874 mg/m^3 were -0.442, 0.983, -0.47, -0.776, 0.0673, respectively.

[d] The Exponential model 2 and model 3, as well as the polynomial 3° models, did not return BMD and/or BMDL values and were excluded from further consideration.

Data source: (Saillenfait et al., 2005).



Note: BMR = 1 SD change from control mean; dose shown in mg/m^3 1,3,5-TMB (Saillenfait et al., 2005)

Figure C-12. Plot of mean response by dose for decreased maternal body weight gain in female Sprague-Dawley rats, with fitted curve for Power model with modeled variance.

Power Model.
(Version: 2.16; Date: 10/28/2009)
The form of the response function is: Y[dose] = control + slope * dose^power
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 1826.86
BMDL at the 95% confidence level = 1302.02

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
lalpha	8.3667	5.41079
rho	-1.04093	0
control	30.0752	-12
slope	-0.00209481	628.225
power	1.14244	-0.427017

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	21	29	30.1	14	11.2	-0.442
497	22	30	27.6	9	11.7	0.983
1471	21	20	21.4	12	13.3	-0.47
2974	17	7	10.6	20	19.2	-0.776
5874	18	-12	-12.3	19	17.8	0.0673

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-314.768805	6	641.537610
A2	-306.803486	10	633.606972
A3	-308.999390	7	631.998779
fitted	-310.618432	5	631.236865
R	-352.099997	2	708.199993

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	90.593	8	<.0001
Test 2 (Are Variances Homogeneous, A2 vs. A1)	15.9306	4	0.003114
Test 3 (Are variances adequately modeled, A2 vs. A3)	4.39181	3	0.2221
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	3.23809	2	0.1981

C.2. BENCHMARK DOSE MODELING SUMMARY – ALTERNATIVE ANALYSIS WITH HIGH DOSES INCLUDED

The modeling summaries included in this section are for comparison purposes only. After calculation of internal blood dose metrics using the animal PBPK model, the high doses were not dropped in these modeling analyses, even though the PBPK demonstrates poor model fit at high doses. These modeling results were not used in any RfC derivations in Volume 1 of the Toxicological Review.

Table C-16. Summary of BMD modeling results for increased latency to paw-lick in male Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean(constant and modeled variance), (Korsak and Rydzyński, 1996)

Constant Variance					
	Goodness-of-fit		**BMD_{1SD}**	**$BMDL_{1SD}$**	
Model[a]	***p*-value**	**AIC**	**(mg/L)**	**(mg/L)**	**Basis for Model Selection**
Exponential (M2)[b] Exponential (M3)	0.00061	190.1611	3.62226	2.73586	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the data were remodeled using a non-homogenous variance model
Exponential (M4)	0.8239	177.4066	0.242222	0.104385	
Exponential (M5)	n/a[c]	179.3571	0.268238	0.105201	
Hill	n/a[c]	179.357065	0.237108	0.0889465	
Linear[d] Polynomial 2° Polynomial 3° Power	0.0009125	189.355645	3.15451	2.22737	
Modeled Variance					
	Goodness-of-fit		**BMD_{1SD}**	**$BMDL_{1SD}$**	
Model[e]	***p*-value**	**AIC**	**(mg/L)**	**(mg/L)**	**Basis for Model Selection**
Exponential (M2)[b] Exponential (M3)	0.000633	191.8156	3.38239	2.34048	No model selected as Test 3 *p*-value was < 0.10. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.
Exponential (M4)	0.8604	179.1164	0.231414	0.09854	
Exponential (M5)	n/a[c]	181.0855	0.252014	0.0990336	
Hill	n/a[c]	181.982905	0.292816	Not calculated	
Linear[d] Polynomial 2° Polynomial 3° Power	0.001014	190.872265	2.8175	1.72529	

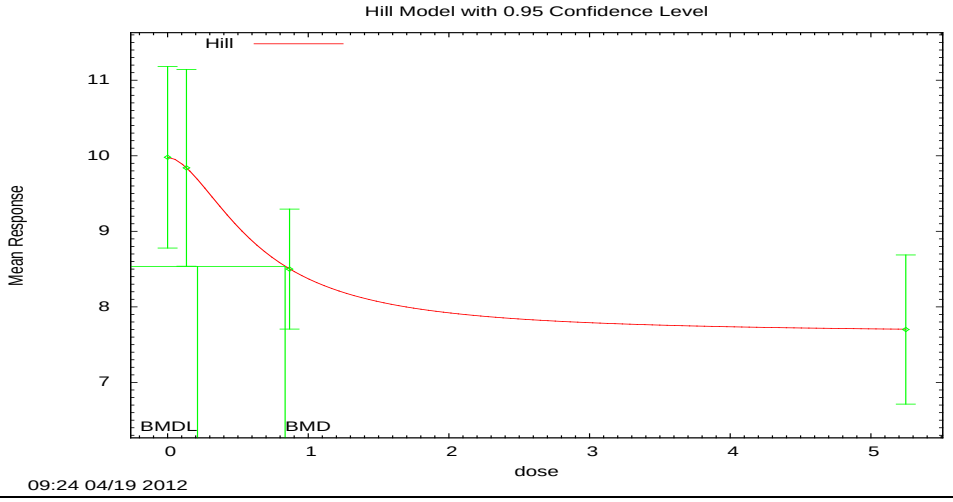
[a] Constant variance case presented (Test 2 *p*-value = 0.07651).
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). Inspection of scaled residuals and visual fit indicated appropriate model fit.
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.
[e] Modeled variance case presented (Test 3 *p*-value = 0.0371)
Data source: (Korsak and Rydzyński, 1996).

Table C-17. Summary of BMD modeling results for decreased red blood cells in male Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean (constant variance), (Korsak et al., 2000a)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b] Exponential (M3)	0.1671	78.98918	3.68518	2.30432	Of the models that provided an adequate fit and a valid BMDL estimate, the Hill model was selected based on lowest BMDL (BMDLs differed by greater than 3-fold)
Exponential (M4)	0.7345	77.52579	0.795033	0.241565	
Exponential (M5)	n/a[c]	79.41075	0.842867	0.249166	
Hill	**n/a[c]**	**79.410749**	**0.835638**	**0.212686**	
Linear[d] Polynomial 2° Polynomial 3° Power	0.1498	79.207001	3.91553	2.5963	

[a]Constant variance case presented (Test 2 *p*-value = 0.4329). Selected model in bold; scaled residuals for selected model for concentrations 0, 0.1339, 0.8671, 5.248 mg/L were -1.93×10^{-08}, 1.75×10^{-08}, $4.83 \times 10^{-08}$and -6.99×10^{-08}, respectively.
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). Inspection of scaled residuals and visual fit indicated appropriate model fit.
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.

Data source: (Korsak et al., 2000a).



Note: BMR = 1 SD change from control mean; dose shown in mg/L 1,2,4-TMB (Korsak et al., 2000a)

Figure C-13. Plot of mean response by dose for decreased red blood cells in male Wistar rats, with fitted curve for Hill model with constant variance.

Hill Model.
(Version: 2.16; Date: 04/06/2011)
The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)
A constant variance model is fit

Benchmark Dose Computations:
BMR = 1 estimated standard deviations from the control mean
BMD = 0.835638
BMDL at the 95% confidence level = 0.212686

Parameter Estimates

Variable	Model	(Default) Initial Parameter Values
alpha	2.08604	2.31783
rho	0	0
intercept	9.98	9.98
v	-2.33466	-2.28
N	1.7672	2.11193
k	0.635516	0.681064

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std	Scaled Resid
0	10	9.98	9.98	1.68	1.44	-1.93e-008
0.1339	10	9.84	9.84	1.82	1.44	1.75e-008
0.8671	10	8.5	8.5	1.11	1.44	4.83e-008
5.248	10	7.7	7.7	1.38	1.44	-6.99e-008

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	-34.705375	5	79.410749
A2	-33.333528	8	82.667056
A3	-34.705375	5	79.410749
fitted	-34.705375	5	79.410749
R	-41.888855	2	87.777711

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1 (Does response and/or variances differ among Dose levels, A2 vs. R)	17.1107	6	0.008885
Test 2 (Are Variances Homogeneous, A2 vs. A1)	2.74369	3	0.4329
Test 3 (Are variances adequately modeled, A2 vs. A3)	2.74369	3	0.4329
Test 4 (Does the model for the Mean fit, A3 vs. fitted)	1.13687e-013	0	n/a

Table C-18. Summary of BMD modeling results for decreased clotting time in female Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean (constant and modeled variance), (Korsak et al., 2000a)

Model[a]	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC			
Constant Variance					
Exponential (M2)[b] Exponential (M3)	0.00311	207.7609	13.2329	4.78502	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance (U.S. EPA, 2012a)*, the data were remodeled using a non-homogenous variance model
Exponential (M4)	0.3078	199.2547	0.119261	0.000258705	
Exponential (M5)	n/a[c]	201.2538	0.12336	0.000534297	
Hill	n/a[c]	201.25379	0.129946	1.20×10^{-10}	
Linear[d] Polynomial 2° Polynomial 3° Power	0.003013	207.824506	12.5899	5.12676	
Modeled Variance					
Model[e]	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)[b] Exponential (M3)	0.0001725	209.2185	16.2811	5.15229	No model selected as the only appropriate fitting models (Exponential model 5) calculated an implausibly low BMDL. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended
Exponential (M4)	0.09227	196.7223	0.297031	0.000698259	
Exponential (M5)	n/a[c]	198.7223	0.235929	7.68×10^{-05}	
Hill	n/a[c]	204.758516	0.138361	Not calculated	
Linear[d] Polynomial 2° Polynomial 3° Power	0.0001675	209.276823	15.0257	5.46511	

[a] Constant variance case presented (Test 2 *p*-value = 0.02286).
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). Inspection of scaled residuals and visual fit indicated appropriate model fit.
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.
[e] Modeled variance case presented (Test 3 *p*-value = 0.2001, except Hill model for which Test 3 *p*-value = < 0.0001).

Data Source: (Korsak et al., 2000a).

Table C-19. Summary of BMD modeling results for decreased reticulocytes in female Wistar rats exposed to 1,2,4-TMB by inhalation for 3 months; BMR = 1 SD change from control mean (constant and modeled variance), (Korsak et al., 2000a)

Constant Variance					
	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	
Model[a]	***p*-value**	**AIC**			**Basis for Model Selection**
Exponential (M2)[b] Exponential (M3)	0.05738	91.21206	5.67056	0.775822	No model selected as Test 2 *p*-value was < 0.10. Therefore, as suggested in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the data were remodeled using a non-homogenous variance model
Exponential (M4)	0.2784	88.67076	0.107641	0.000190582	
Exponential (M5)	n/a[c]	90.67077	0.111117	0.000273446	
Hill	0.3149	88.506257	0.11386	6.85×10^{-15}	
Linear[d] Polynomial 2° Polynomial 3° Power	0.04654	91.631076	6.34191	3.62271	
Modeled Variance					
	Goodness-of-fit		BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)	
Model[e]	***p*-value**	**AIC**			**Basis for Model Selection**
Exponential (M2)[b] Exponential (M3)	0.01667	75.37239	12.0859	4.65557	No model selected as the only appropriate fitting models (Exponential model 5 and Hill models) did not calculate BMDLs. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended
Exponential (M4)[f] Exponential (M5)	0.3582	70.02825	Not Computed	0	
Hill	n/a[c]	89.127269	Not Computed	Not Computed	
Linear[d] Polynomial 2° Polynomial 3° Power	0.009093	76.584735	8.44761	5.29336	

[a] Constant variance case presented (Test 2 *p*-value = < 0.0001).
[b] For Exponential model 3, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 2.
[c] χ^2 test had insufficient degrees of freedom (due to estimated model parameters = dose groups). Inspection of scaled residuals and visual fit indicated appropriate model fit.
[d] For the power model, the power parameter estimate was 1 (boundary). For the polynomial 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary). The models in this row reduced to the Linear model.
[e] Modeled variance case presented (Test 3 *p*-value = 0.253).
[f] For Exponential model 5, the estimate of d was 1 (boundary). The models in this row reduced to exponential model 4.

Data source: (Korsak et al., 2000a).

APPENDIX D. DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law (U.S. Congress, 2011). The report language included direction to EPA for the Integrated Risk Information System (IRIS) Program related to recommendations provided by the National Research Council (NRC) in its review of EPA's draft IRIS assessment of formaldehyde (NRC, 2011). The report language included the following:

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

The NRC's recommendations, provided in Chapter 7 of the review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the tables below. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

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

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

Table D-1. The EPA's implementation of the National Research Council's recommendations in the trimethylbenzenes assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the trimethylbenzenes assessment
General recommendations for completing the IRIS formaldehyde assessment that EPA will adopt for all IRIS assessments (see p. 152)	
1. To enhance the clarity of the document, the draft IRIS assessment needs rigorous editing to reduce the volume of text substantially and address redundancies and inconsistencies. Long descriptions of particular studies should be replaced with informative evidence tables. When study details are appropriate, they could be provided in appendices.	**Implemented.** The overall document structure has been revised in consideration of this NRC recommendation. The new structure includes a concise Executive Summary and an explanation of the literature review search strategy, study selection criteria, and methods used to develop the assessment. The main body of the assessment has been reorganized into two sections, Hazard Identification and Dose-Response Analysis, to help reduce the volume of text and redundancies that were a part of the previous document structure. Section 1 provides evidence tables and a concise synthesis of hazard information organized by health effect, More detailed summaries of the most pertinent epidemiology and experimental animal studies are provided in Appendix B. Information on chemical and physical properties and toxicokinetics is also provided in Appendix B. The main text of the Toxicological Review is approximately 90 pages, which is a major reduction from previous IRIS assessments. Technical and scientific edits were performed to eliminate any redundancies or inconsistencies.

Table D-1 (Continued): The EPA's implementation of the National Research Council's recommendations in the trimethylbenzenes assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the trimethylbenzenes assessment
2. Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various noncancer outcomes. The committee emphasizes that it is not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.	**Implemented.** Chapter 1 has been replaced with a Preamble that describes the application of existing EPA guidance and the methods and criteria used in developing the assessment. The term "Preamble" was chosen to emphasize that these methods and criteria are being applied consistently across IRIS assessments. The new Preamble includes information on identifying and selecting pertinent studies, evaluating the quality of individual studies, weighing the overall evidence of each effect, selecting studies for derivation of toxicity values, and deriving toxicity values. These topics correspond directly to the five steps that the NRC identified in Figure 7-2 of their 2011 report. A new section, Literature Search Strategy and Study Selection, provides detailed information on the search strategy used to identify health effect studies, search outcomes, and selection of studies for hazard identification. This information is chemical-specific and has been designed to provide enough information that an independent literature search would be able to replicate the results. This section also includes information on how studies were selected to be included in the document and provides a link to EPA's Health and Environmental Research Online (HERO) database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited.
3. Standardized evidence tables for all health outcomes need to be developed. If there were appropriates tables, long text descriptions of studies could be moved to an appendix of deleted.	**Implemented.** In the new document template, standardized evidence tables that present key study findings that support how toxicological hazards are identified for all major health effects are provided in Section 1.1. More detailed summaries of the most pertinent epidemiology and experimental animal studies are provided in Appendix B.
4. All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research, for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.	**Partially implemented.** Information in Section 4 of the Preamble provides an overview of the approach used to evaluate the quality of individual studies. Critical evaluation of the epidemiologic and experimental animal studies is included in the evidence tables in Section 1.1. Additional information on study characteristics is found in Appendix B. The study information for TMBs is presented in table format that clearly presents detailed study summary information and key study characteristics. As more rigorous systematic review processes are developed, they will be utilized in future assessments.

Table D-1 (Continued): The EPA's implementation of the National Research Council's recommendations in the trimethylbenzenes assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the trimethylbenzenes assessment
5. The rationales for the selection of the studies that are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.	**Implemented.** The Dose-Response Analysis section of the new document structure provides a clear explanation of the rationale used to select and advance studies that were considered for calculating toxicity values. Rationales for the selection of studies advanced for reference value derivation are informed by the weight-of-evidence for hazard identification as discussed in Section 1.2. In support of the RfC derivations for individual TMB isomers, an exposure-response array was included that compares effect levels for several toxicological effects (Figures 2-1, 2-3, and 2-5). The exposure-response array provides a visual representation of points of departure for various effects resulting from exposure to TMB isomers. The array informs the identification of doses associated with specific effects, and the choice of principal study and critical effects. In the case of TMBs, the database supported development of multiple candidate RfC's. Such values have been developed previously and will be developed in future assessments, where the data allow.
6. Strengthened, more integrative and more transparent discussions of weight-of-evidence are needed. The discussions would benefit from more rigorous and systematic coverage of the various determinants of weight-of-evidence, such as consistency.	**Partially implemented.** A new section, Hazard Identification (Section 1), provides a more strengthened, integrated and transparent discussion of the weight of the available evidence. This section includes standardized evidence tables to present the key study findings that support how potential toxicological hazards are identified and exposure-response arrays for each potential toxicological effect. Weight-of-evidence discussions are provided for each major effect (Section 1.1.1—neurotoxic effects, Section 1.1.2—respiratory effects, Section 1.1.3--reproductive/ developmental effects, and Section 1.1.4—hematological and clinical chemistry effects). A more rigorous and formalized approach for characterizing the weight-of-evidence will be developed as a part of Phase 3 of the implementation process.
General Guidance for the Overall Process (p. 164)	
7. Elaborate an overall, documented, and quality-controlled process for IRIS assessments.	**Implemented.** EPA has created Chemical Assessment Support Teams to formalize an internal process to provide additional overall quality control for the development of IRIS assessments. This initiative uses a team approach to making timely, consistent decisions about the development of IRIS assessments across the Program. This team approach has been utilized for the development of the TMBs assessment. Additional objectives of the teams is to help ensure that the necessary disciplinary expertise is available for assessment development and review, to provide a forum for identifying and addressing key issues prior to external peer review, and to monitor progress in implementing the NRC recommendations.
8. Ensure standardization of review and evaluation approaches among contributors and teams of contributors; for example, include standard approaches for reviews of various types of studies to ensure uniformity.	
9. Assess disciplinary structure of teams needed to conduct the assessments.	

Table D-1 (Continued): The EPA's implementation of the National Research Council's recommendations in the trimethylbenzenes assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the trimethylbenzenes assessment
Evidence Identification: Literature Collection and Collation Phase (p. 164)	
10. Select outcomes on the basis of available evidence and understanding of mode of action.	**Partially implemented.** A new section, Literature Search Strategy and Study Selection, contains detailed information on the search strategy used for the TMBs assessment, including key words used to identify relevant health effect studies. Figure LS-1 depicts the study selection strategy and the number of references obtained at each stage of literature screening. This section also includes information on how studies were selected to be included in the document and provides a link to an external database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited. Each citation in the Toxicological Review is linked to HERO such that the public can access the references and abstracts to the scientific studies used in the assessment. Section 3 of the Preamble summarizes the standard protocols for evidence identification that are provided in EPA guidance. For each potential toxicological effect identified for TMBs, the available evidence is informed by the mode of action information as discussed in Section 1.1. As more rigorous systematic review processes are developed, they will be utilized in future assessments.
11. Establish standard protocols for evidence identification.	
12. Develop a template for description of the search approach.	
13. Use a database, such as the Health and Environmental Research Online (HERO) database, to capture study information and relevant quantitative data.	
Evidence Evaluation: Hazard Identification and Dose-Response Modeling (p. 165)	
14. Standardize the presentation of reviewed studies in tabular or graphic form to capture the key dimensions of study characteristics, weight-of- evidence, and utility as a basis for deriving reference values and unit risks.	**Implemented.** Standardized tables have been developed that provide summaries of key study design information and results by health effect. The inclusion of all positive and negative findings in each health effect-specific evidence table supports a weight-of-evidence analysis. In addition, exposure-response arrays are utilized in the assessment to provide a graphical representation of points of departure for various effects resulting from exposure to TMB. The exposure-response arrays inform the identification of doses associated with specific effects and the weight-of- evidence for those effects.
15. Develop templates for evidence tables, forest plots, or other displays.	**Implemented.** Templates for evidence tables and exposure-response arrays have been developed and are utilized in Section 1.1.
16. Establish protocols for review of major types of studies, such as epidemiologic and bioassay.	**Partially implemented.** General principles for reviewing epidemiologic and experimental animal studies are described in Section 4 of the Preamble. Standardized systematic review is an ongoing process.

Table D-1 (Continued): The EPA's implementation of the National Research Council's recommendations in the trimethylbenzenes assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the trimethylbenzenes assessment
Selection of Studies for Derivation of Reference Values and Unit Risks (p. 165)	
17. Establish clear guidelines for study selection. a. Balance strengths and weaknesses. b. Weigh human vs. experimental evidence c. Determine whether combining estimates among studies is warranted.	**Implemented.** EPA guidelines for study selection, including balancing strengths and weaknesses and weighing human vs. experimental evidence are described in the Preamble (Sections 3-6). These guidelines have been applied in Section 2 of the TMBs assessment to inform the evaluation of the weight-of-evidence across health effects and the strengths and weaknesses of individual studies considered for reference value derivation. In the case of TMBs, the database did not support the combination of estimates across studies. In future assessments, combining estimates across studies will be routinely considered.
Calculation of Reference Values and Unit Risks (pp. 165-166)	
18. Describe and justify assumptions and models used. This step includes review of dosimetry models and the implications of the models for uncertainty factors; determination of appropriate points of departure (such as benchmark dose, no-observed-adverse-effect level, and lowest observed-adverse-effect level), and assessment of the analyses that underlie the points of departure.	**Implemented as applicable.** The rationale for the selection of the point of departure (a 95% lower confidence limit on the benchmark dose; BMDL) for the derivation of the inhalation reference value for 1,2,4-TMB and 1,2,3-TMB is transparently described in Section 2. The determination of sufficient similarity regarding 1,3,5-TMB and 1,2,4-TMB, and the decision to adopt the RfC for 1,2,4-TMB as the RfC for 1,3,5-TMB, is transparently described in Section 2. The rationale for the route-to-route extrapolation in order to use inhalation data for derivation of an RfD for 1,2,4-TMB is transparently described in Section 2. The determination of sufficient similarity regarding 1,2,3-, 1,2,4-, and 1,3,5-TMB, and the decision to adopt the RfD for 1,2,4-TMB as the RfDs for 1,2,3-TMB and 1,3,5-TMB, is transparently described in Section 2. A summary of the benchmark dose modeling for the derivation of the reference values for effects other than cancer, including an alternative analysis with high doses included, is described in Appendix C.
19. Provide explanation of the risk-estimation modeling processes (for example, a statistical or biologic model fit to the data) that are used to develop a unit risk estimate.	**Not applicable.** The TMB assessment concludes that there is inadequate information to assess the carcinogenic potential. Therefore, a unit risk estimate for cancer was not derived.

Table D-1 (Continued): The EPA's implementation of the National Research Council's recommendations in the trimethylbenzenes assessment

NRC recommendations that EPA is implementing in the short term	Implementation in the trimethylbenzenes assessment
20. Provide adequate documentation for conclusions and estimation of reference values and unit risks. As noted by the committee throughout the present report, sufficient support for conclusions in the formaldehyde draft IRIS assessment is often lacking. Given that the development of specific IRIS assessments and their conclusions are of interest to many stakeholders, it is important that they provide sufficient references and supporting documentation for their conclusions. Detailed appendixes, which might be made available only electronically, should be provided when appropriate.	**Implemented.** The new template structure that has been developed in response to the NRC recommendations provides a clear explanation of the literature search strategy, study selection criteria, and methods used to develop the TMBs assessment. It provides for a clear description of the decisions made in developing the hazard identification and dose-response analysis. Information contained in the Preamble and throughout the document reflects the guidance that has been utilized in developing the assessment. As recommended, supplementary information is provided in the accompanying appendices.

Table D-2. National Research Council recommendations that the EPA is generally implementing in the long term

NRC recommendations that the EPA is generally implementing in the long term	Implementation in the trimethylbenzenes assessment
Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (p. 165) 1. Review use of existing weight-of-evidence guidelines. 2. Standardize approach to using weight-of-evidence guidelines. 3. Conduct agency workshops on approaches to implementing weight-of-evidence guidelines. 4. Develop uniform language to describe strength of evidence on noncancer effects. 5. Expand and harmonize the approach for characterizing uncertainty and variability. 6. To the extent possible, unify consideration of outcomes around common modes of action rather than considering multiple outcomes separately.	As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC, including the development of a standardized approach to describe the strength of evidence for noncancer effects. On May 16, 2012, EPA announced (U.S. EPA, 2012b) that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. In addition, EPA will hold a workshop on August 26, 2013, on issues related to weight-of-evidence to inform future assessments.
Calculation of Reference Values and Unit Risks (pp. 165-166) 7. Assess the sensitivity of derived estimates to model assumptions and end points selected. This step should include appropriate tabular and graphic displays to illustrate the range of the estimates and the effect of uncertainty factors on the estimates.	As discussed in Section 1.2, although the nervous system is the primary and most sensitive target of inhaled TMB toxicity, there is evidence of effects in other organ systems. Candidate RfCs for 1,2,4-TMB and 1,2,3-TMB are evaluated together in Figures 2-2 and 2-4 (respectively), including the uncertainty factors applied to individual endpoints.

APPENDIX E. SUMMARY OF AVAILABLE C9 AROMATIC HYDROCARBON FRACTION TOXICITY STUDIES

As part of a testing program mandated under Section 4(a) of the Toxic Substances Control Act (TSCA), a series of toxicity tests were performed that investigated the mutagenicity, developmental and reproductive toxicity, subchronic neurotoxicity, and general inhalation toxicity of the C9 aromatic hydrocarbon fraction (C9 fraction), which is mostly comprised of the ortho-, meta-, and para- isomers of ethyltoluene (2-, 3-, and 4-ethyltoluene, respectively) and the 1,2,4-, 1,2,3- and 1,3,5- isomers of trimethylbenzene (U.S. EPA, 1985). The final testing criteria required that the representative C9 fraction test substance be comprised of no less than 22% ethyltoluene isomers and 15% trimethylbenzene isomers, and required a total ethyltoluene/trimethylbenzene content greater than 75% (U.S. EPA, 1985) (see Tables E-1 and E-2 for detailed descriptions of the final test substances used). The results of these toxicity tests were subsequently published in the following references, and are discussed individually below: mutagenicity (Schreiner et al., 1989); developmental and reproductive toxicity (Mckee et al., 1990); subchronic neurotoxicity (Douglas et al., 1993); and general inhalation toxicity (Clark et al., 1989).

Table E-1. Composition of the C9 fraction test substance used for toxicity testing in Schreiner et al. (1989), McKee et al. (1990), and Douglas et al. (1993)

Compound	Weight percent
o-xylene	3.20
Cumene (isopropylbenzene)	2.74
n-propylbenzene	3.97
4-ethyltoluene	7.05
3-ethyltoluene	15.1
2-ethyltoluene	5.44
1,2,4-trimethylbenzene	40.5
1,2,3-trimethylbenzene	6.18
1,3,5-trimethylbenzene	8.37
≥ C10	6.19
TOTAL	98.74

Source: Schreiner et al. (1989), McKee et al. (1990), and Douglas et al. (1993)

Table E-2. Composition of the C9 fraction test substance used for toxicity testing in Clark et al. (1989)

Compound	Weight percent
non-aromatics	0.46
o-xylene	2.27
n-propylbenzene	4.05
4-ethyltoluene	16.60
3-ethyltoluene	7.14
2-ethyltoluene	7.22
1,2,4-trimethylbenzene	32.70
1,2,3-trimethylbenzene	2.76
1,3,5-trimethylbenzene	9.35
≥ C10	
1-methyl-3-*n*-propylbenzene + 1,2-diethylbenzene	6.54
1-ethyl-3,5-dimethylbenzene	1.77
TOTAL	90.86

Source: Clark et al. (1989)

Schreiner et al. (1989) assessed the mutagenic potential of the C9 fraction (see Table E-1; total trimethylbenzene content = 55.05%) by measuring five genotoxic endpoints: mutation frequency in bacteria, mutation frequency in CHO cells (chinese hamster ovary cells), sister chromatid exchange in CHO cells, chromosomal aberrations in CHO cells, and chromosome aberrations in rat bone marrow cells. In the bacterial mutagenicity assay, five *Salmonella typhimurium* test strains (TA98, TA100, TA1535, TA1537, and TA1538) were exposed to either negative controls (DMSO), positive controls, or to 0.0025-0.50 µl/plate C9 fraction in the presence or absence of the S9 microsomal mixture. After 72 hours of incubation, cells exposed to positive controls exhibited greater rates of gene mutations than negative controls. However, there was no evidence that the C9 fraction induced gene mutations with or without S9 activation in any *S. typhimurium* strain up to the highest test concentration, at which signs of cellular toxicity became apparent. In the CHO forward mutation assay, CHO cells were exposed for 4 hours to either negative controls (DMSO), positive controls, or the C9 fraction at 0.01-0.13 µL/ml (-S9) or 0.02-0.2 µL/ml (+S9). After a seven day post-exposure incubation period, CHO cells exposed to positive controls exhibited statistically significantly greater mutation frequencies compared to negative controls, while no evidence of C9 fraction-induced mutations were observed at any test concentration. To test for the induction of sister chromatid exchange in vitro, CHO cells were exposed to controls or the C9 fraction (2.0-66.7 µg/ml - S9 for 22.5 hours or 0.667-50.1 µg/ml + S9 for 2 hours). Cell-cycle arrest was not observed at exposure concentrations lower than 66.7 or 50.1 µg/ml C9 fraction (-S9 or + S9, respectively), and %

SCE/cell was not increased in cells exposed to any concentration of C9 fraction. The ability of the C9 fraction to induce chromosomal aberrations was similarly investigated in CHO cells: no exposure concentration of the C9 fraction, up to 90.2 µg/mL, induced chromosomal aberrations in the absence or presence of S9. In order to investigate the potential in vivo mutagenicity of the C9 fraction, Sprague-Dawley rats (30 per exposure group, 15 male and 15 female) were exposed via inhalation to 0, 150, 500, or 1500 ppm C9 fraction for 6 hours on 5 consecutive days. Following the termination of exposure, ten rats from each treatment group were sacrificed at 6, 24, and 48 hours, and their bone marrow was harvested, stained, and examined for chromosome/chromatid aberrations. No induction of chromosomal/chromatid aberrations were observed at any exposure concentration in animals sacrificed at 6 or 24 hours. No aberrations were observed in animals sacrificed at 48 hours, but the majority of samples (approximately 66%) were not analyzed due to inadequate staining. In general, the results of Schreiner et al. (1989) indicate that, as tested, the C9 fraction did not induce in vitro or in vivo mutagenicity in multiple assays.

The developmental and reproductive toxicity of the C9 fraction (see Table E-1; total trimethylbenzene content = 55.05%) was investigated by McKee et al. (1990). In the developmental toxicity portion of the study, pregnant CD-1 mice (30 per group) were exposed to 0, 100, 500, or 1500 ppm C9 fraction for 6 hours/day on gestational days (GD) 6-15 (nominal concentrations: 102 ± 3.5, 463 ± 5.3, and 1249 ± 16.5 ppm; actual concentrations: 102 ± 2.6, 500 ± 3.7, or 1514 ± 22.9 ppm). Throughout the exposure period, the dams were examined for clinical signs twice daily, and body weights were taken daily. Blood samples were taken from the dams on GD15, and surviving dams were sacrificed on GD18. The number and location of live and dead fetuses were recorded, as well as the total number of implantations and corpora lutea. Fetuses were weighed, sexed, half of the fetuses examined for external malformations, the remaining fetuses were examined for skeletal malformations. Severe maternal toxicity was observed in the highest exposure group (i.e., 1514 ppm), with 44% of animals dying before sacrifice. Only two dams died in the 500 ppm group, and no animals died in the 102 ppm group. Maternal body weight was statistically significantly decreased at all exposure concentrations on GD15, but only in the 1514 ppm group on GD 18. Maternal body weight gain was decreased in both the 500 and 1514 ppm exposure groups when measured on GD6-15 and GD0-18. Clinical observations of dams revealed some evidence of gross neurobehavioral toxicity, including abnormal gait (18 animals), labored breathing (9 animals), weakness (7 animals), circling (8 animals), and ataxia (8 animals). There were no differences in maternal organ weights in any exposure group compared to controls. Hematocrit and mean corpuscular volume were significantly decreased in dams exposed to 1514 ppm. Exposure to 1514 ppm also resulted in severe developmental toxicity: the number of litters with viable fetuses was decreased (13 vs. 24 in controls, no statistics provided), the number of live fetuses/litter was statistically significantly decreased (7.9 ± 4.3 vs. 10.7 ± 1.8 in controls), and postimplantation loss/dam was

significantly increased (4.3 ± 3.7 vs. 0.9 ± 0.9 in controls). Exposure to both 500 and 1514 ppm also resulted in decreased fetal body weights (1.16 ± 0.11 g [500 ppm] and 0.82 ± 0.17 g [1514 ppm] vs. 1.25 ± 0.14 g in controls). Increased incidence of cleft palate and unossified sternebrae (# 5 and/or 6) was observed in the 1514 ppm group. No other evidence of teratogenicity was observed. The NOAEL identified from the developmental toxicity portion of McKee et al. (1990) was 100 ppm based on decreased fetal weight.

In the reproductive portion of McKee et al. (1990), male and female CD (30 of each sex) rats were randomly assigned to one of four exposure groups (i.e., 0, 103, 495, or 1480 ppm [nominal concentrations: 107 ± 24, 513 ± 12.8, or 1483 ± 33.0 ppm; actual concentrations: 103 ± 2.1, 495 ± 8.0, or 1480 ± 20.5 ppm), and were exposed 6 hours/day, 5 days/week for 10 weeks. Then males and females were co-housed (1:1) for a two week mating period. When mating was confirmed, males were sacrificed. Females were additionally exposed to the C9 fraction 6 hours/day, 7 days/week from GD0 to GD20. Dams were then allowed to deliver. F1 pups were culled to 8/litter on post-natal day (PND) 8. Exposure was restarted on PND 5 and continued until PND21 (weaning), at which point dams were sacrificed and F1 pups were counted, sexed, and weighed. F1 pups were randomly selected for further use in the study (i.e., one week after weaning, they were exposed for 10 weeks and then mated for 2 weeks to produce the F2 generation). The F2 litters were treated similarly to the F1 litters, but to investigate the effects of exposure immediately after weaning; exposure of the animals used to produce the F3 generation began immediately after weaning (i.e., PND22). All parental animals were examined twice daily for clinical signs of toxicity, and body weights were measured weekly. At sacrifice, organs and tissues were microscopically examined in the control and high exposure groups. Litters were examined immediately after delivery for litter size, stillbirths, live births, and gross anomalies. Culled pups and any pups that died spontaneously were necropsied. Pups were weighed on PND0, 4, 7, and 14.

All F_0 males survived exposure, whereas seven F_0 females in the 1480 ppm group died (3 prior to mating, 3 during gestation, and 1 during lactation). Weight gain in both F_0 males and females was statistically significantly decreased in the 495 and 1480 ppm groups. No pathological lesions in the reproductive organs were noted in F_0 generation animals (or in any F_1, F_2, or F_3 animals). There were no observed alterations in female or male fertility, number of females delivering a litter, or litter size at birth. There was a small, but not statistically significant, increase in time necessary for successful mating. In the F_1 generation, there were no decreases in birth weight, or body weights at PND4, compared to controls. Starting on PND7, and continuing until weaning, body weights were significantly decreased in the 1480 ppm exposure group relative to control. No differences in post-natal survival were observed. The decreased body weights of F_1 males and females in the 1480 ppm group was still manifest when exposure was reinitiated (i.e., 10 days after weaning), and during the pre-mating period, body weight gains were lower than controls in males at 495 ppm and in males and females at 1480

ppm. F_1 males and females in the 1480 ppm group also exhibited some signs of neurotoxicity demonstrated by ataxia (18 males, 23 females) and/or decreased motor activity (11 males, 8 females). Male fertility (number of fertile males/number of mated males) was significantly decreased at 1480 ppm. Lastly, six females in the 1480 died (three during exposure, one during gestation, one during delivery, and one on PND2). There were statistically significant reductions in the number of live F_2 offspring delivered per litter and the percentage of live F_2 births. F_2 birth weights were also decreased, but not significantly. The authors report that among mated F_1 females, mating of 24 females (six in the control group, eight at 103 ppm, one at 495 ppm, and nine at 1480 ppm) was not confirmed, and exposure was carried out until delivery, rather than being terminated on GD20. When the dams were analyzed as separate groups, the F_2 litter size was only statistically significantly decreased in litters delivered from the dams that were exposed until delivery. In dams whose exposure was terminated on GD20, F_2 litter size was slightly decreased, but not significantly so. The percentage of live births was decreased in both groups of dams; among the dams that were exposed until delivery, pup survival was still decreased at PND4. As with the F_1 generation, F_2 body weights at birth through PND4 were not affected by treatment, but starting on PND7 and continuing until weaning, F_2 body weights were statistically significantly decreased in the 1480 ppm group.

As stated above, the pre-mating exposure of F_2 animals selected to produce the F_3 generation was begun immediately after weaning (i.e., PND21). A majority of animals in the 1480 ppm group died during the first week of exposure (36/40 males, 34/40 females). Of the high exposure group animals that survived, body weights were substantially reduced throughout the pre-mating exposure period (31-40% below controls in males and 21-35% below in females). Additionally, body weights were slightly decreased in the 103 ppm (10% males, 6% females) and 495 ppm (16% males and females) exposure groups. There were no observed effects on the mean number of live F_3 births or post-natal survival. Birth weights of the F_3 generation were statistically significantly decreased in the 1480 ppm group. Birth weights at PND7 were decreased in the 1480 ppm group, and beginning on PND14 through weaning, body weights were statistically decreased in the 495 and 1480 ppm group. In general, the results of McKee et al. (1990) indicate that exposure to the C9 fraction can induce developmental toxicity (decreased live fetuses, increased postimplantation loss, increased malformations [cleft palate], and decreased fetal weight) and possibly reproductive toxicity (decreased male fertility). Multigenerational exposures were also observed to induce decrements in postnatal weights that occurred at lower doses in later generations compared to earlier generations. Consequently, the NOAEL identified from the reproductive portion of McKee (1990) was 100 ppm based decreased fetal weights in the F_3 generation. Lastly, pre-mating exposures of adult animals was observed to elicit some measures of neurotoxicity (ataxia and decreased motor activity).

Douglas et al. (1993) investigated the neurotoxic potential of the C9 fraction (see Table E-1; total trimethylbenzene content = 55.05%) following subchronic exposure to the mixture. Male CD rats (20 per group) were exposed to 0, 100, 500, or 1500 ppm C9 fraction for 6 hours/day, 5 days/week for 13 weeks (nominal concentration: 94 ± 1.0, 481 ± 5.1, and 1334 ± 17.0 ppm; actual concentration: 101 ± 2.5, 432 ± 2.8, 1320 ± 13.0 ppm). Body weights were recorded weekly during the exposure period, and animals were examined for clinical signs at these time points. Following termination of exposure, animals were sacrificed and tissues were removed for histopathology. Specific testing for neurotoxicity was performed 5, 9, and 13 weeks after exposure was begun. Specific neurotoxicity tests included examination of motor activity (frequency of movement within a cage), and a functional observation battery (fore and hind limb grip strength, audio startle response, thermal response [hot plate stimulus test], and hind limb foot splay). Histopathological examinations were performed on the central and peripheral nerve tissue, including the proximal sciatic nerves, dorsal root ganglia, spinal cord, and specific regions of the brain. No animals died during the exposure period, and the only reported clinical signs reported were urogenital staining, urination, defecation, and vocalization (none of which were considered treatment related). Animals in the high exposure group (i.e., 1320 ppm) exhibited statistically decreased body weights at every time point during exposure; animals in the 432 ppm group had decreased body weights early during exposure, with a statistically significant decrease at week 4. However, by the end of the exposure period, these animals weighted more than controls. No consistent treatment-related effects on motor activity were reported. When analyzed in 10 minute blocks, horizontal activity and total activity during minutes 10-20 of the test were statistically significantly increased in the 1320 ppm exposure group during week nine of the exposure period. However, motor activity in this exposure group returned to control levels during minutes 20-30 of the test, and no effects were observed at the termination of exposure (i.e., week 13). When results were summarized across the entire 30 minute test period, no effects on motor activity were reported at any time during the 13 week exposure period. The results of all the neurotoxicity tests comprising the functional observation battery were generally negative, except for a transient and non-treatment related decrease in auditory startle response in the 432 ppm exposure group at week nine of exposure. Additionally, there appeared to be a statistically significant increase in thermal response time when the endpoint was measured immediately prior to the exposure period. However, this was most likely a statistical artifact due to an unusually low control group response measure at this time point. No exposure-related incidences of neuropathological lesions were reported following termination of exposure. In general, the results of Douglas et al. (1993) indicate that the C9 fraction is not neurotoxic to adult rats: as no consistent neurotoxic effects were noted, the NOAEL for this study was identified as 1320 ppm. However, this finding appears to be in disagreement with the reported neurotoxic effects noted in the McKee et al. (1990) developmental and multigenerational reproductive study, in which pregnant and non-pregnant

adult animals exposed to similar levels of C9 exhibited gross signs of neurotoxicity, including abnormal gait, weakness, ataxia and decreased motor activity.

Clark et al. (1989), investigated the inhalation toxicity of the C9 fraction (see Table E-2; total trimethylbenzene content = 44.81%) following exposure of male and female Wistar rats (50 animals per sex per group) to 0, 450, 900, or 1800 mg/m^3 for 6 hours/day, 5 days/week for up to 12 months (actual concentration: 470 ± 29, 970 ± 70, 1830 ± 130 mg/m^3). Ten males and females were sacrificed halfway through the exposure period (i.e., at 6 months), 25 males and females were sacrificed at 12 months (i.e., at the end of exposure), and 15 males and females were sacrificed after a four month recovery period. Animals were examined twice daily for overt signs of toxicity, and body weights were recorded weekly through the first month of exposure and monthly thereafter. Tail vein blood was taken periodically during exposure (weeks 1, 2, 3, 4, 6, 8, 12, 20, 24, 28, and 32) from 10 males and females in the control and high dose group, and cardiac blood was collected from 10 males and females in all groups at the 6 and 12 month necropsies, and after the recovery period. Both types of blood samples were analyzed for common hematological parameters (e.g., erythrocyte count, hemoglobin concentration). Cardiac and tail vein blood was additionally drawn from 10 males and females at the 6 and 12 month necropsies and at the end of the recovery period and analyzed for clinical chemistry parameters (e.g., total protein, alkaline phosphatase). Urine samples were collected from 12 males and females at 0, 3, 6, 9, and 12 months' exposure and analyzed for common urinalysis parameters (e.g., glucose, protein). All animals underwent complete necropsies after sacrifice. The only reported treatment-related clinical sign was an increase in aggression (i.e., difficulty in handling) in males in the high exposure group. Three control (two male, one female) and two males in the low exposure group died during exposure. Body weights were slightly decreased (2-3%) relative to control during the first 4 weeks in male rats exposed to 1830 mg/m^3 and females exposed to 970 mg/m^3 and during the first 12 weeks of exposure in females exposed to 1830 mg/m^3. No consistent trends were reported for any of the hematological parameters analyzed from the tail vein samples. In the interim (i.e., 6 month) and terminal (i.e., 12 month) cardiac blood samples, the only treatment-related effects reported were decreased eosinophil counts (30 to 55%, all exposure groups) in female rats at 6 months and decreased osmotic fragility (5%, all exposure groups) and increased lymphocyte counts (29%, 1830 mg/m^3) in male rats at 12 months. Clinical chemistry effects were generally mild, with high exposure group females exhibiting increased potassium (6 months), increased sodium (12 months), and decreased albumen (6 months); the only clinical chemistry effect noted in males was increased creatinine in the high exposure group at 12 months. There were no urinalysis parameters affected by treatment. At the end of exposure, liver and kidney weights were statistically significantly increased (11% and 10%, respectively) in high exposure group males. Gross and histopathological examination generally revealed no consistent treatment-related lesions. A slight increase in pulmonary macrophage infiltration and alveolar

wall thickening was observed in male and female rats at 12 months, with the average severity grade for these effects increasing with dose. Although there were no clear treatment-related increases in tumors at 12 months, one high exposure female had a leiomyoma on the left uterine horn, one high exposure male had a lymphoma of the spleen, and one low exposure male had a glioblastoma of the cerebellum. In general, the results of Clark et al. (1989) indicate that the C9 fraction has low systemic toxicity (NOAEL = 1830 mg/m^3) following chronic exposure.

In summary, the results of Schreiner et al. (1989), McKee et al. (1990), Douglas et al. (1993), and Clark et al. (1989) are all well-conducted studies that investigate relevant toxicological endpoints in appropriate in vitro and in vivo systems. These toxicity tests were mandated by Section 4(a) of TSCA to investigate the mutagenicity, neurotoxicity, teratogenicity, reproductive toxicity, and general toxicity of the C9 fraction, and indicated that the C9 fraction elicited limited toxicity in the test systems used. It must be acknowledged that the specific test compound used in the C9 fraction was a complex aromatic hydrocarbon mixture containing between 45-55% TMB isomers, with the remaining mixture primarily consisting of ethyltoluene isomers. Tertiary constituents (xylene, n-propyl- and isopropylbenzene, and unspecified C10 aromatic hydrocarbons) comprised as much as 16% of the test compound. Although a conclusion of sufficient toxicokinetic and toxicological similarity is used in the Toxicological Review to support the adoption of consistent, cross-isomer reference values, such a conclusion has not been reached, nor attempted, for the other constituents of the C9 mixture. For some constituents (i.e., the C10 compounds), such a comparison is not possible as they were not specifically identified in the compositional analysis. Regarding possible toxicokinetic similarities, the EPA is currently unaware of any detailed data on the ADME of the C9 fraction (particularly information regarding the distribution of TMB isomers in the C9 fraction to the brain and other organ systems). As such, given this particular data gap, an assumption that the C9 fraction would be an adequate surrogate for individual TMB isomers is not justified.

Additionally, the C9 mixture studies failed to observe clearly adverse effects, except for the developmental and reproductive toxicity observed in McKee et al. (1990). However, multiple peer-reviewed studies investigating the toxic effects of individual isomers exist, and serve as the basis for hazard identification, dose-response analysis, and reference value derivation in the Toxicological Review of Trimethylbenzenes. These studies include those observing neurotoxicity (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak et al., 1995), respiratory toxicity (Korsak et al., 2000a, b; Korsak et al., 1997; Korsak et al., 1995), developmental toxicity (Saillenfait et al., 2005), and hematological toxicity (Korsak et al., 2000a, b). Given the availability of these studies, and the general lack of observed toxicity in the C9 studies, it is appropriate for the individual isomer studies to serve as the scientific foundation for the Toxicological Review. Therefore, although there are available, peer reviewed studies investigating the toxicity of the

C9 fraction, the uncertainty regarding any interactive effects other C9 constituents may have on the ADME of TMB isomers and the general lack of reported toxic effects limit their utility for the assessment of the human health risk of individual TMB isomers. For these reasons, these studies were not included in the Toxicological Review.

Additionally, two other industry reports regarding the toxicity of mixtures containing the isomers were located (Industrial Bio-Test Laboratories, 1992; Chevron, 1985). These documents were excluded from the Toxicological Review following careful consideration as they were not peer-reviewed and did not investigate the toxicity of individual TMB isomers. Ultimately, the decision was made to not seek external peer review for these documents as these studies would not qualitatively enhance hazard identification, quantitatively enhance dose-response analysis, or substantially decrease uncertainty in the assessment. Two peer-reviewed studies investigating the effects of complex mixtures containing TMB isomers were also found (Lehotzky et al., 1985; Ungvary and Tatrai, 1985). However, these studies also did not study TMB isomers individually, and unlike the C9 fraction studies above, provided no information on the compositional makeup of the test substance. For these reasons, the above studies were not included in the Toxicological Review of Trimethylbenzenes.

APPENDIX F. RESOLUTION OF PUBLIC COMMENTS

The Toxicological Review of Trimethylbenzenes was released for a 60-day public comment period in June 2012. After the close of the public comment period, a listening session was held on August 1st, 2012. EPA received comments on the draft assessment from one public reviewer: the Hydrocarbon Solvents Panel of the American Chemistry Council (ACC). The major comments received have been synthesized and paraphrased below with a reference to the complete comment also provided. EPA's responses to the comments as well as information regarding how the assessment has been revised are also included.

Comment: The Draft IRIS Assessment is subject to EPA and OMB Information Quality Guidelines, and, as the Draft IRIS Assessment is influential information, it must adhere to a rigorous standard of quality. EPA must employ "a higher degree of transparency regarding (1) the source of the data used, (2) the various assumptions employed, (3) the analytic methods applied, and (4) the statistical procedures employed." As currently presented, the Draft Assessment has failed to comport with the Information Quality Guidelines (Comments I.1 and I.2, pp 4-6)

EPA Response: In response to NRC recommendations, EPA has increased the transparency of IRIS assessments, particularly in regard to (1) the source of the data used (i.e., inclusion of evidence tables in the main body of the Toxicological review, and study summary tables included in Appendix B); (2) the various assumptions used in the document (i.e., extensive discussions of the interpretation of study data used in the assessment, especially neurotoxicological data); and (3) the analytic methods applied and (4) the statistical procedures employed (i.e., explicit discussion of modeling methodologies in the Toxicological Review and Appendix C). Further, this assessment has been through the Interagency Science Consultation review step (Step 3 of the IRIS Process) which includes OMB.

Comment: In the Draft IRIS Assessment, EPA has included a section titled "Preamble to the IRIS Toxicological Reviews" that includes a summary discussion of the scope of the IRIS program, process for developing IRIS assessments, study selection, data evaluation and derivation of toxicity values. As currently written, the preamble offers an abbreviated view of EPA policies, guidance and standard practices but fails to include the detail necessary to provide useful information on how the Agency reviews or weighs the scientific information for inclusion in its toxicological review as discussed in the NAS recommendations. (Comment I.3, p 6)

EPA Response: The Preamble to the IRIS Toxicological Reviews has been developed in response to NRC recommendations to concisely summarize EPA policies, guidance and practices employed in IRIS assessment development. It is not intended to provide a detailed application of procedures to the TMB isomers. Rather the Preamble is complemented by evaluation of the available scientific information found in the body of the assessment document. The EPA will seek comments from the external peer review panel as to the effectiveness of this structure in IRIS assessments. *Comment*: Although the Draft Assessment identified a solid core set of databases to search for relevant data, EPA has failed to conduct a thorough literature search which has resulted in the omission of data from two TSCA 4(a) test rules (U.S. EPA, 1993, 1985). The omission of the TSCA data suggests EPA may have additionally missed other studies (Comment II.1, p 7)

EPA Response: The studies published as a result of the 1985 TSCA 4(a) test rule (Douglas et al., 1993; Mckee et al., 1990; Clark et al., 1989; Schreiner et al., 1989) were identified in the initial set of references considered for inclusion in the Toxicological Review. However, as these studies use the C9 fraction as the test substances, they were excluded from further consideration (see next comment/response and Appendix E for further information).

Comment: EPA's decision to consider the TMB isomers toxicokinetically and toxicologically equivalent was appropriate. However, given this decision, then data on any of the isomers or on TMB-containing mixtures (predominantly TMBs with other similar hydrocarbons [e.g. C9 aromatic including ethyltoluene] can be used to characterize the hazards of TMBs individually or collectively. This includes the data submitted, and accepted by the EPA under TSCA Section 4(a) test rules (U.S. EPA, 1993, 1985). Inclusion of this data would greatly enhance the database available on TMB isomers individually, and address many of the uncertainties raised in the Draft IRIS Assessment. ACC encourages EPA to review all available data on TMBs and C9 mixtures and to reevaluate those studies in regard to the calculations for the RfC and RfD. (Comment II.2, pp 8-10; Comment V, pp 17-18)

EPA Response: The 1985 TSCA 4(a) test rule (U.S. EPA, 1985) required that "manufacturers and processors of the C9 aromatic hydrocarbon fraction … test the C9 aromatic hydrocarbon fraction for neurotoxicity, mutagenicity, developmental toxicity, reproductive effects, and oncogenicity." EPA issued the final testing requirements that the C9 fraction be tested based on the findings that (1) there were there no data to suggest that exposure to individual TMB isomers posed a threat to human health, that (2) there was no evidence of substantial releases of TMB isomers to the environment, and that (3) there was adequate data to suggest that TMB isomers would not persist in the environment (U.S. EPA, 1985).

However, much of this information is dated and no longer correct. Information does exist currently that occupational and residential exposures to TMB isomers do occur (HSDB, 2011a, b, c; Martins et al., 2010; Choi et al., 2009; Guo et al., 2009; Jiun-Horng et al., 2008) and that substantial quantities

of 1,2,4-TMB are released to the environment (TRI, 2008) (see Preface). Lastly, TMB was nominated to the IRIS program due to its presence at Superfund sites, indicating that individual TMB isomers, once released to the environment, are capable of persisting in the environment at contaminated locations. Therefore, while testing the C9 fraction was originally deemed sufficient given the lack of evidence that exposure to individual isomers of TMB was likely, current information demonstrates that TMB isomers are released to and persist in the environment and that human populations are exposed to TMBs in occupational and residential settings.

In the Federal Register Notice announcing the C9 fraction testing requirements, EPA agreed with public comments that, in the absence of toxicological information on individual ethyltoluene or TMB isomers, "assessing the toxicity of the C9 mixture as a complete entity should provide a reasonable upper bound for the toxicity of the individual ethyltoluene and TMB [isomers] in the C9 mixture" (U.S. EPA, 1985). However, this assumption has been shown to be inaccurate given current information. In the time since the promulgation of the C9 fraction testing requirements and subsequent conduct and publication of the C9 fraction toxicity studies, multiple peer-reviewed studies have been published that demonstrate that individual TMB isomers do elicit clearly adverse toxicological effects. These include neurotoxicity (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b; Gralewicz et al., 1997a; Korsak et al., 1995), respiratory toxicity (Korsak et al., 2000a, b; Korsak et al., 1997; Korsak et al., 1995), developmental toxicity (Saillenfait et al., 2005), and hematological toxicity (Korsak et al., 2000a, b). Generally, the C9 fraction studies failed to observe clear measures of toxicity in the systems investigated. The ultimate reason for the discrepancy between the individual isomer and C9 fraction studies is unknown.

However, it must be acknowledged that the specific test compound used in the C9 fraction was a complex aromatic hydrocarbon mixture containing between 45-55% TMB isomers, with the remaining mixture primarily consisting of ethyltoluene isomers. The test compound also contained xylene, n-propyl- and isopropylbenzene, and unspecified C10 aromatic hydrocarbon constituents. These tertiary compounds comprised as much as 16% of the test compound. Additionally, in Clark et al. (1989), up to 9% of the test compound was unidentified impurities. For the purposes of setting a reference value for trimethylbenzenes, it is preferable to analyze the trimethylbenzene isomers themselves, and not complex mixtures that include other compounds. For these reasons, these studies were not included in the Toxicological Review. A more comprehensive discussion of this subject has been provided in Appendix E of the Supplement Information document.

Comment: The Draft IRIS Assessment states that "no chronic, subchronic, or short-term oral exposure studies were found in the literature" for 1,3,5-TMB. This is incorrect; there are oral toxicity studies performed by the request of EPA Office of Water Chemicals Final Test Rule (U.S. EPA, 1993). EPA's exclusion of these studies (Koch Industries, 1995a, b) is not justified, as inclusion

of the studies provides direct results for oral exposure to 1,3,5-TMB in rats and does, in fact, enhance both the hazard identification and dose response analysis. (Comment II.2, pp 10-11)

EPA Response: After careful reconsideration, EPA agrees that the 14- and 90-day oral gavage 1,3,5-TMB toxicity studies should be incorporated into the document. Accordingly, the hazard identification and dose-response sections of the Draft Assessment have been updated to include information on and discussion pertaining to the Koch Industries studies (1995a, b). One other industry report investigating the oral toxicity of 1,2,4-TMB was further considered for inclusion in the Toxicological Review (Borriston, 1983). In this study, male F344 rats (n = 10) were exposed to either 0.5 or 2.0 g/kg 1,2,4-TMB daily for 28 days. All rats in the high dose and one rat in the low dose group died during exposure (no times given). Other reported effects were enlarged adrenal glands, mottled and red thymuses, and congested lungs. Given the limited toxicological information provided by this report (other than total mortality in the high dose group), this report was not included in the Toxicological Review.

Comment: EPA has selected decreased pain sensitivity (expressed as increased latency to response) as the critical effect for TMB toxicity, and Korsak and Rydzyński (1996) as the principal study. Exposure to TMB isomers resulted in an increased latency in response when measured immediately after treatment but found no effects 2 weeks post-exposure for animals in the repeat dose study. The most likely explanation is that exposure to TMB isomers results in acute, reversible responses. Acute effects are related to the most recent exposures, and are not the consequence of repeated exposures. In this regard, it is unclear how the Korsak and Rydzyński (1996) study can be selected as the principal study. Furthermore, results for the pain sensitivity endpoint in the neurotoxicity study with C9 aromatics (Douglas et al., 1993) found no adverse effects in animals examined at 5, 9 and 13 weeks during and after exposure to higher levels than employed by Korsak and Rydzyński (1996). The discussion of pain sensitivity should be revised to accurately emphasize that decreases in pain sensitivity and increases in response latency were observed only when animals were tested immediately after 90 days of treatment (Korsak and Rydzyński, 1996), but not when the animals were held without treatment for any extended period of time indicating the transient nature of the response. (Comment III, pp 11; Comment IV.1, p 13; Comment VI.2, p15)

EPA Response: For the reasons discussed previously, the C9 aromatics studies, including Douglas et al. (1993), are not considered in this assessment. In the sections pertaining to the selection of the proposed overall RfCs for 1,2,4-TMB and 1,3,5-TMB (Sections 2.1.5 and 2.2.5, respectively), a detailed discussion of the suitability of the decreased pain sensitivity endpoint is included. This discussion has been expanded. Specifically, the U.S. EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998) do note that effects that are reversible in minutes, hours, or days after the end of exposure and appear to be associated with the pharmacokinetics of the agent and its presence in the body may be of less concern than effects that persist for longer periods of time after the end of exposure (pg. 8). However, this is subsequently clarified to indicate that reversible

effects occurring in occupational settings may be of high concern, particularly if they diminish a person's ability to survive or adapt to the environment (U.S. EPA, 1998) (pg. 8); such is the case for exposure to TMBs in occupations with potentially dangerous surroundings and/ or heavy equipment, such as dockyard painters or asphalt workers.

As pointed out in *A Review of the Reference Dose and Reference Concentration Process (U.S. EPA, 2002)*, "[i]t is also important to keep in mind that effects that may initially appear to be reversible may re-appear later or be predictive of later adverse outcomes." (pg. 4-16). Additionally, the *Neurotoxicity Guidelines* (U.S. EPA, 1998) state that "latent effects (those that become evident only after an environmental challenge [e.g., in this case, footshock]) have a high level of concern." The hot plate test is a relatively simple assessment that may not be sensitive enough to detect subtle changes (U.S. EPA, 1998), suggesting that the large changes observed immediately after TMBs exposure may reflect gross effects. It is possible that, at longer durations after exposure, an environmental challenge is necessary for the more subtle perturbations that persist to become manifest at a detectable level using this test. The latent decrements in pain sensitivity following foot shock appear to reflect a prolongation of the numbing effects of foot shock following exposure to TMBs weeks earlier, as the immediate increases in latency due to foot shock were unchanged by prior TMB exposure. This indicates that some aspect(s) of the altered pain sensitivity phenotype may fail to resolve following termination of exposure. No environmental challenge was applied in the subchronic study by Korsak and Rydzyński (1996); such an experiment may have uncovered similar latent responses. Conversely, the short-term TMB exposure studies testing pain sensitivity failed to analyze hot plate latency with a foot shock challenge shortly after exposure, as these evaluations only occurred at ≥ 50 days post-exposure.

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

Although it is important to consider the potential for reversibility of neurological effects, "for chronic lifetime exposures, designation of an effect as irreversible or reversible is academic, as exposure is presumed to be lifetime (i.e., there is no post-exposure period)" (U.S. EPA, 2002; pg. 3-27). Thus, the nature of an RfC precludes the possibility of recovery of the critical effect and supports the choice of the principal study, even if all aspects of the pain sensitivity phenotype were found to be transient (which does not appear to be the case). Taken together, the database supports

the characterization of decreased pain sensitivity associated with exposure to TMB isomers as an effect of high concern, and an appropriate endpoint on which to base the RfC derivation. However, EPA agrees that the observation of reversibility of the decreased pain sensitivity endpoint is an important factor to consider. As such, EPA has determined that a full 10-fold uncertainty factor for extrapolation from a subchronic to chronic duration is not warranted, and has instead applied a 3-fold uncertainty factor (see discussion of uncertainty factors, below).

Comment: Although Korsak and Rydzyński (1996) was identified as the key study, significant emphasis was placed on subsequent studies in which animals were exposed for only 4 weeks duration and held for longer periods and foot shock was introduced (Wiaderna et al., 2002; Gralewicz and Wiaderna, 2001; Wiaderna et al., 1998; Gralewicz et al., 1997b) to support a position that the observed pain sensitivity was not an acute response but that exposure to TMB isomers results in persistent impairment as long as 50-51 days post exposure, long after TMB had been eliminated from the body. However, the studies actually demonstrated that pain sensitivity per se was not persistent. Moreover, these studies show some inconsistencies in their findings:
[Note: numbering of the bullets provided as comments is used to frame the EPA responses below]

(1) Korsak and Rydzynski (1996)… 1,2,3- and 1,2,4-TMB… tested them for pain sensitivity after 90 days of exposure… increased latency… immediately after termination of exposure… tested the rats 2 weeks post-exposure and there were no differences.

(2) Gralewicz et al. (1997b)…1,2,4 TMB for 4 weeks… tested at days 50-51 using the hot plate assay and found no effects. They then shocked the animals… finding no effects. They then tested the rats 24 hours after foot shock, finding a significant increased time to response in the 100 and 250ppm groups.

(3) Wiaderna et al. (1998)… 1,2,3 TMB for 4 weeks, tested them at 50 and 51 days after exposure using a hot plate assay only and no effects were seen… after foot shock was administered, latency [was unchanged]… when tested 24 hours after foot shock a significant increase in latency… was found at 100ppm [only].

(4) Gralewicz and Wiaderna (2001)…1,2,3-, 1,2,4-, and 1,3,4-isomers of TMB for 4 weeks…and then tested them on days 50-51 for pain response, finding no effects. Then they shocked the animals and tested for pain sensitivity immediately after foot- shock and 24, 72, and 120 hours post-shock. Increased latency time was observed at 24 hours for 1,2,4 TMB and 1,3,5 TMB but … significant reductions in latency time to response were found in experiments with 1,3,5 TMB at 72 hours post-shock and 1,2,4- and 1,3,5- at 120 hours.

(5) Wiaderna et al. (2002)…1,3,5-TMB… for 4 weeks… tested on days 50-51 and found no effects in the hot plate test and no effects immediately after foot shock or at any intermediate point before the 240 hours post-shock assessment at which point a significant reduction in latency time was found at all exposure levels… Results did not replicate significant differences reported by Gralewicz and Wiaderna (2001)… at 72 and 120 hours post-shock. (Comment III, pp 11-12)

EPA Response: Additional details and clarifying discussions have been added to the Toxicological Review, and are summarized here. Specifically:

(1-3) The comments submitted (above) are accurate. Immediately following 90 days of exposure, increased latency in the hot plate test (decreased pain sensitivity) was observed (Korsak and Rydzyński, 1996); however, this effect did not persist 2 weeks after termination of exposure. A statistically significant increased latency in the hot plate test was observed only 24 hr post foot-shock at 100 or 250ppm 1,2,4-TMB and 100ppm (non-significantly increased at 250ppm) 1,2,3-TMB (Wiaderna et al., 1998; Gralewicz et al., 1997b).

(4&5) The data described in the submitted comments (above) do not relate to the results of performance in the hot plate test [i.e., Fig. 4 in Gralewicz et al. (1997b); Fig. 2 in Wiaderna et al. (Wiaderna et al., 2002); Fig. 4 in Gralewicz and Wiaderna (Gralewicz and Wiaderna, 2001). Rather, the evidence presented in the submitted comments reflects observations of reduced step-down latency in passive avoidance tests [i.e., Fig. 3 in Gralewicz et al. (1997b); Fig. 1 in Wiaderna et al. (Wiaderna et al., 2002); Fig. 3 in Gralewicz and Wiaderna (Gralewicz and Wiaderna, 2001)]. Importantly, although these passive avoidance tests do not directly assess pain sensitivity (these tests are usually interpreted as measures of impulse control and memory retention), a reduction in the latency to step down could also reflect decreased pain sensitivity to the negative reinforcement (i.e., foot shock), as the animals may be exhibiting less fear memory of stepping down onto the platform where they previously received what was intended to be painful foot shocks (the foot shocks employed in these tests have a much shorter duration than those used to induce reductions in pain sensitivity in hot plate tests). Notably, there is no use of a hot plate to detect pain sensitivity in the passive avoidance tests. This misattribution of the passive avoidance tests as measures of pain sensitivity is apparent when looking at descriptions of the timing of the endpoint assessment: e.g., the comment in (4) "Then they shocked the animals and tested for pain sensitivity immediately after foot- shock and 24, 72, and 120 hours post-shock". Pain sensitivity (the hot plate test) was only conducted a few seconds or 24 hours after foot shock; impulse control and memory retention (passive avoidance tests) were conducted at 0, 24, 72, and 168 hours (7 days) after foot shock.

To address the comments related to lack of consistency, the results of the hot plate tests in these studies report an increased latency (decreased pain sensitivity) at 24 hr post foot-shock at 100 ppm, but not 250 ppm (slightly increased latency only), 1,2,3-TMB (Wiaderna et al., 1998); at 100ppm, but not 250ppm (slightly increased latency only), 1,3,5-TMB (Wiaderna et al., 2002); and at 100 ppm 1,2,4- or 1,3,5-TMB [latency increases ~75% over controls by 1,2,3-TMB were not statistically significant; (Gralewicz and Wiaderna, 2001)]. Thus, the results are consistent.

The text, evidence tables, and arrays relating to hot plate tests of pains sensitivity and passive avoidance tests of cognitive function (Section 1.1.1) have been revised and expanded to more clearly describe the results of these very different tests. The discussion of the hot plate tests, in

particular, now includes a greater emphasis on both the general lack of differences in pain sensitivity observed in non-shocked rats at 50 days post-exposure as well as the lack of inconsistencies in the decreased pain sensitivity (increased hot plate latency) at 51 days following TMB exposure when an environmental challenge (foot shock) is applied 24 hr earlier.

Comment: Evidence of persistence in response of the pain sensitivity endpoint was found only after foot shock administration. No agreed guidelines for study conduct and rationale for administering foot shock were cited in the Draft Assessment and thus the varied protocols lead to a lack in clarity regarding whether or not the testing conducted is scientifically valid and reproducible. The Draft Assessment acknowledged that incorporation of foot shock complicates the interpretation of these studies: "[m]ost of the neurotoxicity tests in animals incorporated the application of foot shock which, depending on the procedure, can involve multiple contributing factors and can complicate interpretations regarding effects on discrete neurological function." Discussions of the effects of the neurotoxicity studies demonstrating persistence of the pain sensitivity endpoint should be expanded to qualify that significant persistent effects were only reported after foot shock was introduce[d]. (Comment III, pp 12-13; Comment IV.1, p 13)

EPA Response: In rats, it is well accepted that foot shock induces short-lived analgesia. This is a scientifically valid test and a reproducible effect. In the experiments using foot shock in concert with analyses of pain sensitivity (i.e., hot plate tests), the protocols are near-identical (i.e., ¾ studies used 2mA, 100ms pulses every 2 seconds for 2 minutes; the other used 4mA). Protocols employing foot shock in passive avoidance tests (which, as stated previously, is not a test of pain sensitivity) or active avoidance tests are different, as the stimulus is intentionally shorter. The limitations regarding the interpretation of pain sensitivity experiments when the hot plate test is coupled with foot shock has been clarified to focus on the pain sensitivity endpoints alone, rather than "neurotoxicity tests",

The consistently observed effect of increased latency to paw lick 24 hours after foot shock was reported at one or more concentrations for all isomers across studies with the exception of one study of 1,2,3-TMB by Gralewicz and Wiaderna (2001) [effects of 1,2,3-TMB were significant in Wiaderna et al., (1998)], where the 75% increase relative to controls was not statistically significant. As described in the text, the most likely explanation for this finding is that prior TMB exposure potentiates the duration, but possibly not the magnitude, of the short-lived analgesia caused by foot shock. However, as outlined in the text, it cannot be completely ruled out that TMB exposure may alter cognition such that contextual clues related to the sequential combination of foot shock and hot plate testing are differentially processed between groups. Thus, control groups may better associate the hot plate environment with the previously-applied aversive stimulus and more quickly withdraw their paws than their TMB-exposed counterparts who may exhibit a decreased fear response or shorter retention of that fear-associated memory. Alternatively, since this test paradigm can cause the hot plate test apparatus to become associated with foot shock,

inducing stress-related responses in the shocked animal such that subsequent exposure to the hot plate test apparatus alone can reduce sensitivity to pain (possibly via the release of endogenous opiods), prior TMBs exposure could amplify this effect. These possible alternative explanations underlie why the responses were indicated as difficult to interpret as effects on a discrete neurological function (e.g., on pain sensitivity or memory alone). Importantly, despite the possible overlap between contributing neurological processes in this test paradigm, these observations are still regarded as significant and adverse, and clearly indicate a persistence of neurological effects long after TMB exposures have ceased.

Comment: Increased clarity is needed regarding selection of the critical effect for derivation of the reference values for the TMB isomers. In discussion at the Listening Session [August 1st, 2012] it was stated that IRIS used the "step down" technique to develop the assessment. This appears to be incorrect as the document itself indicates pain sensitivity is the key endpoint. If the "step down" data are key, then EPA should consider revising the Draft IRIS Assessment as this distinction is not currently clear from the document. (Comment VI.1, p 14)

EPA Response: In the discussion at the Listening Session, EPA stated that the public comments included erroneous descriptions of data relating to tests of passive avoidance (i.e., decreased step down latency) as measures of pain sensitivity; specifically, as the previous comments reflect, decreases in step down latency (in passive avoidance tests of cognition) were interpreted by the commenters as inconsistent with the observations of increased paw lick latency (in hot plate tests of pain sensitivity). EPA has not stated that the results of the passive avoidance tests (i.e., decreased step down latency) were used as the key endpoint. Rather, these "step down" data have been clarified by EPA as distinct from those resulting from pain sensitivity assays and that the results of these two different tests were complementary rather than inconsistent (see comments above for details). Revisions to the text have been made and additional clarifying information is now included in the evidence tables (**Section 1.1.1.**) to more clearly portray the findings from, as well as the differences between, these two, distinct test paradigms, and to more transparently convey the lack of inconsistencies in the conclusions drawn from the results of each.

Comment: Gralewicz and Wiaderna (2001) reported large individual differences in each group in step down latency for pain sensitivity and foot shock. "In order to reduce the with-in group variability, data from two rats with the lowest and highest mean step-down latency in the first post shock trial were excluded from data sets for each group of rats". This suggests it was necessary to adjust the data to get significance in the Gralewicz and Wiaderna (2001) study raising further questions about the suitability of these data for risk assessment purposes. (Comment VI.1, p 14)

EPA Response: The comment relates only to data derived from tests of passive avoidance (cognitive effects), and not to data from tests of hot plate behaviors (pain sensitivity). No corrections were indicated by Gralewicz and Wiaderna (2001) in regards to the hot plate tests. The data used by EPA

for quantitative dose-response analyses are from tests of hot plate latency, not passive avoidance; the suitability of the pain sensitivity data is not questioned in the above comment.

As to the specific interpretation of the passive avoidance tests performed by Gralewicz and Wiaderna (2001), the modification cited above does not appear to apply to the significance of the observations of decreased step-down latency at day 7 after the foot shock: "Statistical comparisons of the data from all animals revealed differences between groups in trial 6, i.e. on day 7 after the footshock ($F(4,282) = 2.86$, $P < 0.05$); in the MES [1,3,5-TMB] group the step-down latencies were significantly shorter than in the C [control] group. In order to reduce…". However, because the modified analysis quoted in the comment above was somewhat unclear in the paper by Gralewicz and Wiaderna (2001), EPA has decided to revise the evidence tables to reflect that the data presented graphically appear to represent groups with excluded rats, drawing uncertainty regarding statistical significance. Thus, the indication of statistical significance at 7 days after foot shock for 1,3,5-TMB is the only significance indicator that will remain in the evidence tables (Section 1.1.1.; the modified statistical analyses are now included as notes only), as this analysis, at least, was clearly based on all animals tested. As the direction and approximate magnitude of these responses remain consistent across the database, this clarification does not substantially change EPA's interpretation of the results of the passive avoidance tests.

Comment: In developing the RfC for 1,3,5 TMB, IRIS chose to discount the developmental toxicity study performed by Saillenfait et al. (2005) as the key study even in the absence of adequate neurotoxicity data for this isomer (i.e., neurotoxicity data from an appropriate sub-chronic or chronic study). EPA should carefully consider the study which provides the most robust response on which to base the RfC derivation for 1,3,5-TMB. (Comment VI.1, p 14)

EPA Response: The RfC derivation section contains an extensive discussion of the developmental and maternal toxicity endpoints observed in the Saillenfait et al. (2005) study, and the Draft Assessment has been revised so that candidate RfCs based these effects are derived for 1,3,5-TMB: 1 mg/m^3 based on decreased maternal weight gain and 7 mg/m^3 based on decreased male and female fetal body weight. The most sensitive RfC derived from 1,3,5-TMB-specific data is 20-fold higher than the RfC derived for 1,2,4-TMB based on neurotoxicity data (1 mg/m^3 vs. 5×10^{-2} mg/m^3). The RfC section for 1,3,5-TMB also includes an extensive discussion of the toxicokinetic and toxicological similarities between 1,2,4-TMB and 1,3,5-TMB, especially the similarities in toxicity between the isomers observed in short-term neurotoxicity studies. It appears that the major factor driving the derivation of an RfC for 1,3,5-TMB that is so much greater than the RfC for 1,2,4-TMB is the lack of a subchronic 1,3,5-TMB neurotoxicity test, and not some intrinsic difference in toxicity between the two isomers. Given the observed similarities in toxicity and toxicokinetics between the two isomers, EPA concluded that it was not scientifically justified to derive an overall RfC value for 1,3,5-TMB that is so much higher than derived for 1,2,4-TMB. As such, the decision to adopt the overall RfC value for 1,2,4-TMB (based on decreased pain sensitivity) as the RfC for

1,3,5-TMB is retained in the Draft Assessment. The candidate RfC values for 1,3,5-TMB based on maternal and developmental effects are presented alongside the overall RfC value for comparison purposes, and for potential further uses such as subsequent cumulative risk assessments that assess the combined effect of multiple agents acting at a common site.

Comment: EPA applies an uncertainty factor of 10 to account for extrapolation from subchronic exposure to chronic exposure (UF_S) based on the "assumption that effects observed in a similar chronic study would be observed at lower concentrations for a number of possible reasons, including potential cumulative damage occurring over the duration of the chronic study or an increase in the magnitude or severity of effect with increasing duration of exposure." However, the critical effect observed in the principal study (Korsak and Rydzyński, 1996) does not demonstrate any cumulative damage from exposure to TMB as effects are not seen two weeks after exposure is terminated. In consideration of the fact that pain sensitivity is reversible upon termination of exposure, EPA should consider a UF_S of 3 or less. (Comment VI.2, pp 14-15)

EPA Response: After careful consideration, EPA agrees that a full 10-fold UF_S is not supported by the available data. Given the observation of reversibility in neurotoxicity endpoints reported in subchronic inhalation studies, an uncertainty factor of 3 has been applied in the Draft Assessment. Lowering the UF_S to 1 was not supported as, in the case of neurotoxicity endpoints, chronic exposure may overwhelm the adaptive responses observed after termination of subchronic exposure, resulting in a lack of reversibility for the pain sensitivity endpoint at 1,230 mg/m^3, a greater magnitude of this response, and/ or manifestation of more severe latent responses associated with this effect. Additionally, hematotoxicity endpoints were also observed to exhibit reversibility, and the inflammatory nature of observed respiratory effects suggests that adaptive mechanisms may alleviate these effects following termination of exposure. Therefore, a UF_S of 3 was also applied to these endpoints.

Comment: In determining the uncertainty factor for database deficiencies (UF_D), EPA cites the absence of multi-generation and developmental neurotoxicity studies for all three isomers as contributing to the rationale for application of a 3-fold UF_D. Inclusion of the available 3-generation C9 fraction study (Mckee et al., 1990) and Aromatol (blended C9 aromatic hydrocarbon mixture) developmental neurotoxicity study (Lehotzky et al., 1985) would provide sufficient data to overcome any deficiencies in the developmental/reproductive area and eliminate the need for any additional uncertainty factors to account for database deficiencies, reducing the uncertainty factor to 1. (Comment VI.3, pp 15-16)

EPA Response: Given the decision to exclude the C9 fraction studies from the Draft Assessment (see above, and Appendix E), the McKee et al. (1990) study has been removed from the discussion regarding the selection of the UF_D. As explained above and in Appendix E, the C9 fraction studies were excluded from the Draft Assessment because they are complex solvent mixtures that at most

only contain 55% TMB isomers. Thus, there is considerable uncertainty regarding how their compositional make-up influences the observed general lack of C9-induced developmental toxicity compared to the individual TMB isomer study (which does observe developmental toxicity following exposure to either 1,2,4-TMB or 1,3,5-TMB). The Lehotzky et al. (1985) study was excluded based on the same rationale. Therefore, as the C9 fraction studies have been excluded from the Draft Assessment, the lack of TMB isomer-specific multigenerational reproductive and developmental toxicity and developmental neurotoxicity studies remains a weakness of the TMB database.

Comment: The Panel agrees that the database for TMBs provides "inadequate information to assess carcinogenic potential" of these isomers. The database for TMBs, however, supports the likelihood that TMBs are not mutagens and are unlikely to be genotoxic carcinogens. In the only study investigating TMB-induced genotoxicity, only 1,2,3-TMB was reported to elicit positive results in the Ames assay (Janik-Spiechowicz et al., 1998). The observation that 1,2,3-TMB was genotoxic in the absence of metabolic activation and the manner in which the data were presented call into question the conclusion of positive mutagenicity for this particular isomer. Further, although Janik-Spiechowicz et al. (1998) reports increased sister chromatid exchange in the bone marrow of male mice following exposure to each individual TMB isomer, no alterations in the frequency of micronucleus formation was noted. As micronucleus formation is a definitive endpoint for cytogenetic damage, this indicates that clastogenicity is not expressed following exposure to TMB isomers. Consideration of the available C9 fraction mutagenicity study (Schreiner et al., 1989) supports the conclusion that TMB isomers are not likely to be mutagens. (Comment IV.4, pp 16-17)

EPA Response: There is only one available study (Janik-Spiechowicz et al., 1998) that investigates the mutagenic potential of individual TMB isomers. The EPA concludes in the Draft Assessment that this study provides at best limited information regarding the mutagenic potential of TMB isomers, and that the database is inadequate to conclude that any isomer is directly genotoxic. In the absence of any further evidence that individual TMB isomers do not result in gene mutations or chromosomal aberrations, a definitive conclusion that TMB isomers are not mutagenic is not currently supported.

Comment: The most useful study for the determination of the RfC is Clark et al. (1989), a one year inhalation study in rats at doses of 450, 900 and 1800mg/m^3. This study provides a longer duration of exposure and the outcome is consistent with the 90 day inhalation study of 1,2,3 TMB (Korsak et al., 2000b), and the 90 day oral toxicity study of 1,3,5-TMB (Koch Industries, 1995b). The 90 day neurotoxicity study with C9 aromatics (Douglas et al., 1993) which was performed at higher doses than Clark et al. (1989) and evaluated standard neurotoxicity endpoints; motor activity, functional observation battery including the hot plate latency response [without foot shock] at 5, 9 and 13 weeks of exposure is also useful as supporting information as no adverse effects were identified. (Comment V, p 17)

EPA Response: As discussed above and in Appendix E, the available C9 fraction studies have not been included in the Draft assessment for a number of reasons. Primarily, the C9 fraction is a complex mixture containing at most 55% TMB isomers. Currently, it is unclear why the results of the C9 fraction studies disagree with the results of individual TMB isomer studies, although the possibility exists that interactive effects between the constituents of the C9 fraction and biological systems alter the ADME of TMB. Therefore, the Clark et al. (1989) study is not suitable as the basis for the derivation of RfCs values for the TMB isomers. Therefore, the methodology used in the assessment to identify the RfCs for the isomers (i.e., derivation of RfC values for 1,2,4-TMB and 1,2,3-TMB using isomer-specific data, and setting the RfC for 1,3,5-TMB equal to the RfC for 1,2,4-TMB based on toxicological and toxicokinetic similarities between the isomers) is retained in the Draft Assessment. The sections outlining the derivation of the RfC for each individual TMB isomer have been thoroughly edited to more clearly delineate the process by which the values were derived.

Comment: For the RfD determination the 90 day oral study with 1,3,5 TMB (Koch Industries, 1995b) is preferable to extensive extrapolation from inhalation data. Results have been accepted by EPA to characterize the hazards of 1,3,5 TMB. Reliance on this study would obviate the need for pharmacokinetic analysis and route to route extrapolation. The more extensive data base accompanying this study reduces the uncertainties identified with the current investigation and avoids reliance on studies with interpretational difficulties. Furthermore, since IRIS acknowledges the similarity in toxicological responses among the TMB isomers, an RfD based on animal data for 1,3,5 TMB could reasonably be extrapolated to the other 2 isomers. (Comment II.2, pp 10-11; Comment V, p 17)

EPA Response: As stated above, discussion of the 90-day oral gavage Koch Industries (1995b) study has been added to the Draft Assessment, and it was considered as a possible principal study on which to derive an RfD. However, although the Koch Industries (1995b) study was submitted to EPA under a TSCA 4(a) test rule, it had not undergone an independent external peer review. As stated in Section 3.1 of the Preamble, "[i]f a study that may be critical to the conclusions of the assessment has not been peer-reviewed, EPA will have it peer-reviewed. As such, EPA sought an independent external review of the Koch Industries (1995b) study by three experts in neurotoxicology, human health risk assessment, and general laboratory animal toxicology studies (Versar, 2013). All three external reviewers concluded that the Koch Industries (1995b) study was well-written, followed GLP or standard protocols (with only minor deviations) for the time period in which the study was conducted (i.e., mid-1990s), and that the conclusions of the study were supported by the reported findings. However, two reviewers specifically commented that Koch Industries (1995b) study was not an appropriate study on which to base the derivation of a reference dose for a number of reasons (detailed below). The third reviewer, while not explicitly

stating the study was not suitable for RfD derivation, did provide comments that addressed multiple shortcomings of the study.

Two reviewers questioned the human relevancy of the chosen route of exposure (oral gavage), with one reviewer noting that, as the toxicity of 1,3,5-TMB was investigated due to it being a water contaminant, exposure via drinking water would be preferable over exposure via gavage. Further, this reviewer noted that the dosing regimen of the Koch Industries (1995b) study (5 days/week) was not optimal as toxicokinetic studies demonstrate rapid clearance of TMB and its metabolites (< 24 hours). Dosing only 5 days a week results in 48 hours of non-exposure and extended clearance; this reviewer suggested a dosing regimen of 7 days/week would have been more appropriate. One reviewer expressed strong concern that the NOAEL identified in the study was most likely an artifact of the study investigating insensitive endpoints (i.e., body weights, gross pathology). This reviewer expressed confidence that a lower NOAEL would have been identified had the study investigated endpoints "more pertinent to human health" (e.g., "behavioral, respiratory, or electrophysiological" endpoints). A second reviewer commented that, as demonstrated by the available peer-reviewed literature on TMBs, neurotoxicity is a critical endpoint for the evaluation of TMB-induced toxicity. This reviewer ultimately concluded that the Koch Industries (1995b) study is not reliable "for assessing noncancer risk, because the endpoint of concern for TMB exposure, neurotoxicity, was not evaluated". This reviewer acknowledged that although clinical signs were observed, these markers of effect were "too general to be predictive of neurotoxicity". This reviewer notes that although the Koch Industries (1995b) study could be used to quantitatively derive an RfD, the endpoint of concern (neurotoxicity) may not be protected against.

Given the result of the external peer review noted above, and the critical shortcomings of the Koch Industries (1995b) study (no testing for neurotoxicity and the general lack of any other observed toxicity), this study has limited utility for the derivation of an RfD for 1,3,5-TMB. Therefore, the methodology used in the assessment to identify an RfD for 1,3,5-TMB (i.e., setting the RfD equal to the RfD for 1,2,4-TMB based on toxicological and toxicokinetic similarities between the isomers) is retained in the Draft Assessment. The sections outlining the derivation of the RfD for each individual TMB isomer have been edited to more clearly delineate the process by which the values were derived.

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