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United States
Environmental Protection
Agency

Toxicological Review of Trimethylbenzenes

(CAS No. 25551-13-7, 95-63-6, 526-73-8, and 108-67-8)

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

Supplemental Information

June 2012

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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

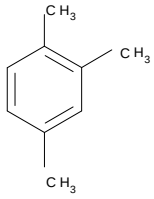
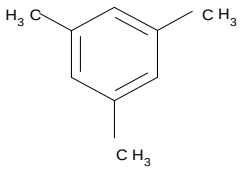
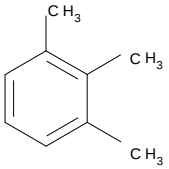
Table A-1. Other national and international health agency assessments for TMBs

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., 1956a)
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., 1997a; 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

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}		
Molecular weight	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		

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).

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 exposed to 25 ppm (1,2,3-TMB mg/m^3) 1,2,4-TMB, 1,2,3-TMB, or 1,3,5-TMB for 2 hours exhibited similar maximum capillary blood concentrations (6.5 and 6.2 µM, respectively), whereas absorption for 1,2,3-TMB was observed to be higher (7.3 µM) (Järnberg et al., 1998, 1997a; Järnberg et al., 1996). Kostrewski et al. (1997) observed equivalent maximal capillary blood concentrations in humans exposed to 30.5 ppm (150 mg/m^3) 1,2,4-TMB or 1,3,5-TMB for 8 hours (8.15 and 6.3 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 µM. In humans exposed to 25 ppm (123 mg/m^3) 1,3,5-TMB for 4 hours, venous blood concentrations were markedly lower (0.85 µM), 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 fairly similar in humans (60 ± 3%, 48 ± 3%, and 55 ± 2%, respectively) and approximate equivalency is observed between the respiratory uptake of 1,2,4-TMB between humans and rats (60 ± 3% and 44–50%, respectively) (Järnberg et al., 1996; Dahl et al., 1988).

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, 221, and 64.6 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. 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. 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, 66.5, and 59.1, respectively) were similar in humans, 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 µg/g) and adipose tissue (200 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. In humans, both 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. Greater amounts of urinary benzoic and hippuric acid metabolites (73%) were observed after exposure to higher amounts of 1,3,5-TMB (up to 30.5 ppm) for 8 hours (Kostrzewski et al., 1997; Kostrewski and Wiaderna-Brycht, 1995).

Following occupational exposure to 1,2,4-TMB or 1,3,5-TMB, urinary benzoic acid and hippuric acid metabolites were highly correlated with TMB isomer air concentrations (Jones et al., 2006; Fukaya et al., 1994; Ichiba et al., 1992). Following oral exposures, the total metabolism of the different isomers differs somewhat, with the total metabolism of 1,3,5-TMB being fairly complete (73%), the total metabolism of 1,2,3-TMB being much less (33.0%), and the total metabolism of 1,2,4-TMB ranging from incomplete to almost totally metabolized (37–86%) (Huo et al., 1989; Mikulski and Wiglusz, 1975). The major terminal metabolites for 1,2,4-TMB and 1,3,5-TMB are dimethylhippuric acids (24–38% and 59% total dose, respectively). Dimethylhippuric acid metabolites represent a smaller fraction (10.1%) of the metabolites produced following 1,2,3-TMB exposure.

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.

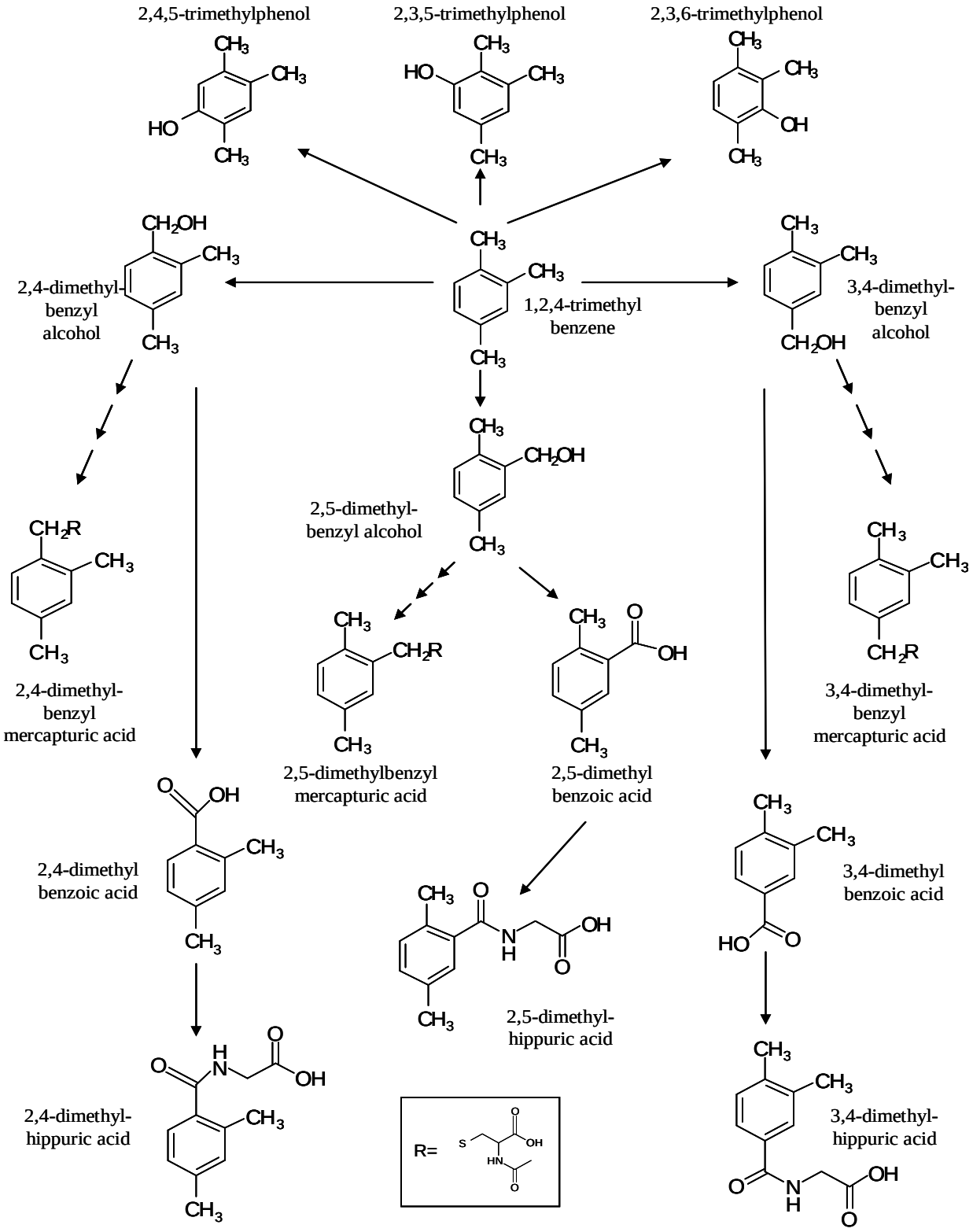


Figure B-1. Metabolic scheme for 1,2,4-TMB.

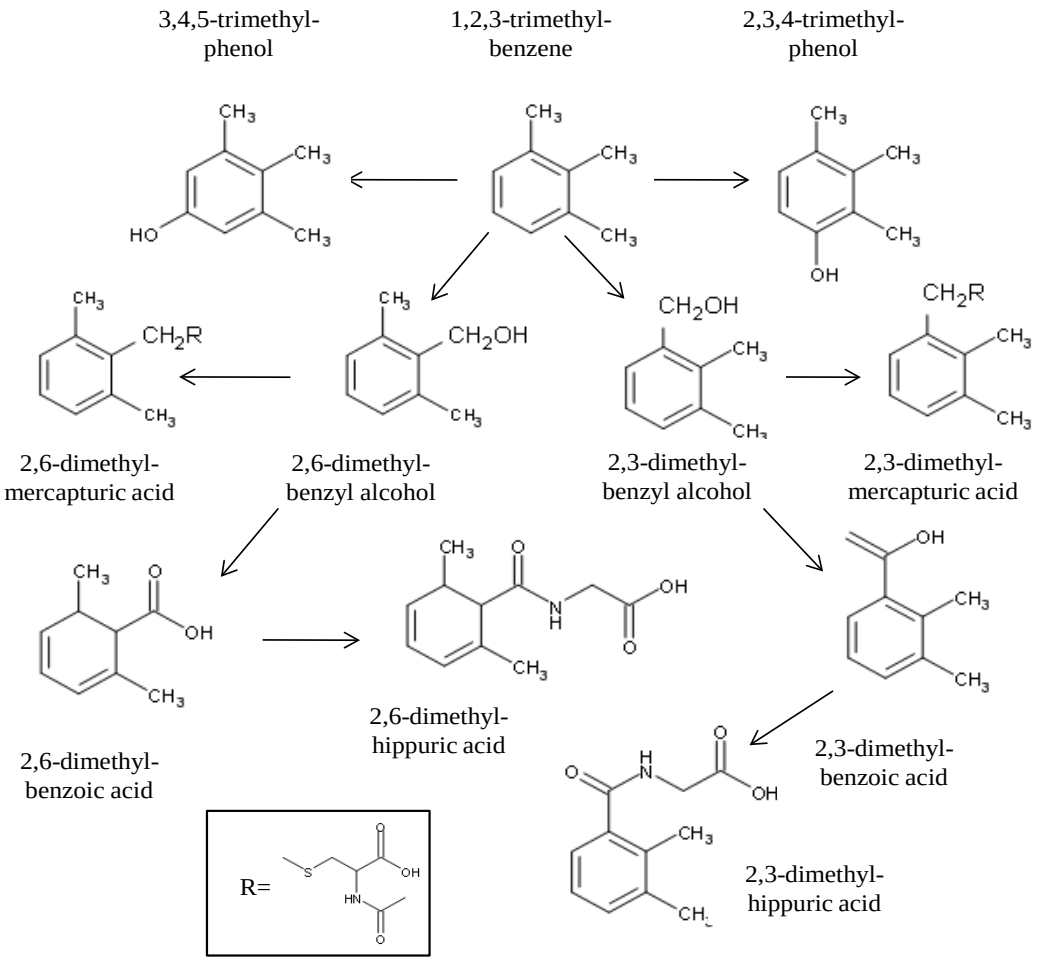


Figure B-2. Metabolic scheme for 1,2,3-TMB.

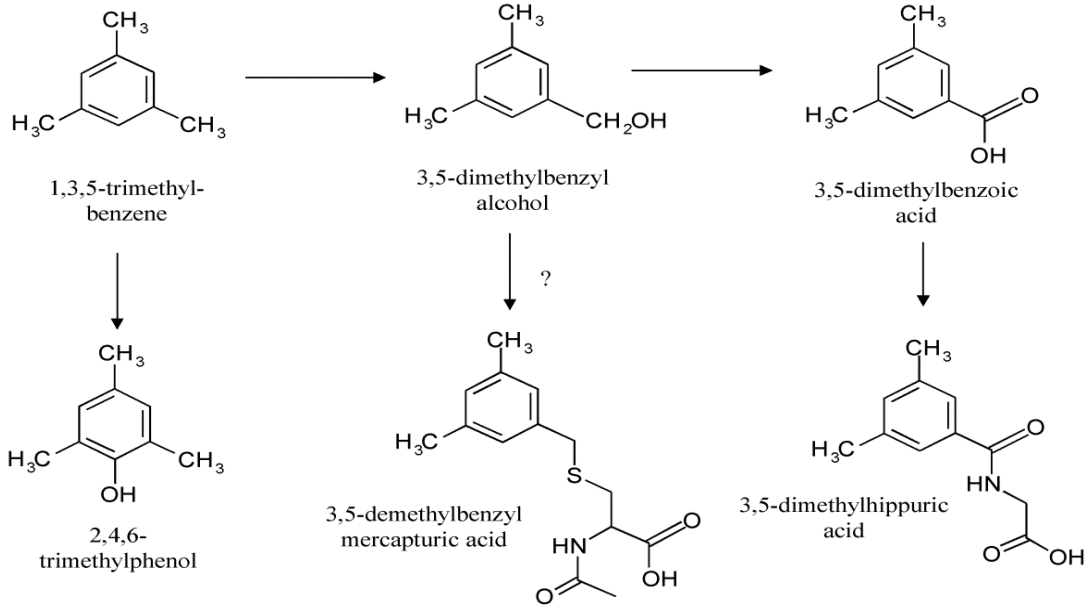


Figure B-3. Metabolic scheme for 1,3,5-TMB.

B.2.4. Excretion

In humans 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%) 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

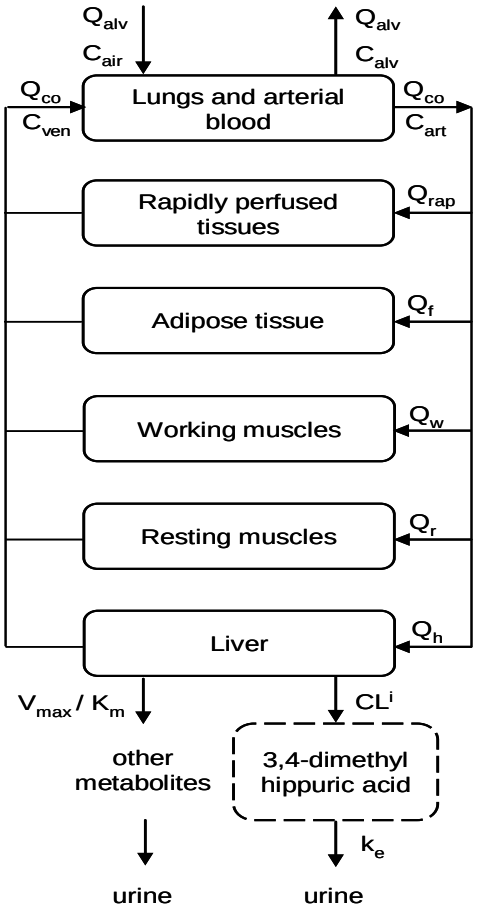
Järnberg and Johanson (1999)

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.



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		
Working muscles	0.55		
Resting muscles	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).

Emond and Krishnan (2006)

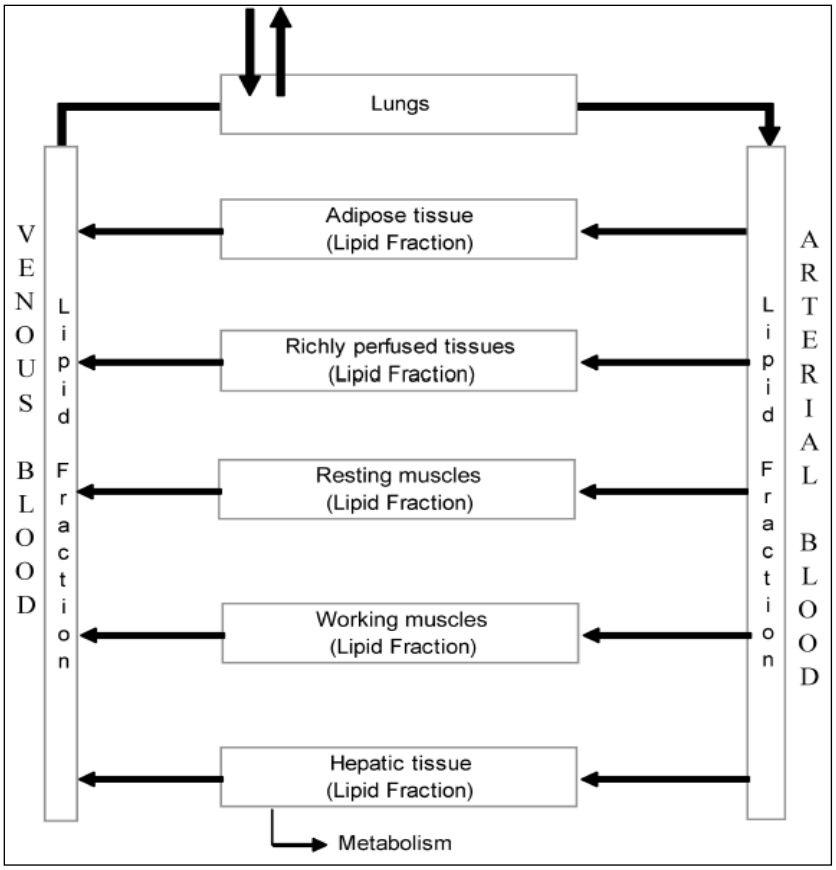
The Emond and Krishnan (2006) model was not developed specifically for 1,2,4-TMB, but rather to test a modeling concept. The PBPK model developed was to test the hypothesis that a model could be developed for highly lipophilic volatile organic chemicals (HLVOCs) using the neutral lipid-equivalent (NLE) 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.



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.

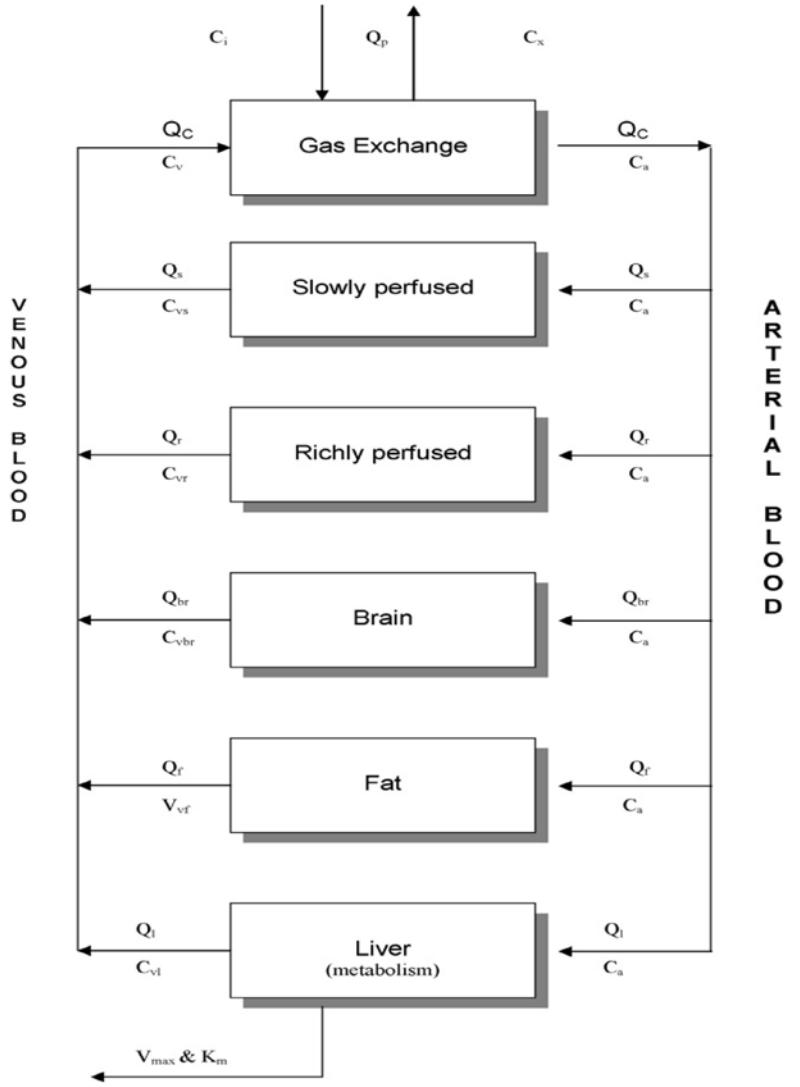
The PBPK model developed by Emond and Krishnan (2006) is used to test the hypothesis that a model could be developed for HLVOCs using the NLE content of tissues and blood as the basis. To test this NLE-based approach, the uptake and distribution kinetics in humans for several chemicals including 1,2,4-TMB 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.

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 parrafins, 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).



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 Arms and Travis (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.

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 Arms and Travis (1988).
[b]Assuming a standard 250 g rat.
[c]Hissink et al. (2007) value outside of literature range, but acceptable (see discussion in text).

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-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 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 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).

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).

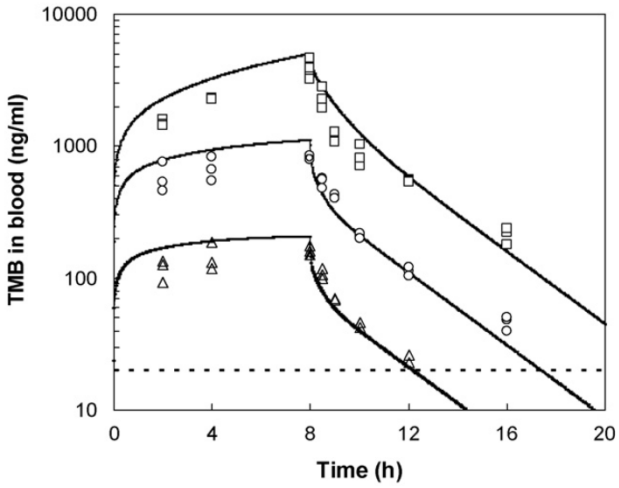
Where data were available, the agreement is generally acceptable. While the rat-derived K_m is less than the lower 95% confidence interval value for the human K_m, the human $V_{max}C/K_m$ ratio is in acceptable agreement. When considering sufficiently low exposure concentrations, the performance of 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)



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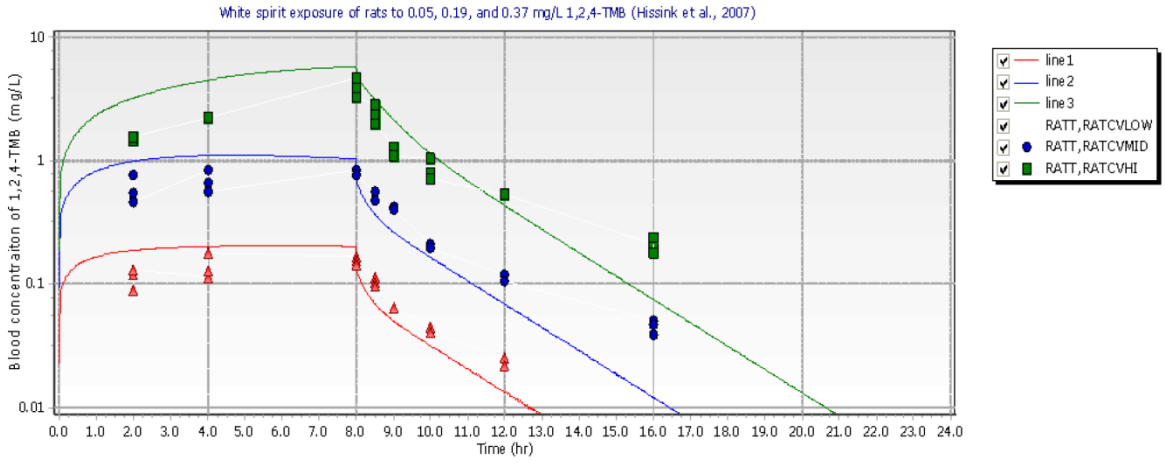
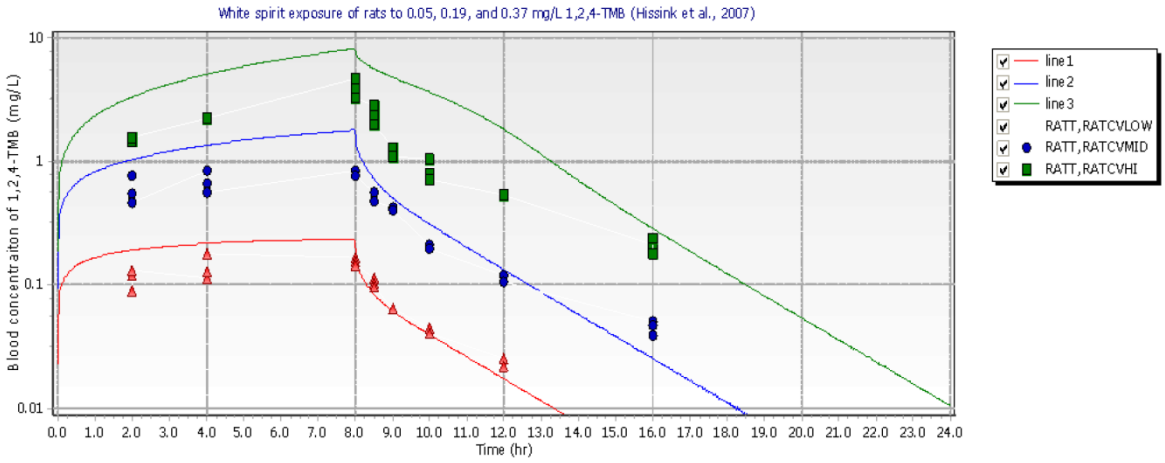
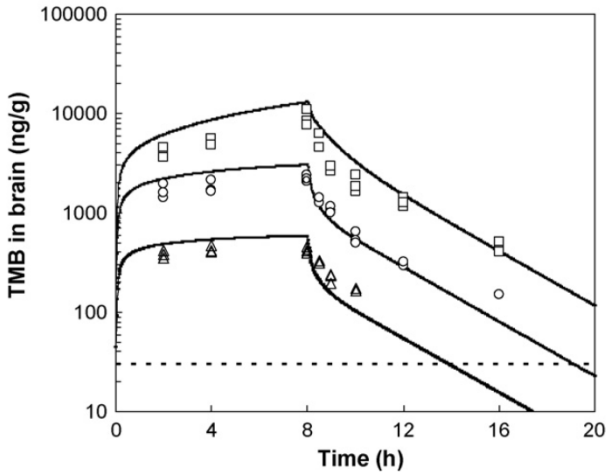


(c)

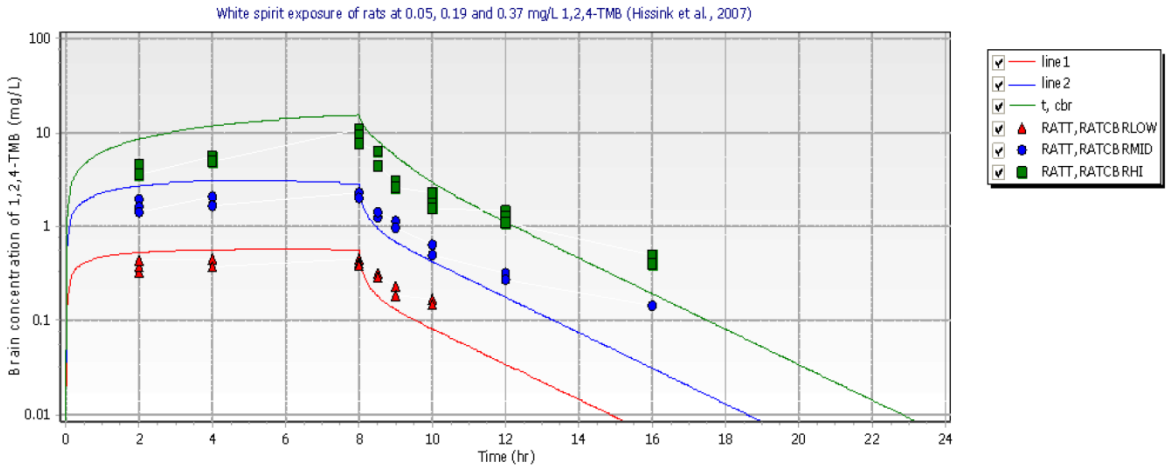


(a) Hissink et al. (2007), Figure 2, lower panel (b) variable V_{max}, (c) constant V_{max}.

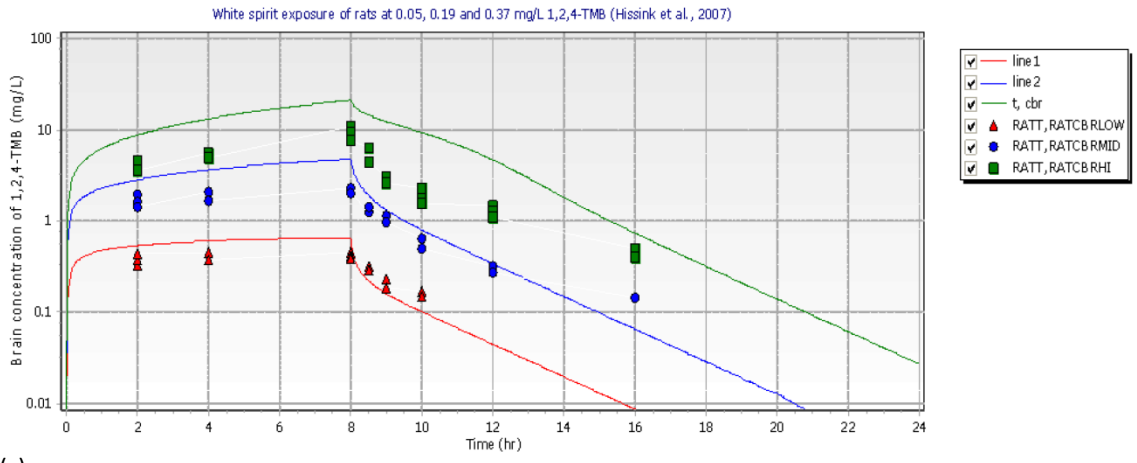
Figure B-7. Simulated and measured blood concentrations of 1,2,4,-TMB in rats exposed to 600, 2,400, or 4,800 mg/m³ WS for 8 hours.



(a)



(b)

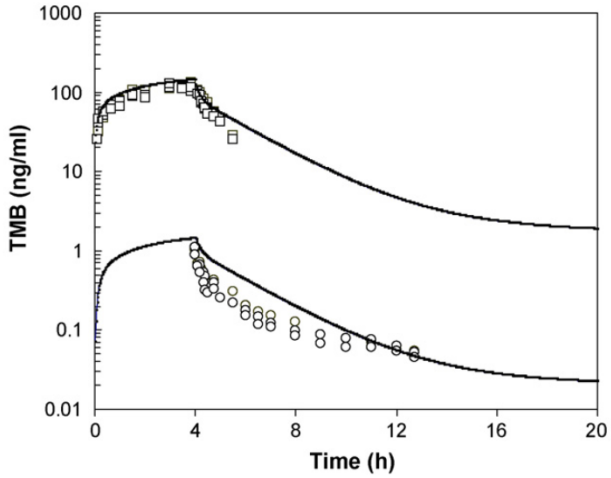


(c)

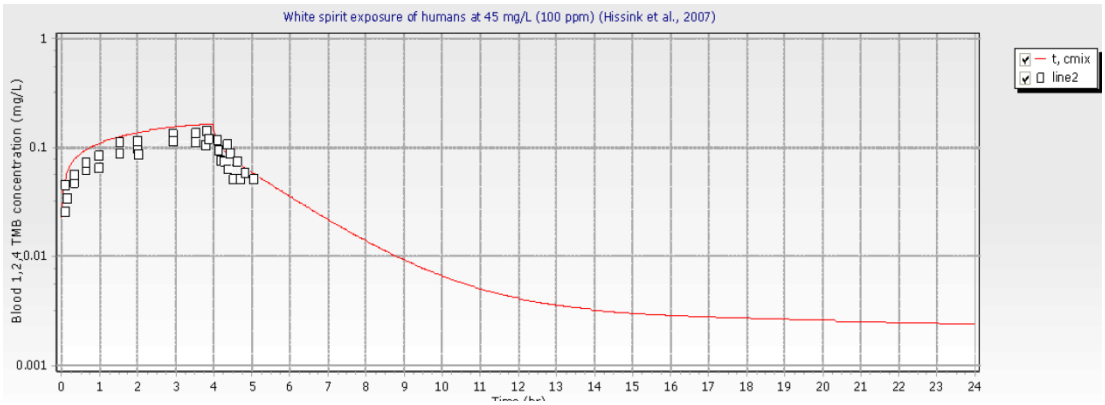
(a) Hissink et al. (2007), Figure 3, lower panel. (b) variable V_{max} (c) constant V_{max}.

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.

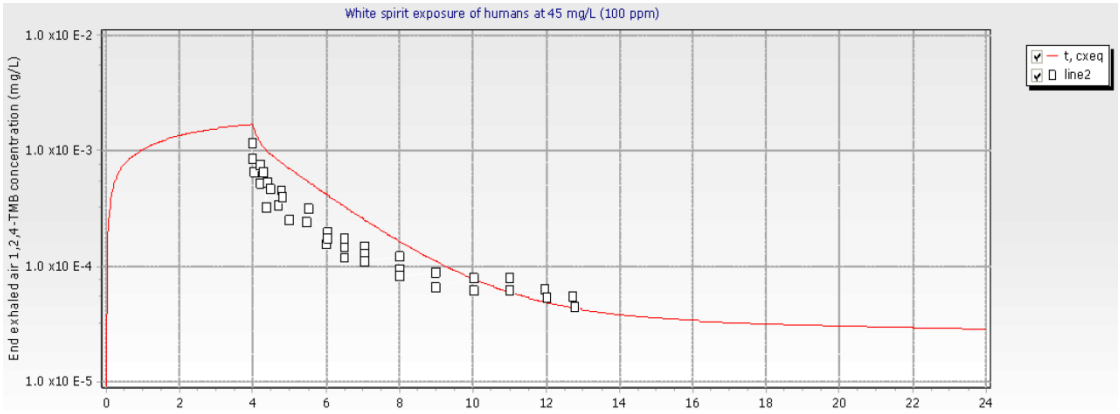
(a)



(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/m^3 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, 2011).

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/h/kg$^{0.70}$)	4.17	
K_m (mg/L)	0.322	

[a]Study specific.

Rat Model Optimization

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

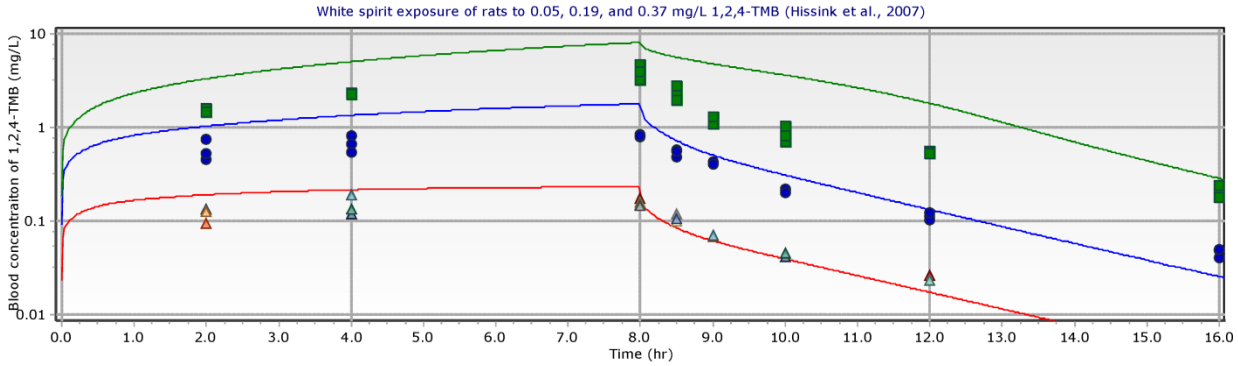
Table B-8. Rat 1,2,4-TMB kinetic studies used in model development and testing

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/d, 5 d/wk 4 wks	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/d 14 d	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/d 3 d	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).

(a)



(b)

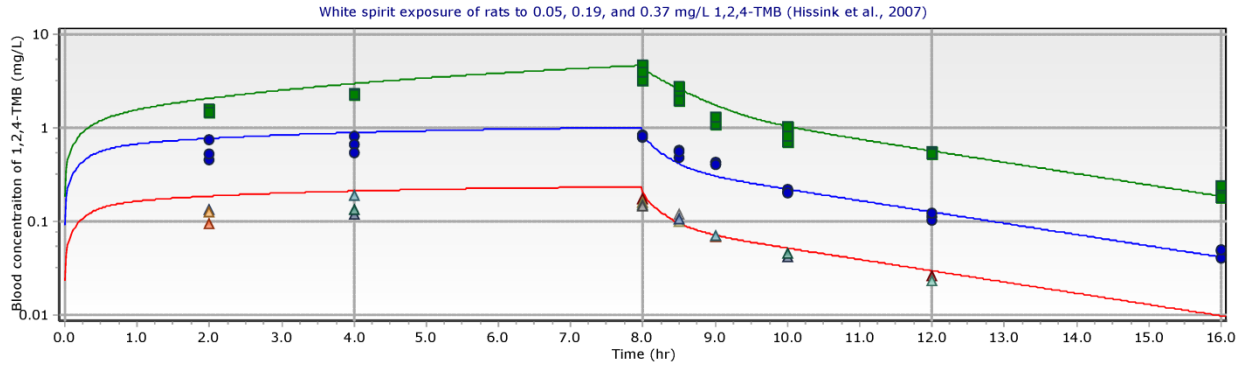


Figure B-10. Comparisons of model predictions to measured blood concentrations in rats exposed to 1,2,4-TMB in WS (Hissink et al., 2007) (a) before and (b) after numerical optimization.

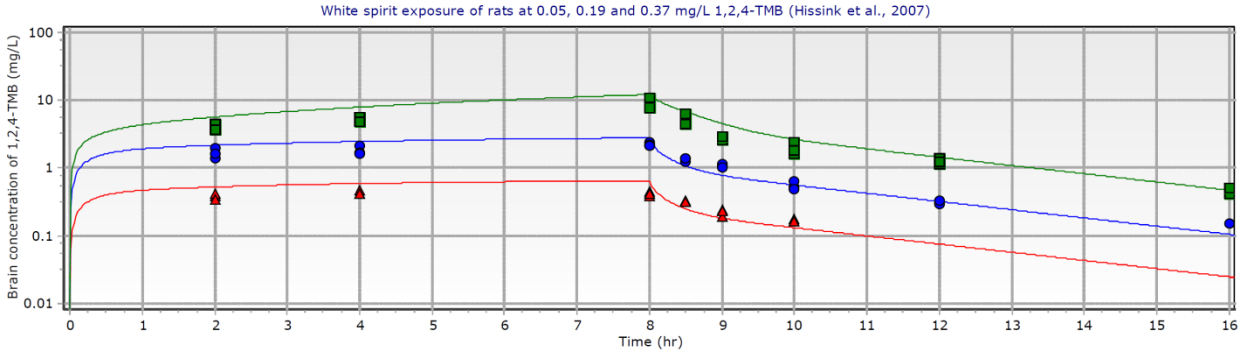
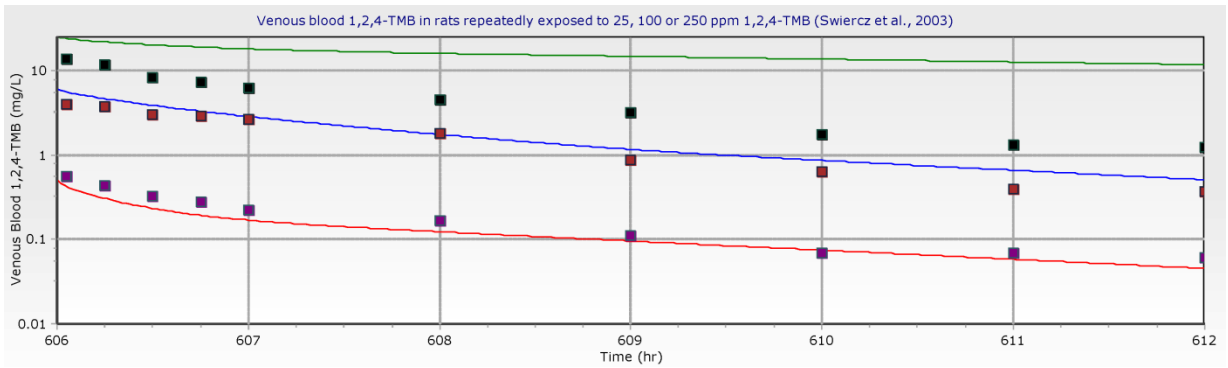
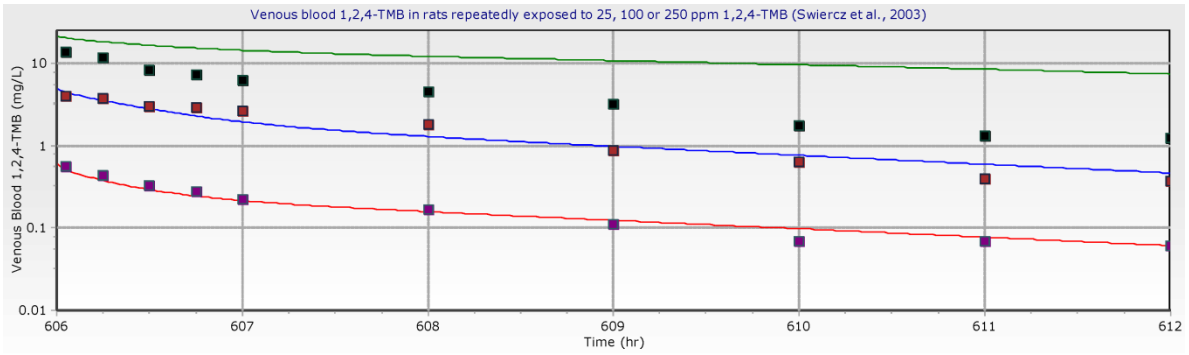


Figure B-11. Comparisons of model predictions to measured brain concentrations in rats exposed to 1,2,4-TMB in WS (Hissink et al., 2007) using model parameters optimized for fit to Hissink et al. (2007) rat blood data.

(a)





(b)

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 (a) before and (b) after numerical optimization.

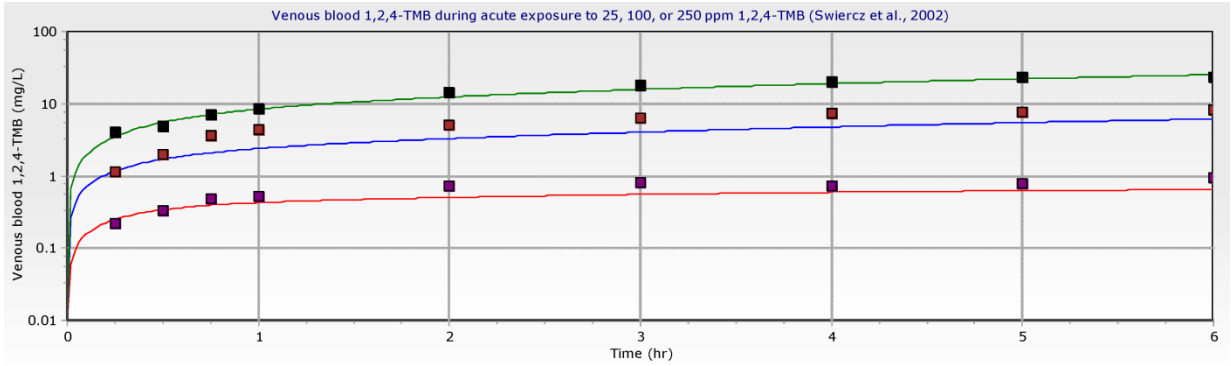
The $V_{max}C$ and K_m values derived from optimization to the Hissink et al. (2007) rat data were used as the starting values for optimizing fit to the venous blood data of Swiercz

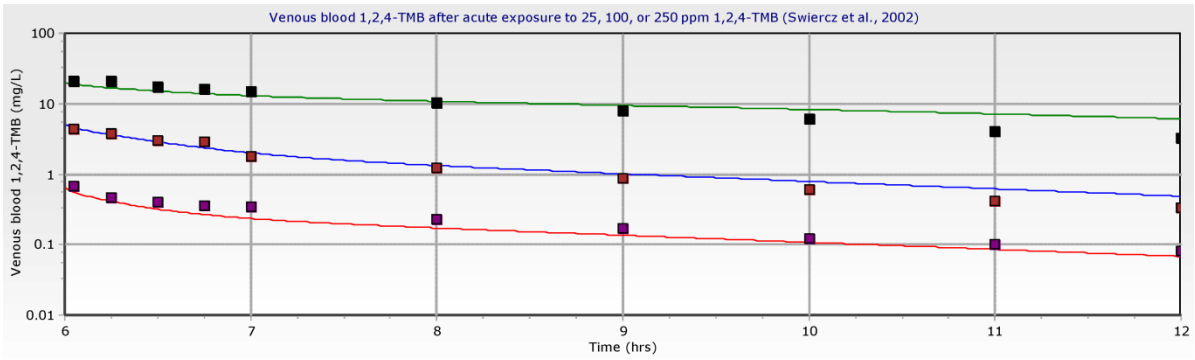
et al. (2003), in which exposure was to 1,2,4-TMB (only) repeatedly for 4 weeks. Venous blood samples were collected from the tail vein. The best fit parameters of $V_{max}C$ = 4.17 mg/hr/kg$^{0.7}$ and K_m= 0.322 mg/L produced an increase in the LLF from -28.1 to -15.6, a statistically significant improvement, 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)

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

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

	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/m^3)	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 from 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/d. 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/d, for 3 d) 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	1.70	5.0
	2	8.71	1.51	5.8
	3	8.72	2.05	4.2
	Recovery[b]	1.08	0.024	7.6
Brain	1	22.6	4.57	4.9
	2	23.1	4.19	5.5
	3	23.1	4.38	5.3
	Recovery[b]	0.46	Nondetect	Not calculated
Liver	1	18.2	4.92	3.7
	2	18.7	3.66	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	17.0	1.4
	3	23.1	12.4	1.9
	Recovery[b]	0.46	0.24	1.9
Fat	1	491	210	2.3
	2	503	165	3.1
	3	504	128	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 and1.69 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/d, for 14 d) 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 section regarding rat model optimization, page B-29).

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) (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).

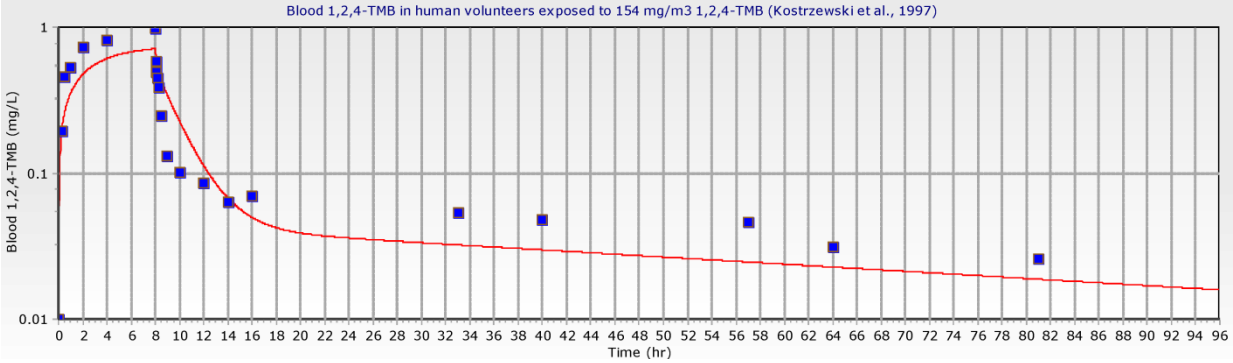


Figure B-14. Comparisons of model predictions to measured human venous blood concentrations of Kostrzewki et al. (1997) in human volunteers exposed to 154 mg 1,2,4-TMB/m³ for 8 hours.

For comparisons between the Järnberg et al. (1999; 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/m³), 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/m³), 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.

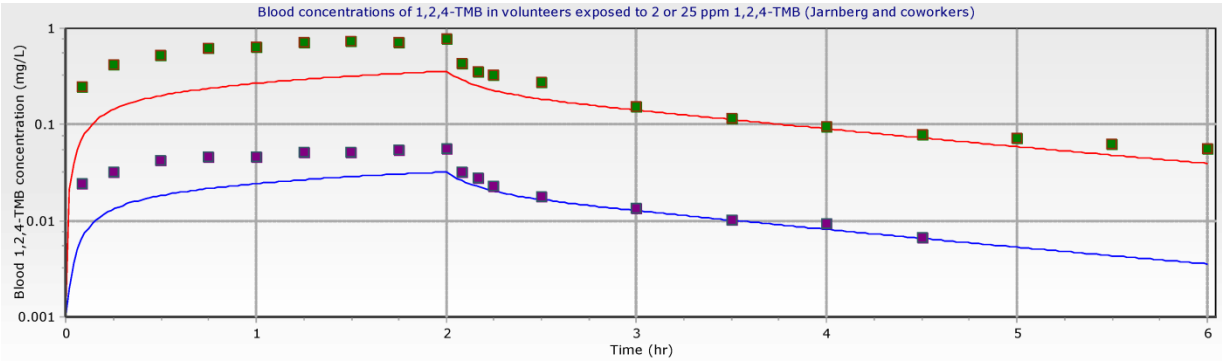
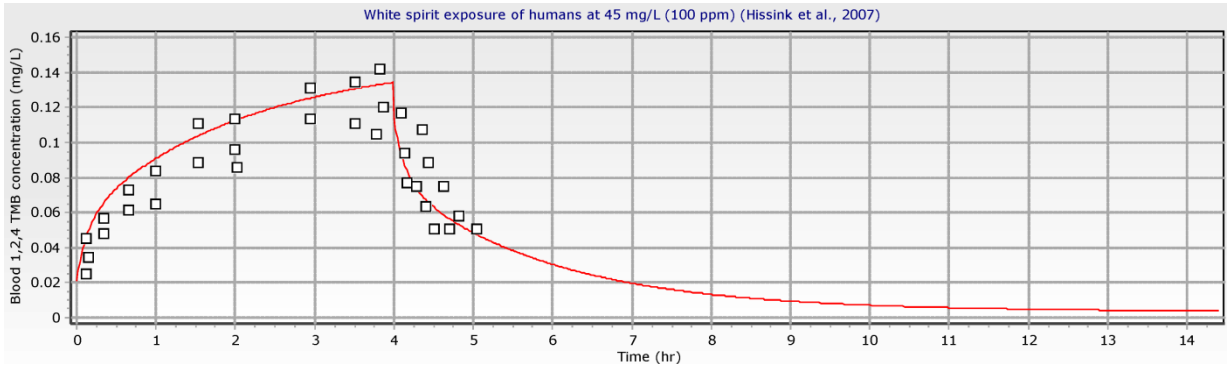
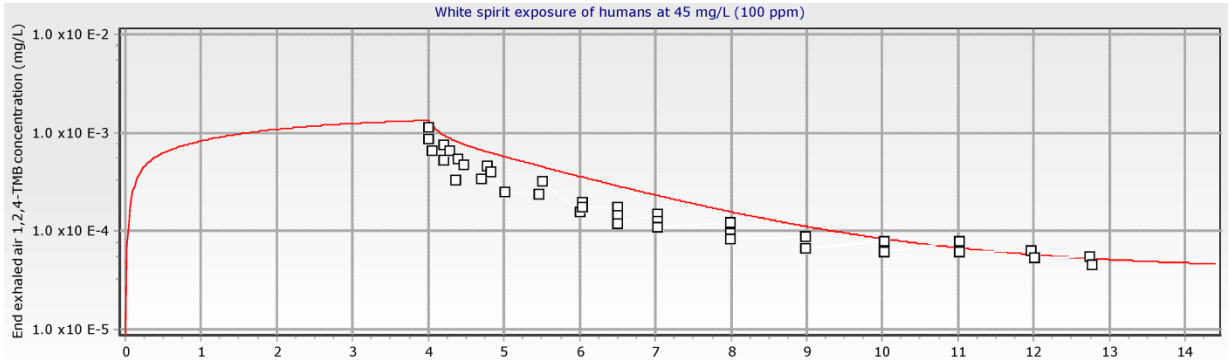


Figure B-15. Comparisons of model predictions to measured human venous blood concentrations of Järnberg et al. (1998, 1997a; 1996) in volunteers exposed to 2 or 25 ppm (~10 or 123 mg/m³) 1,2,4-TMB for 2 hours while riding a bicycle (50 W).

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)

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) (Hissink et al., 2007).

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)

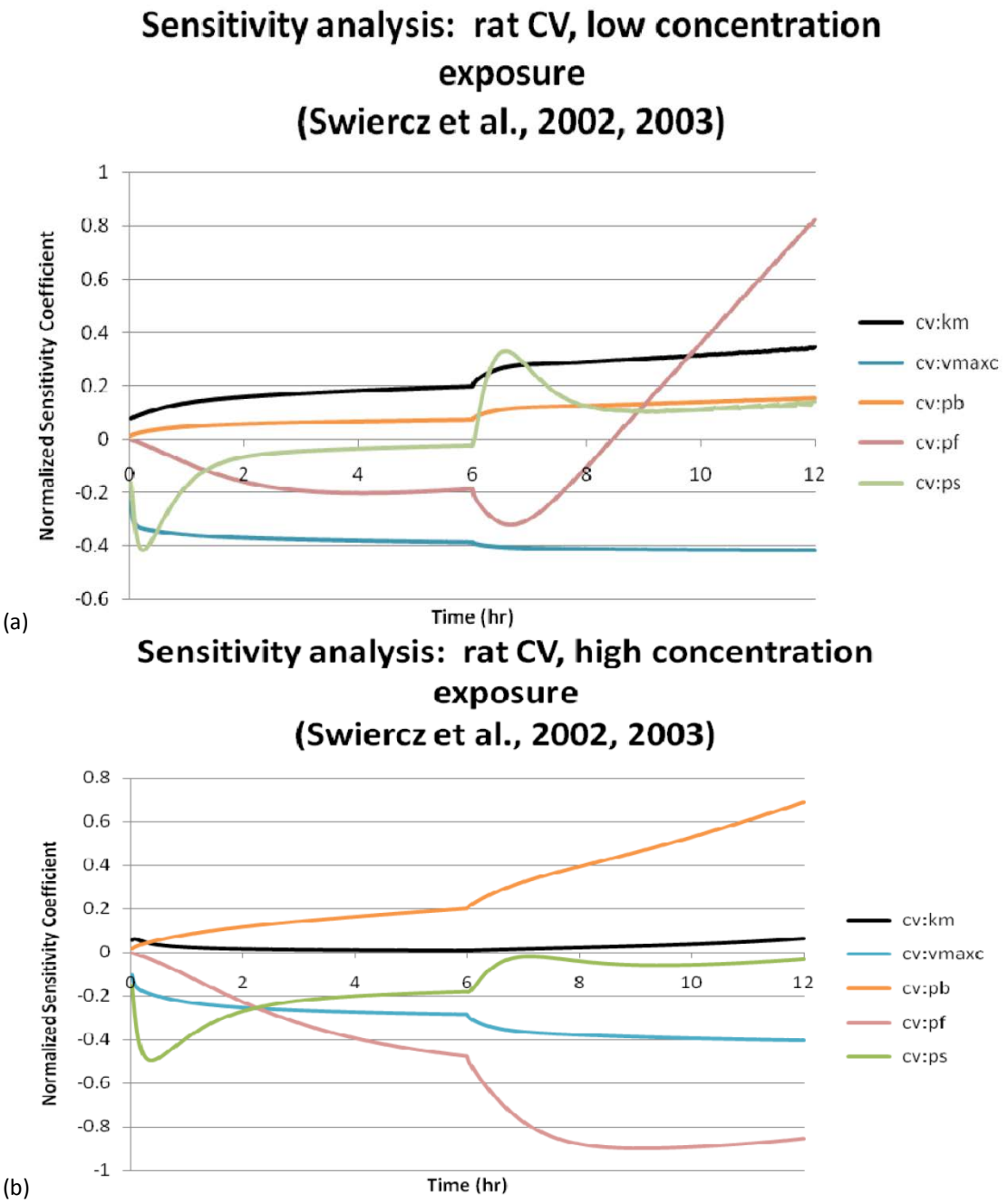


Figure B-17. Time course of normalized sensitivity coefficients of moderately sensitive chemical-specific parameters (response: venous blood concentration) in rats exposed to (a) 25 ppm (123 mg/m^3) or (b) 250 ppm (1,230 mg/m^3) of 1,2,4-TMB via inhalation for 6 hours (Swiercz et al., 2003; Swiercz et al., 2002).

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, the assumption that 100% of the dose would enter the liver is a common assumption.

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.

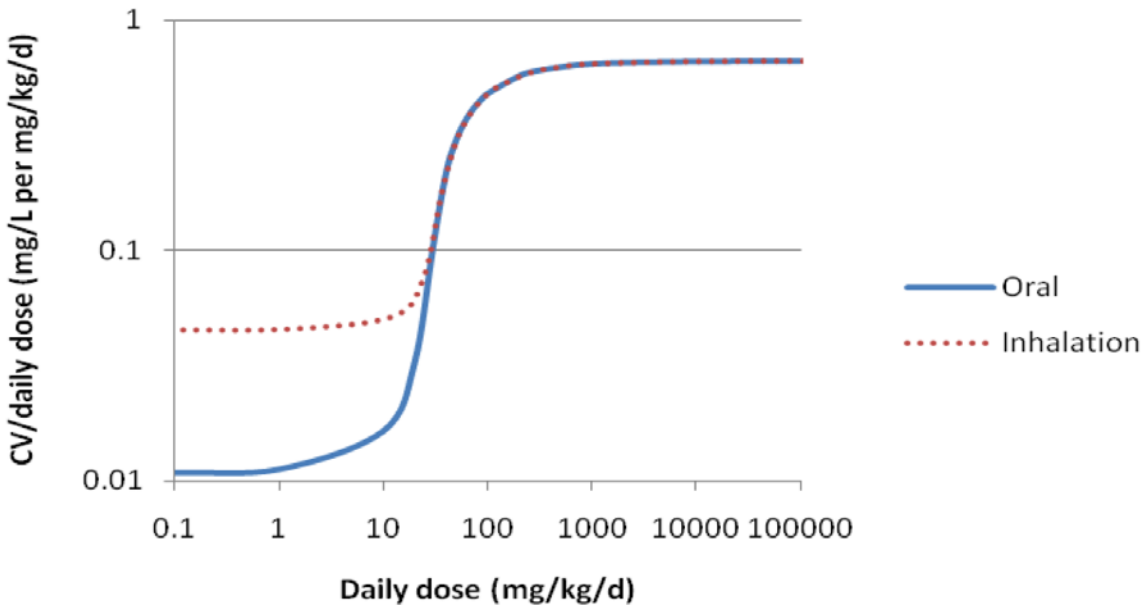


Figure B-18. Effect of route of exposure and dose rate on steady-state venous blood concentration (t = 1,200 hr) for continuous human exposure to 1,2,4-TMB.

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.

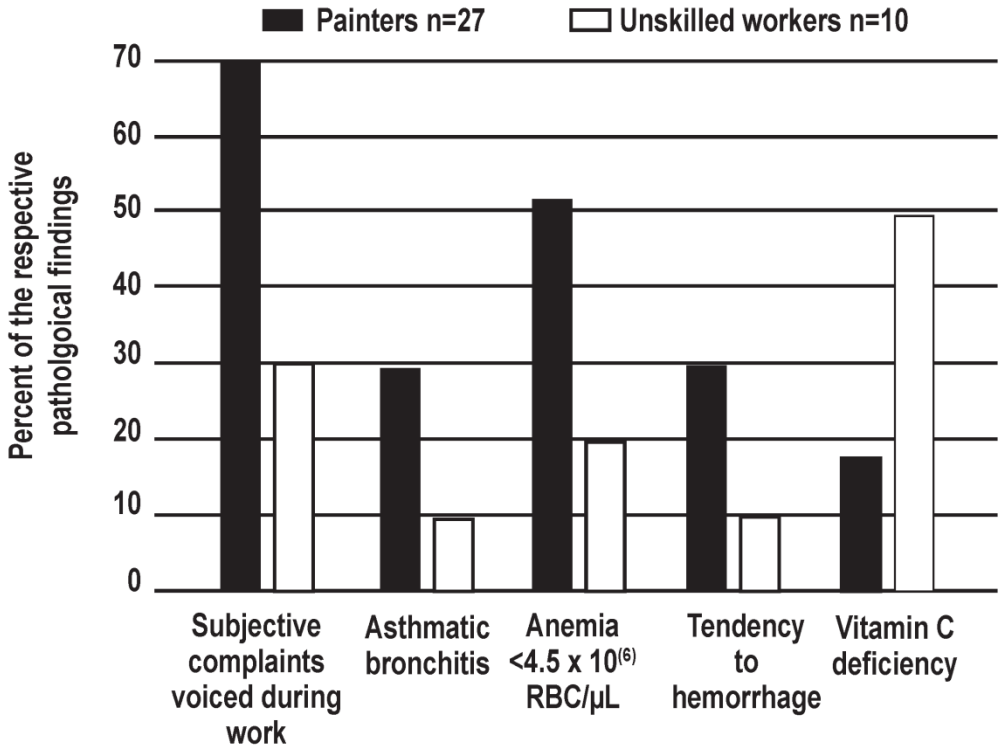
B.4. HUMAN STUDIES

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

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 (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.

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.

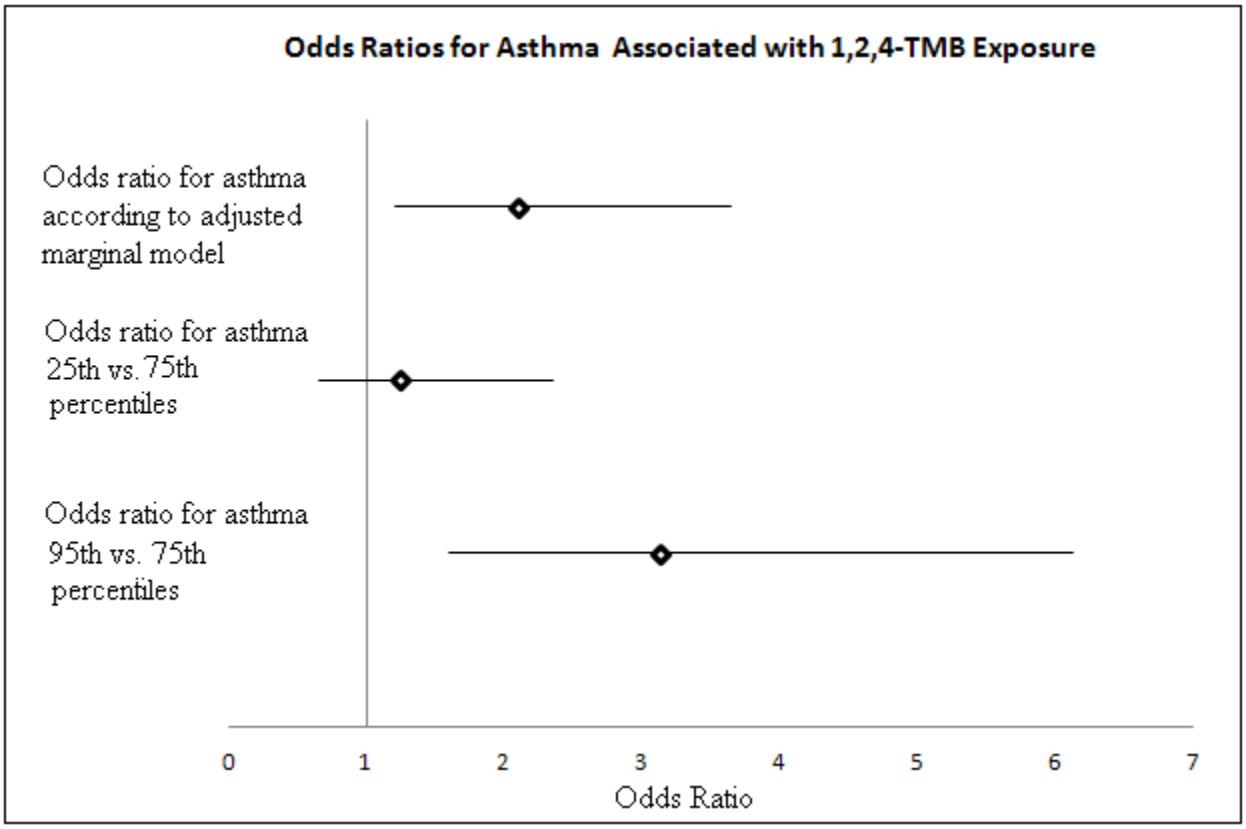


Table B-18. Characteristics and quantitative results for epidemiologic cohort study of exposure to 1,2,4-TMB. Chen et al. (1999)

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.

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, $p<0.00001$.

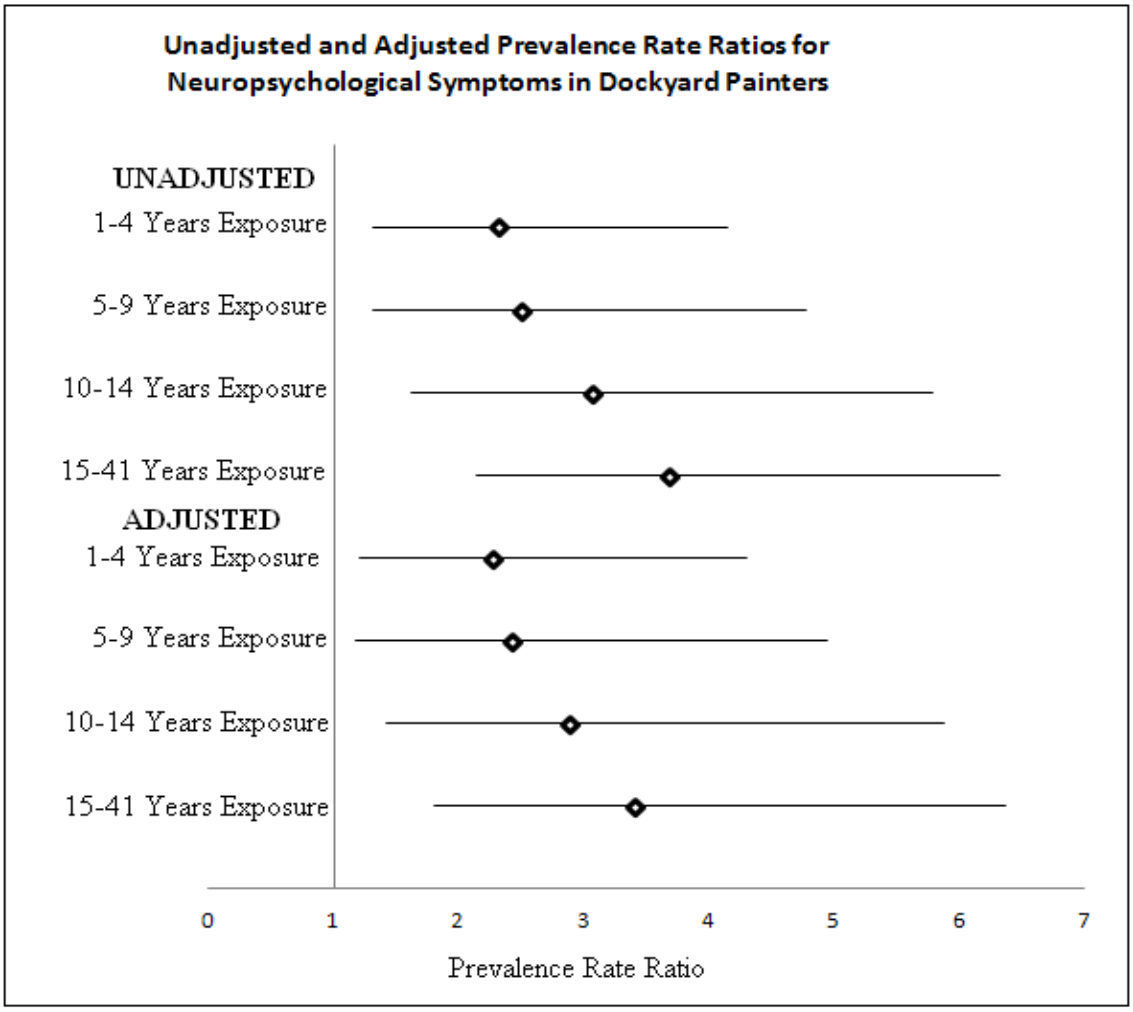


Figure 2. The effect of elapsed time since cessation of painting on all symptoms. Values reported are prevalence rate ratios for painters vs. non-painters. No significant decrease in risk with increasing post-exposure time was found.

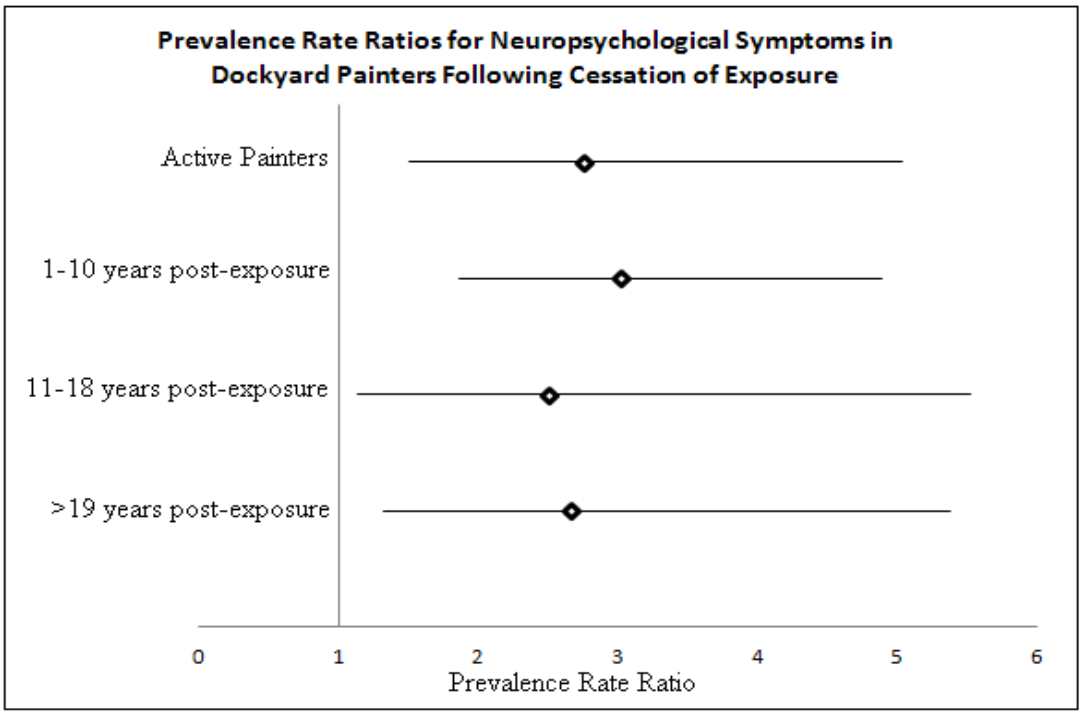


Figure 3. The effect of exposure duration on odds ratio for neuropsychological symptoms. With increasing years of exposure, odds ratios were found to increase.

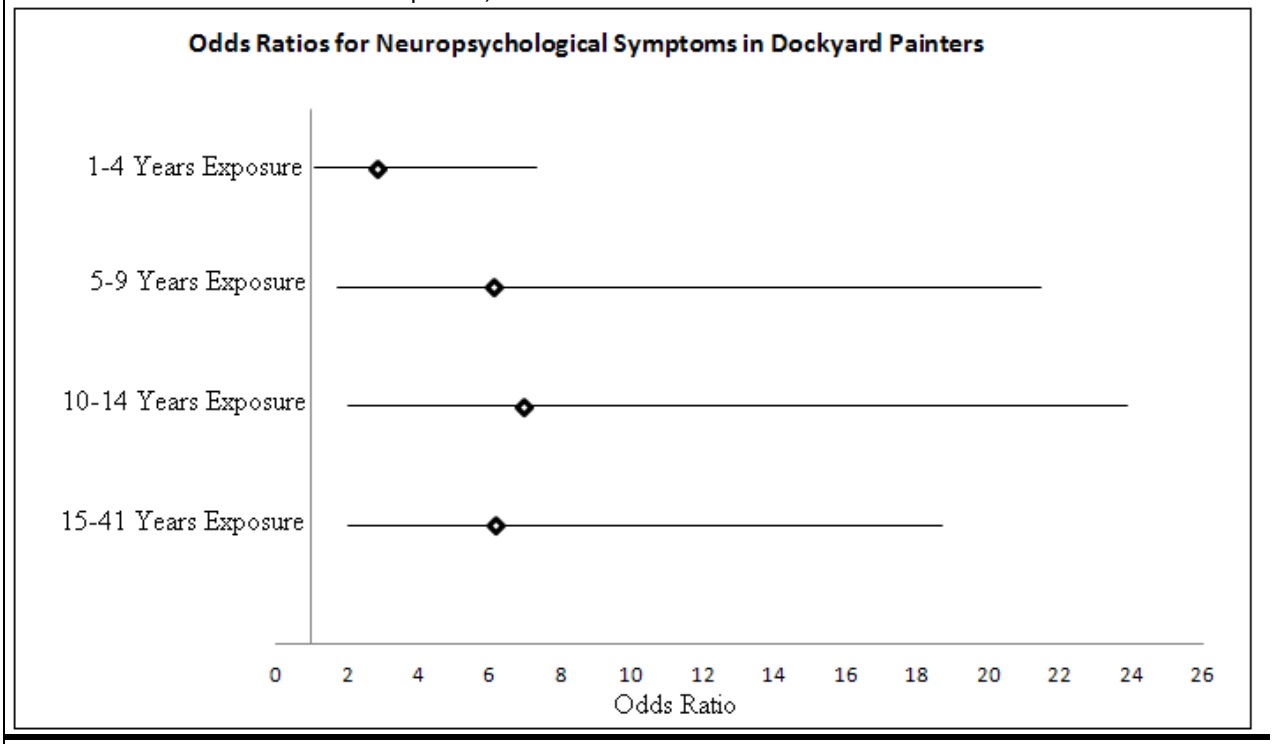


Table B-19. Characteristics and quantitative results for controlled human exposure study of exposure to 1,2,4-TMB in WS Lammers et al. (2007)

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 wk, 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					

Observation	**Test scores (mean ± SD) at various time points in humans exposed to 57 or 570 mg/m^3 WS for 4 hrs**	
	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

	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

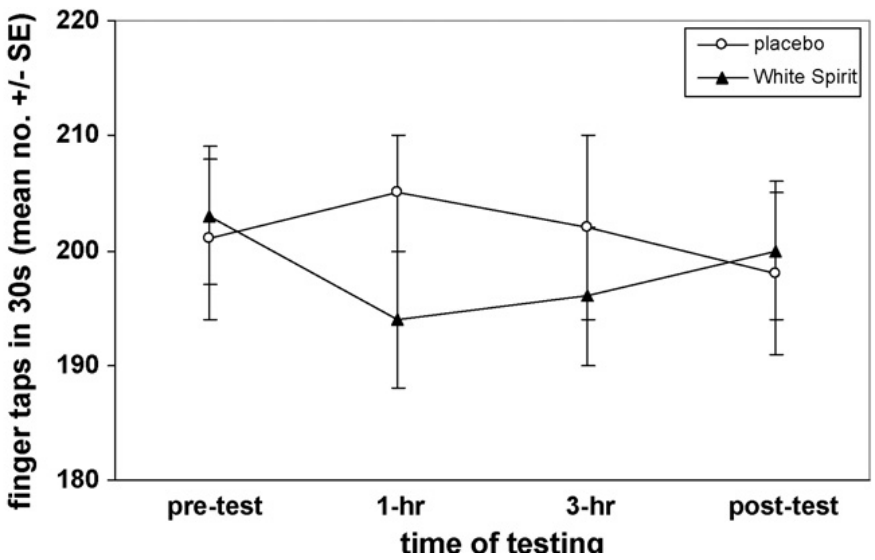


Figure 2. Performance on finger tapping test with the dominant hand at different time points during and after exposure.

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.

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.
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	

Observation	Results of Neurobehavioral Test of Study Subjects					
	Unadjusted Mean ±Std Dev			Adjusted[a] Mean (S.E.)		
	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

Observation	Neurobehavioral Test Results by Duration of Work, Adjusted for Age and Education		
	<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
*, ** $p < 0.05$, $p = 0.052$

Table B-20. 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.

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 hroups 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	22.1	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 ($p<0.05$).

Table B-21. 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-22. 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/d, 5/wk

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.

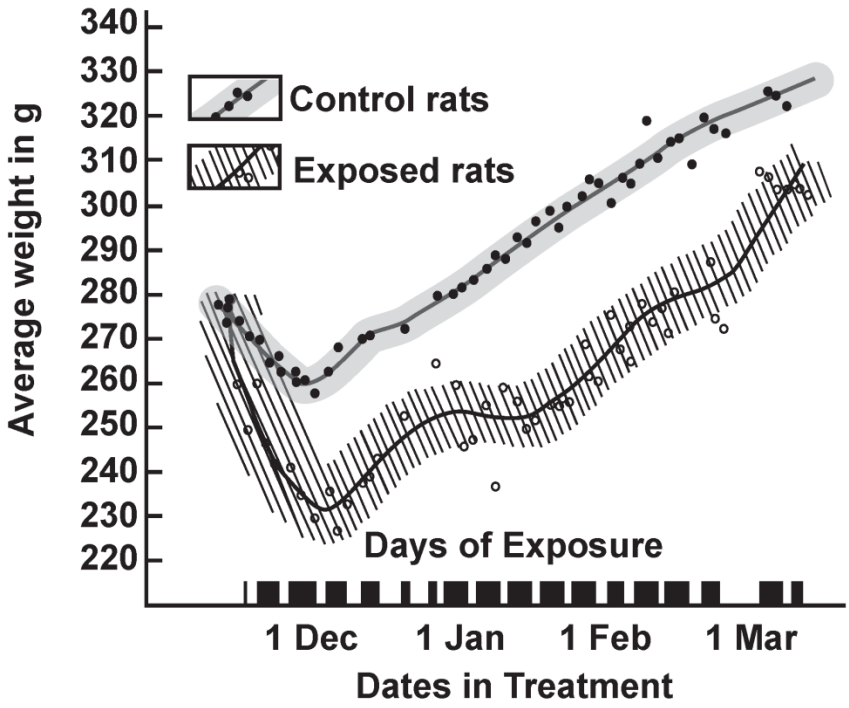


Figure 2. Effect of long-term exposure to trimethylbenzene (about 1,700 ppm [8,364 mg/m^3]) on the growth of rats. Open circles: Average body weights of the exposed rats. Closed circles: Average weights of the control rats. Hatched [and dotted] area[s]: Double square deviation from the mean values plotted.

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

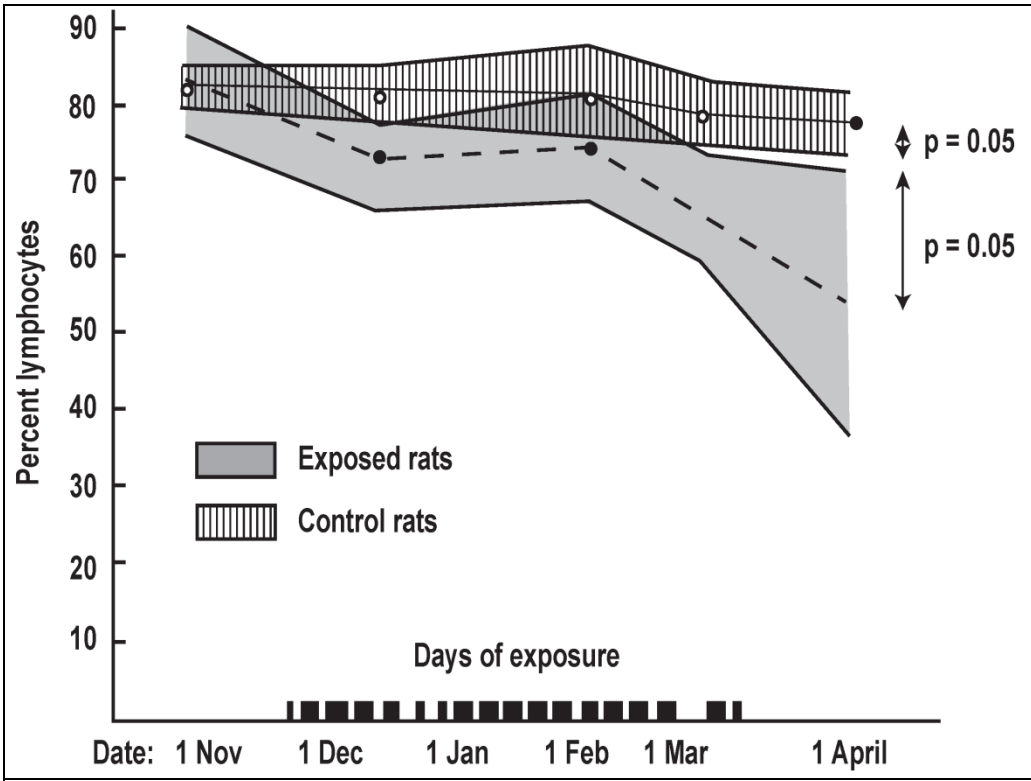


Figure 3. Behavior of the relative number of lymphocytes in trimethylbenzene-exposed rats (exposure: about 1,700 ppm [8,364 mg/m^3]).

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

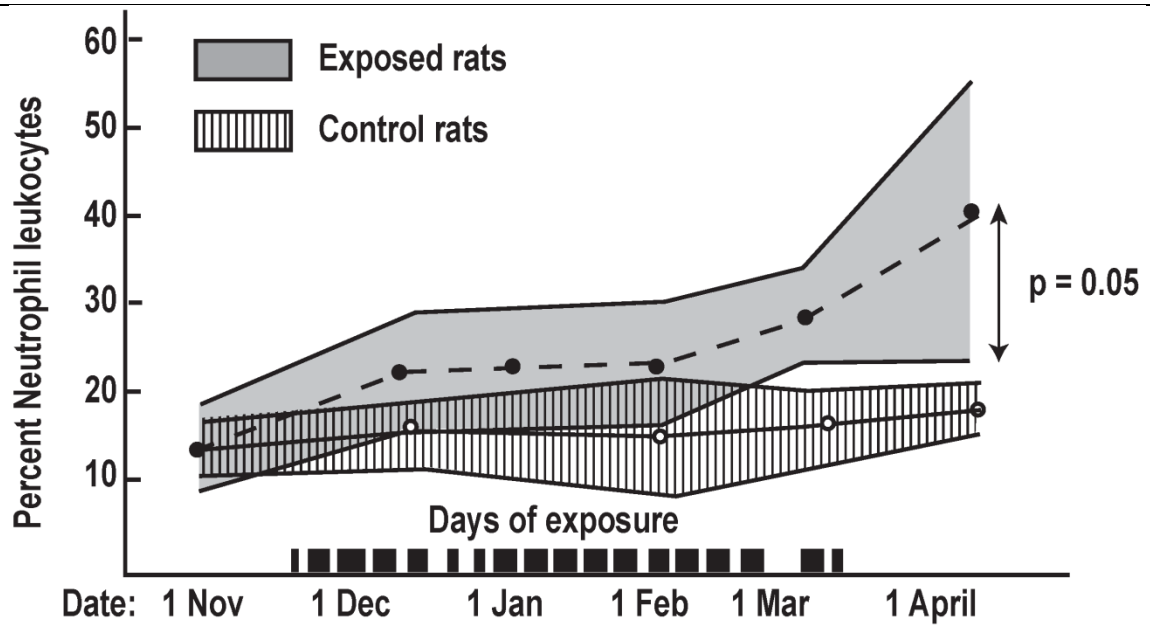


Figure 4. Behavior of the relative number of neutrophil leukocytes in trimethylbenzene exposed rats (exposure: about 1,700 ppm [8,364 mg/m^3]).

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

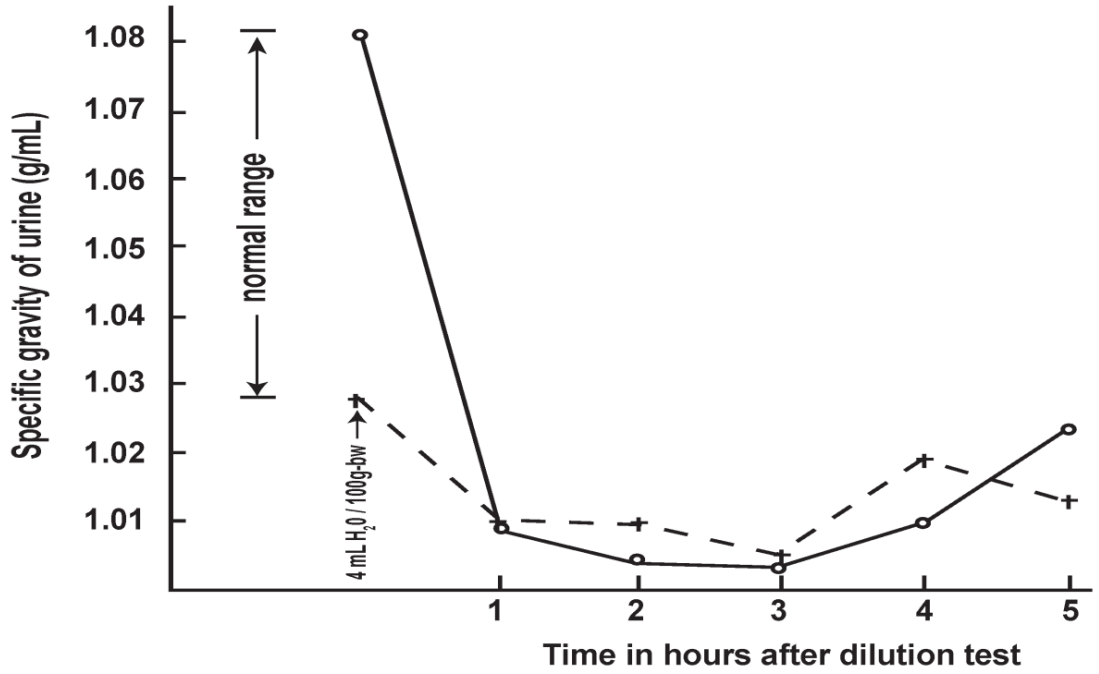


Figure 5. Specific gravity of spontaneous and dilution urines in TMB-exposed rats (exposure: about 1,700 ppm [8,364 mg/m^3]).

Source: Reproduced with permission of Springer-Verlag (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 (Baettig 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. (1956a) is published in German. However, Baettig et al. **(**1958**)** presents an English-translation of the results originally presented in Battig et al. (1956a). As such, a separate study summary table is not provided for Battig et al. (1956a). 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. (1997a)

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

Additional study details

- Animals were exposed to 1,2,4-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/d, 5 d/wk for 4 wks. 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.

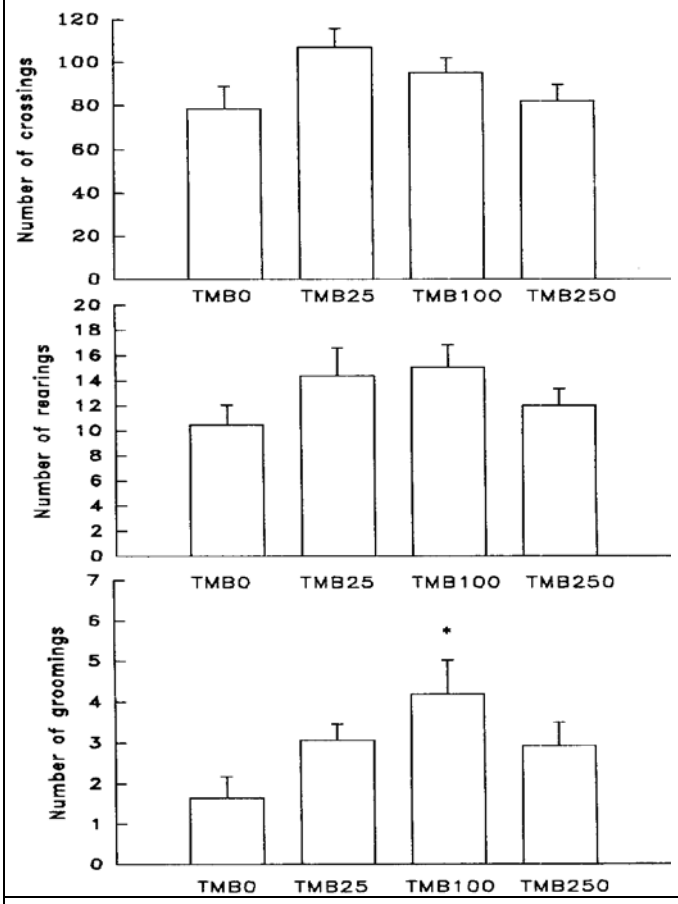


Figure 1. A comparison of spontaneous locomotor (upper diagram), exploratory (middle diagram, and grooming (lower diagram) activity of rats in an open field during a 5-min observation period. The test was performed 25 days after a 4-week exposure to TMB. The bars represent group means and SE (n = 15 for each group). **p*<0.05 compared with TMB0 group (0 ppm control group).

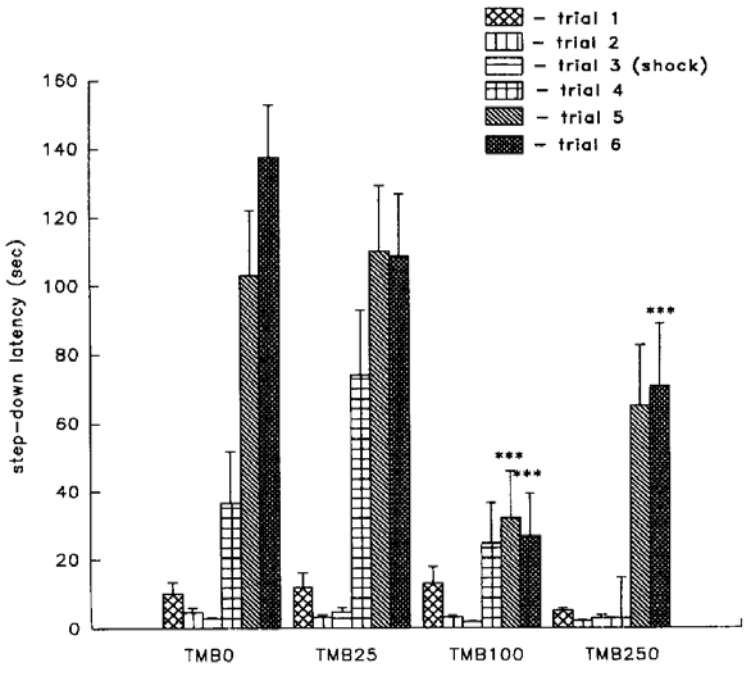


Figure 2. Diagrams illustrating the effect of a 4-week exposure to 1,2,4-TMB on the step-down passive avoidance learning in rats. The test was performed on days 35–45 after exposure. Trials 1, 2, and 3 were performed at 24-hr intervals. The step-down response was punished by a 10-s foot shock only in 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).

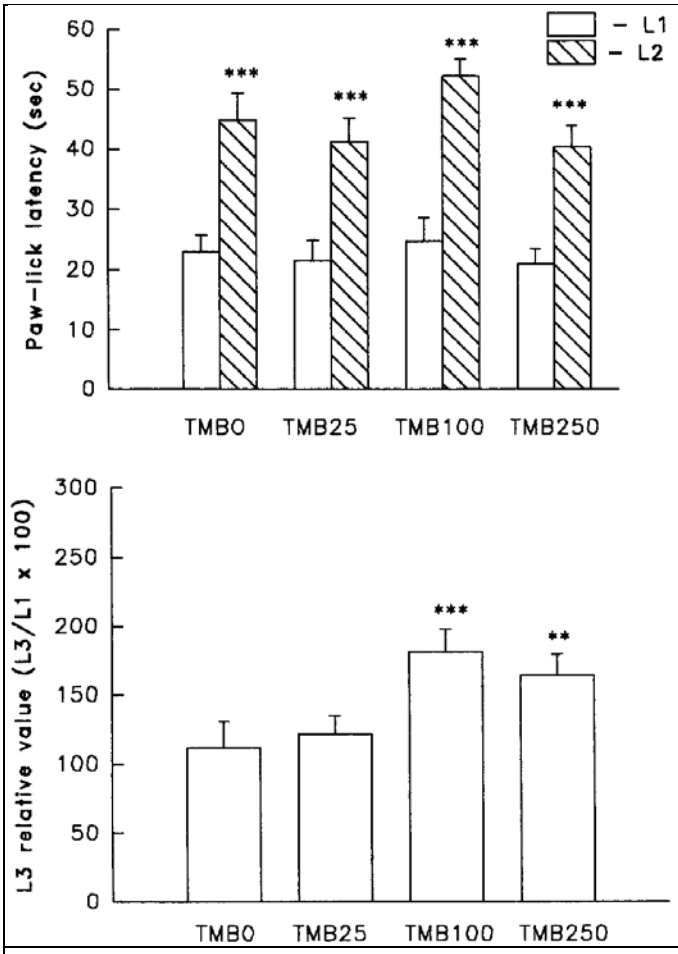


Figure 3. Hot plate behavior tested in rats on day 50 (trials 1 and 2) and day 51 (trial 3) after 4-week exposure to 1,2,4-TMB. Bars represent group means and SE (n = 15 for each group).

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).

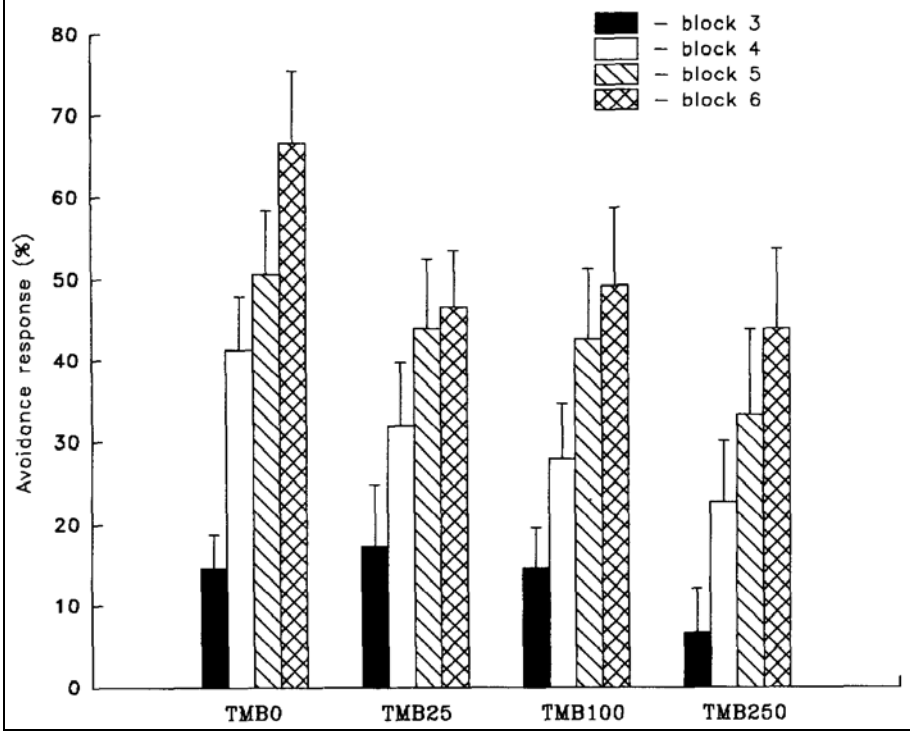


Figure 4. A comparison of the active avoidance performance increment during a single 30-trial training session in consecutive groups of rats. The testing was performed on day 54 after 4-week exposure to 1,2,4-TMB. Bars represent the percentage (group mean and SE, n = 15 for each group) of avoidance response 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.

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.		

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

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

Additional study details

- Animals were exposed to 1,2,4-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/d, 5 d/wk for 4 wks. 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.

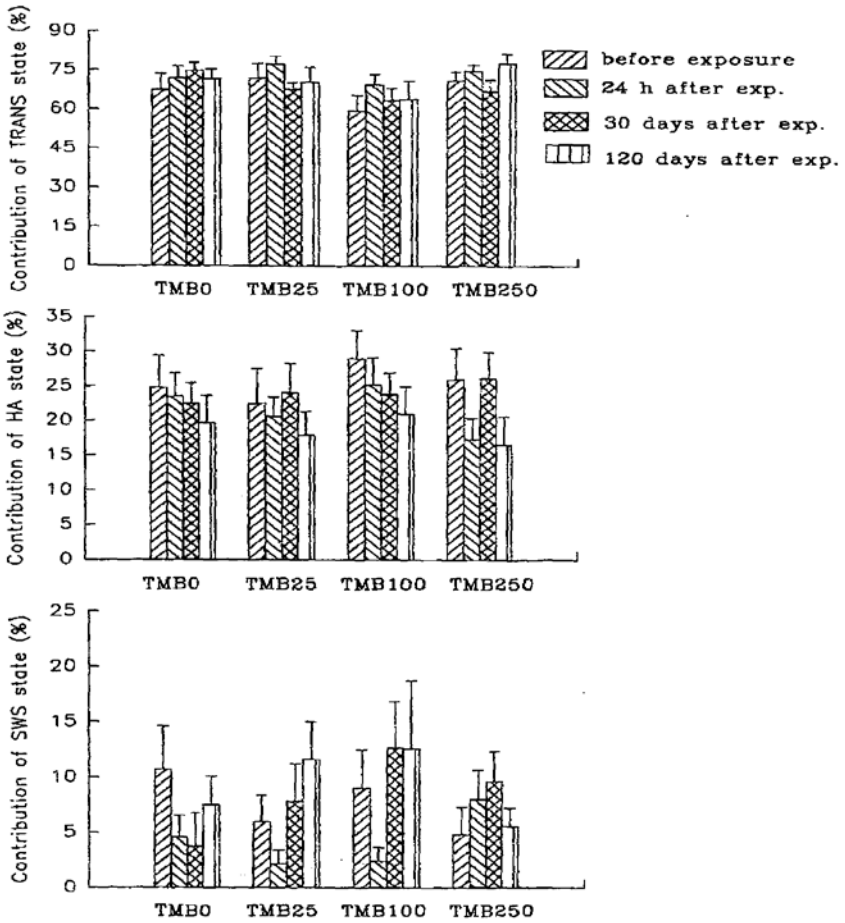


Figure 1. Diagrams showing the effect of a 4-week inhalation exposure to 1,2,4-TMB on the contribution of transitional (upper diagram, high arousal (middle diagram), and slow-wave sleep (lower diagram)) states in the rat EEG during successive 1-hour recording periods. The bars represent group means and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

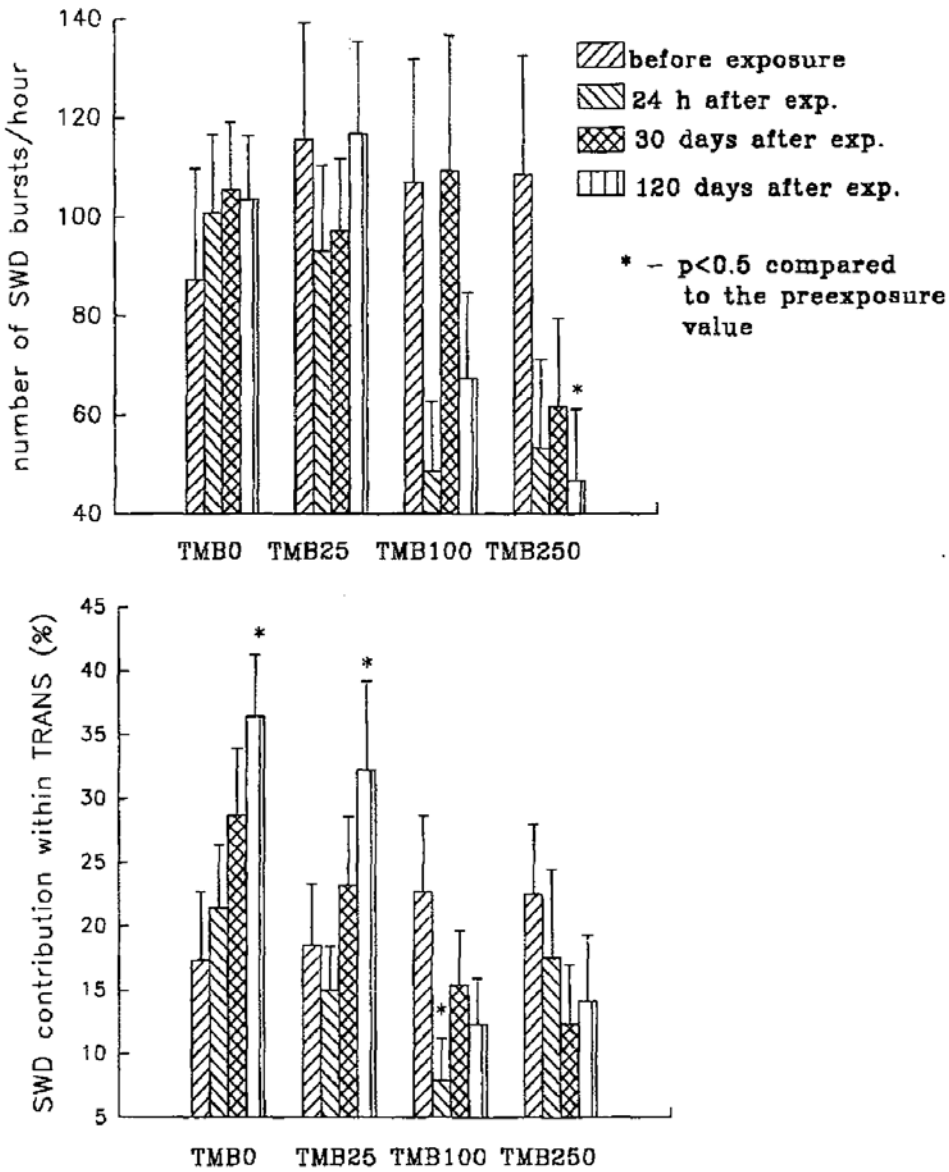


Figure 2. Diagram showing the effect of a 4-week inhalation exposure to 1,2,4-TMB on the SWD burst occurrence (upper diagram) and on the percent contribution of SWD activity within TRANS state (lower diagram) during successive 1-hour recording periods. The bars represent group means and SE. * denotes $p<0.05$ in comparison to the preexposure value in the same group.

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.

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/d, 5 ds/wk)	0 or 100 ppm (0 or 492 mg/m^3) 1,2,3-, 1,2,4-, or 1,3,5-TMB	4 wks

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/d, 5 d/wk for 4 wks. 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 wks 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.

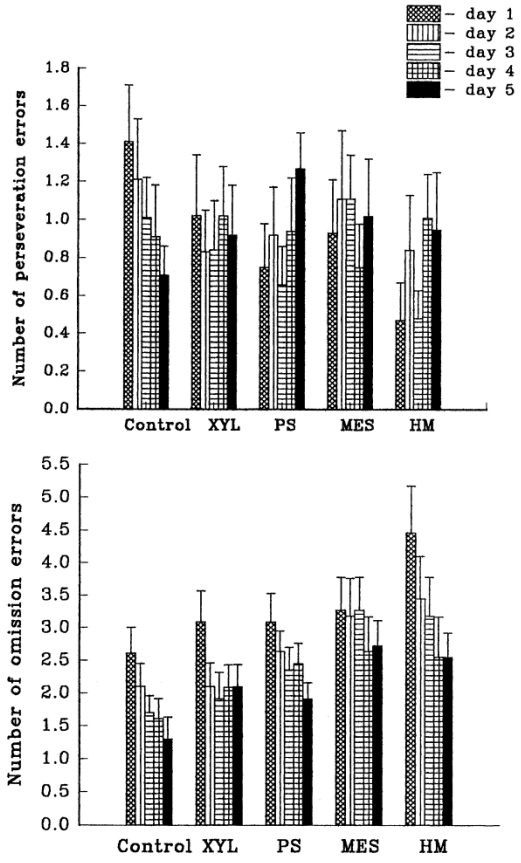


Figure 1. Radial maze performance of rats exposed for 4 weeks to *m*-xylene or a TMB isomer at a concentration of 100 ppm (492 mg/m^3). The test (one trial a day) was performed on days 14–18 after exposure. The diagrams illustrate the number of perseveration (upper diagram) and omission (lower diagram) 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.

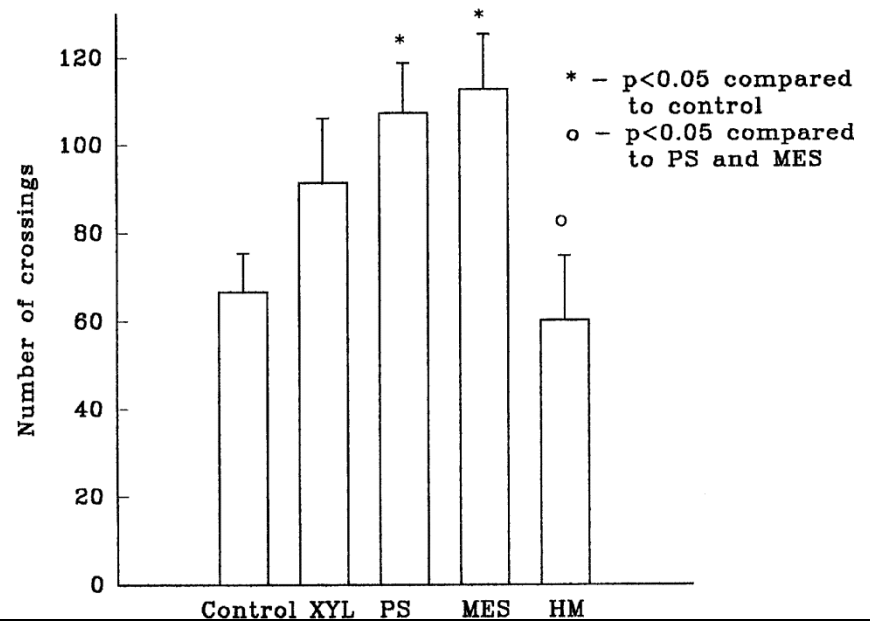
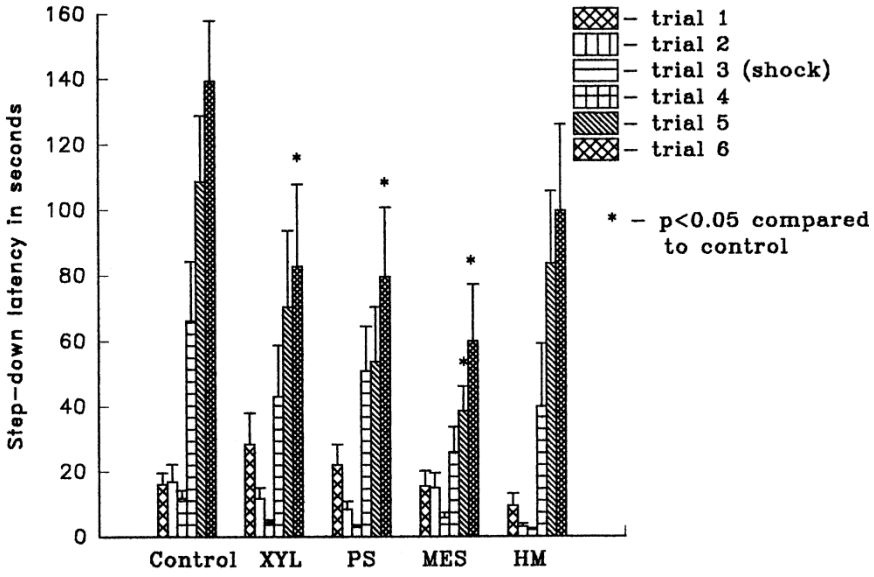


Figure 2. A comparison of open-field locomotor activity in sham-exposed and solvent-exposed rats. The test was performed on day 25 after a 4-week exposure to *m*-xylene or a TMB isomer at concentration of 100 ppm (492 mg/m^3). Bars represent group means and SE.

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 h 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.



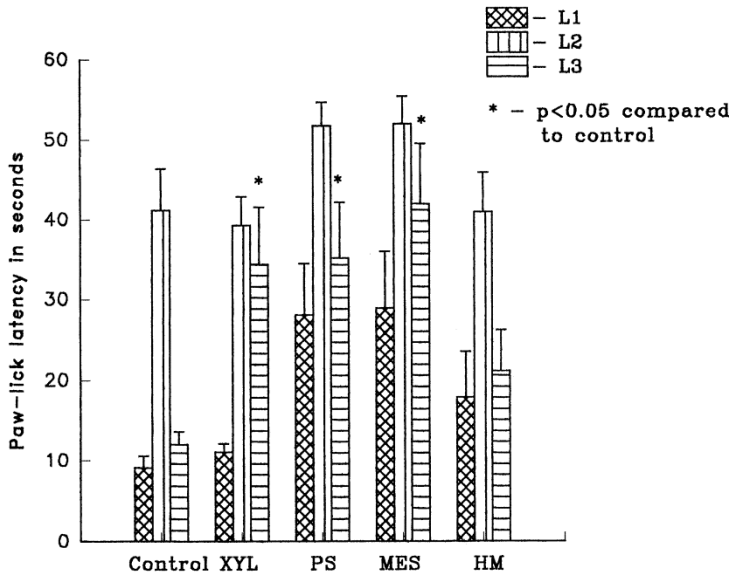


Figure 4. A comparison of sham-exposed and solvent-exposed rats with respect to the latency of the paw-lick response to heat (54.5°C) before (L1), several seconds after (L2), and 24 hr after a 2 min intermittent footshock. The test was performed on days 50 and 51 after a 4-week inhalation exposure to *m*-xylene or a TMB isomer at a concentration of 100 ppm (492 mg/m^3). Bars represent group means and SE.

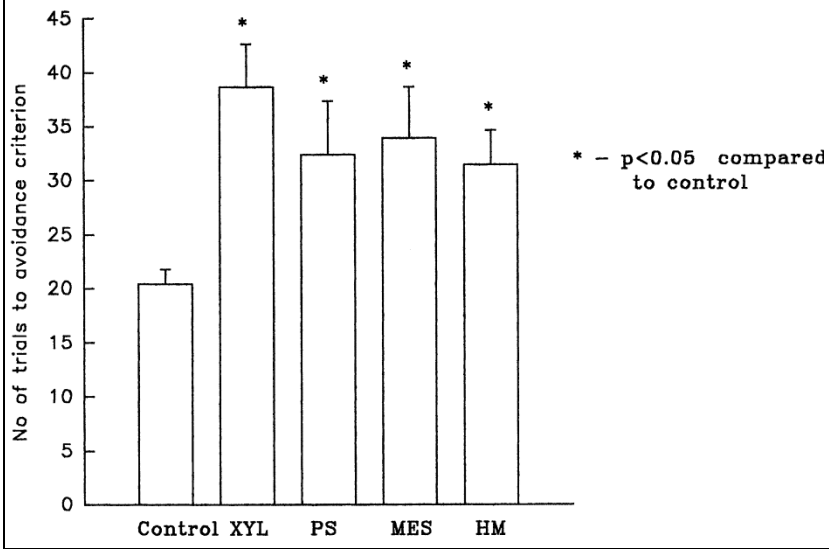


Figure 5. Active avoidance learning in rats after a 4-week inhalation exposure to *m*-xylene or a TMB isomer at a concentration of 100 ppm (492 mg/m^3). In one massed-trial session (inter-trial interval 20–40 s; maximum number of trials 60) the rats learned to shuttle between two neighboring compartments in order to avoid a footshock. The test was performed on day 54–60 after exposure. Bars represent group means and SE of the number of trials.

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.

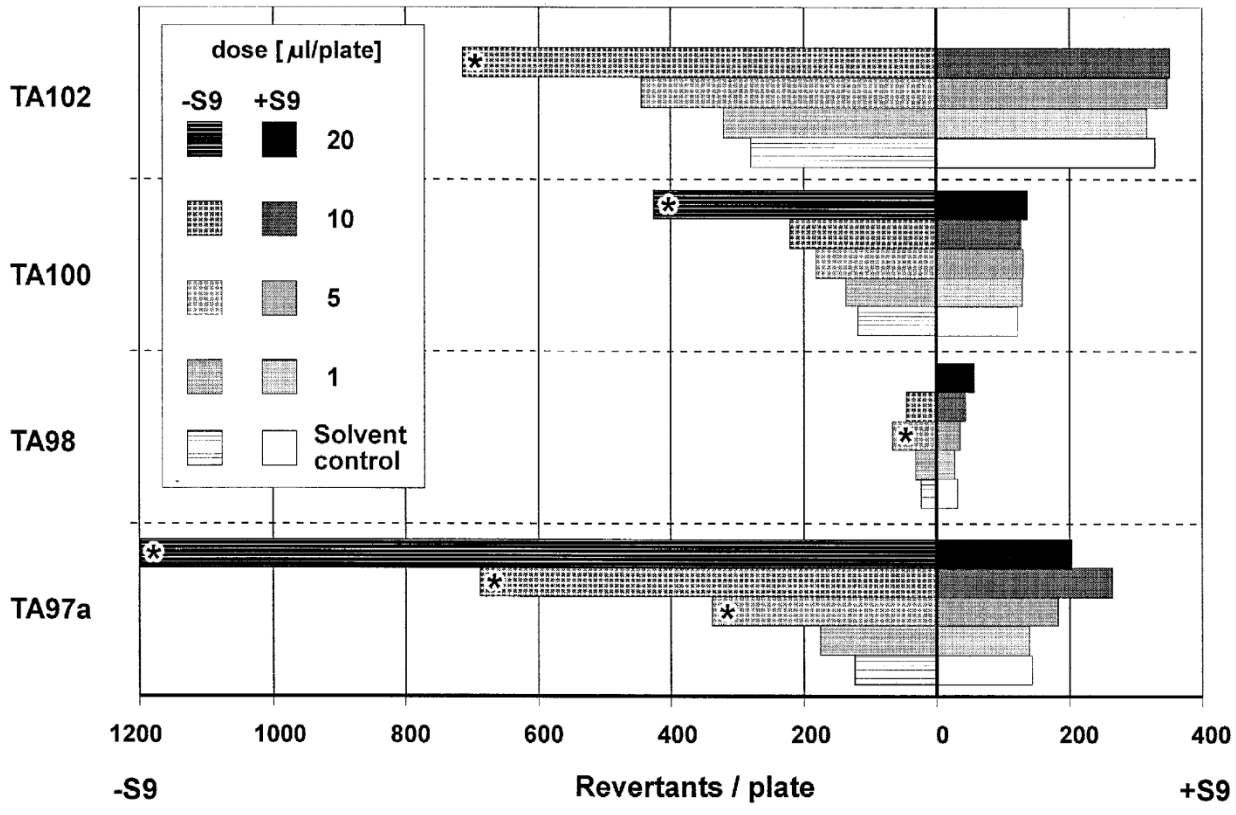
Table B-27. Characteristics and quantitative results for Janik-Speichowicz (1998)

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 d 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 .

Figure 1. 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)

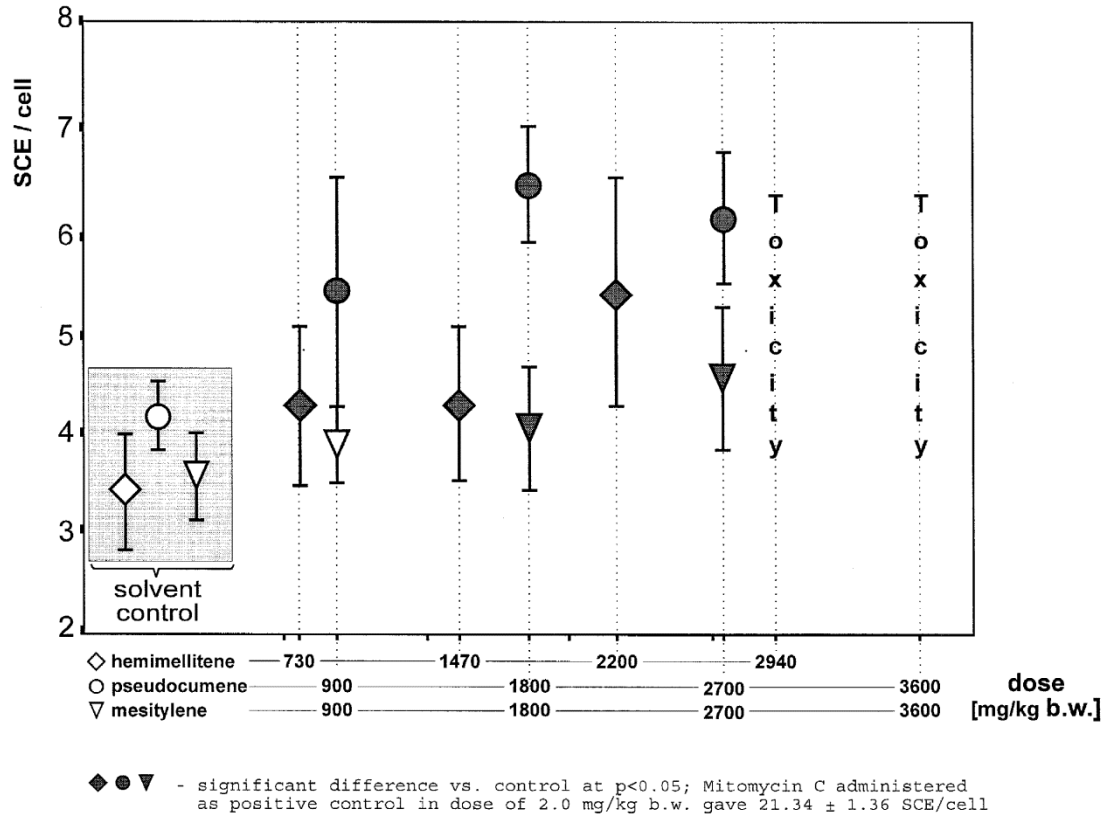
Observation	Exposure to 1,2,4-TMB (µg or µL)						
	0	100 (Solvent control)	1	5	10	20	30
TA97a (-S9)	212±7	126±13	148±23	158±10	165±8	141±25	115±3
TA97a (+S9)	145±5	141±12	152±7	168±8	176±21	155±20	106±7
TA98 (-S9)	24±3	23±3	24±3	29±5	41±7	27±8	TOX[a]
TA98 (+S9)	31±3	31±5	35±4	28±1	29±4	30±3	29±6
TA100(-S9)	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

	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

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

Observation	Exposure to 1,2,4-TMB (mg/kg body weight)			
	0	**2000**	**3280**	**4000**
	% of Polychromatic Erythrocytes with Micronuclei (± SD)			
Males 30 h harvest time	--	0.15±0.10	--	0.23±0.10
Males 48 h harvest time	0.18±0.07	0.18±0.10	--	0.16±0.8
Males 72 h harvest time	--	0.20±0.08	--	0.16±0.07
Females 30 h harvest time	--	--	0.23±0.5	--
Females 48 h harvest time	0.23±0.05	--	0.18±0.05	--
Females 72 h harvest time	--	--	0.13±0.05	--
	Ratio of polychromatic to normochromatic erythrocytes			
Males 30 h harvest time	--	1.18	--	1.16
Males 48 h harvest time	0.95	1.02	--	0.74
Males 72 h harvest time	--	1.02	--	0.68*
Females 30 h harvest time	--	--	0.98	--
Females 48 h harvest time	0.95	--	1.01	--
Females 72 h harvest time	--	--	0.85	--
Observation	**Exposure to 1,3,5-TMB (mg/kg body weight)**			
	0	**1800**	**2960**	**3600**
	% of Polychromatic Erythrocytes with Micronuclei (± SD)			
Males 30 h harvest time	--	0.20±0.00	--	0.24±0.11
Males 48 h harvest time	0.21±0.08	0.17±0.09	--	0.17±0.05
Males 72 h harvest time	--	0.17±0.09	--	0.14±0.05
Females 30 h harvest time	--	--	0.17±0.09	--
Females 48 h harvest time	0.20±0.08	--	0.20±0.00	--
Females 72 h harvest time	--	--	0.22±0.05	--
	Ratio of polychromatic to normochromatic erythrocytes			
Males 30 h harvest time	--	0.62	--	0.40*
Males 48 h harvest time	0.61	0.56	--	0.33
Males 72 h harvest time	--	0.58	--	0.42*
Females 30 h harvest time	--	--	0.51	--
Females 48 h harvest time	0.60	--	0.60	--
Females 72 h harvest time	--	--	0.58	--

Figure 1. Sister chromatid exchanges induced in bone marrow cells of Imp:*Balb/c* mice.



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

Table B-28. 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.

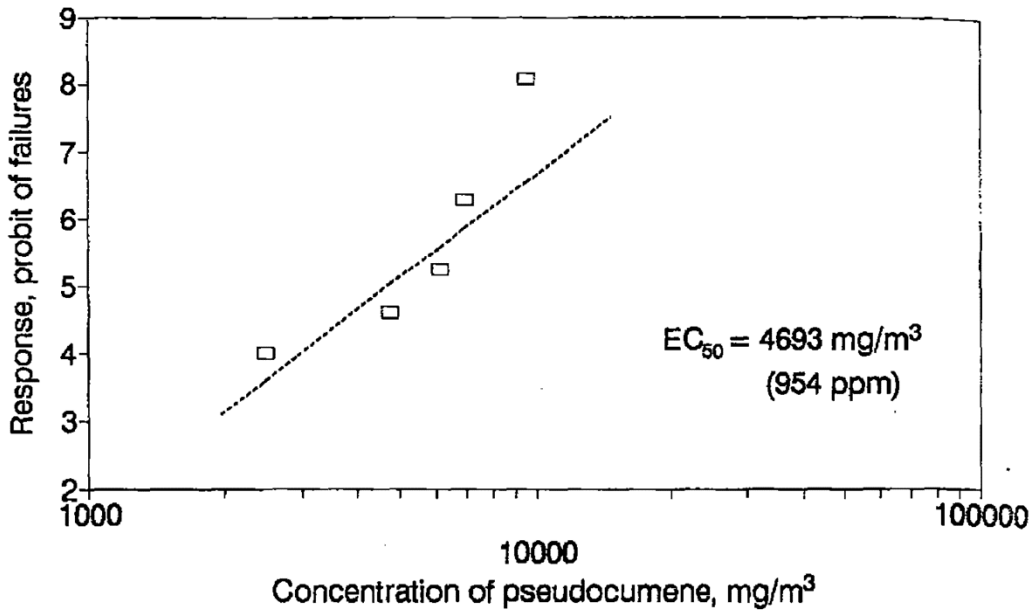


Figure 2. Hot-plate behavior in rats exposed to 1,2,4-TMB (i.e., pseudocumene). Rats were exposed to vapors of solvent for 4 hrs. Hot-plate behavior was tested immediately after termination of exposure. Each point represents the mean value of separate measurements of latency over the control in 10 rats.

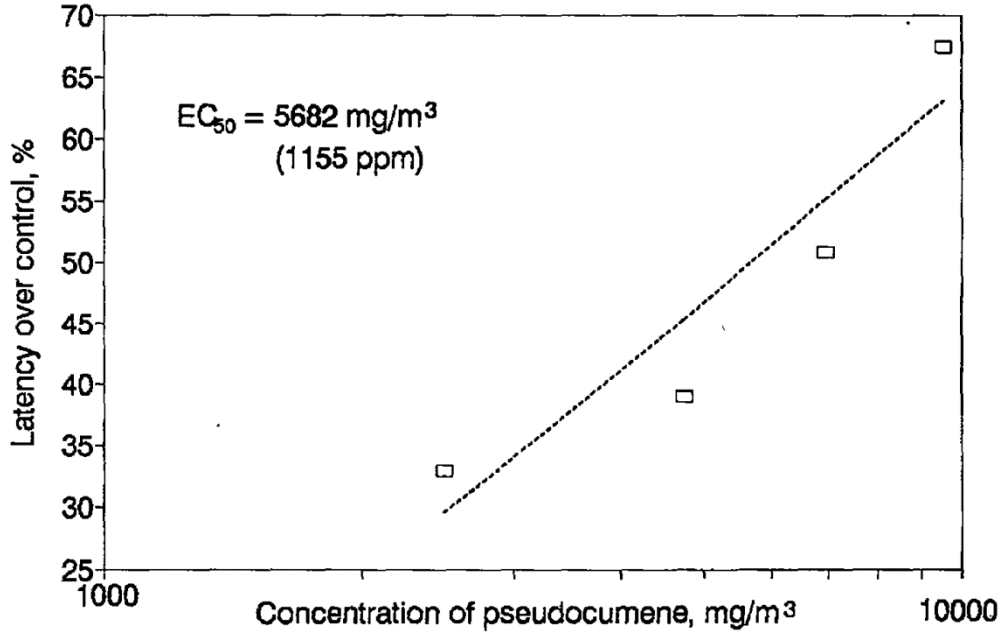


Figure 3. Time-response relationship for the effect of 1,2,4-TMB (i.e., pseudocumene) on respiratory rate in mice. Each point represents the mean value in 8–10 mice. After termination of 6 min exposure recovery of respiratory rate was observed.

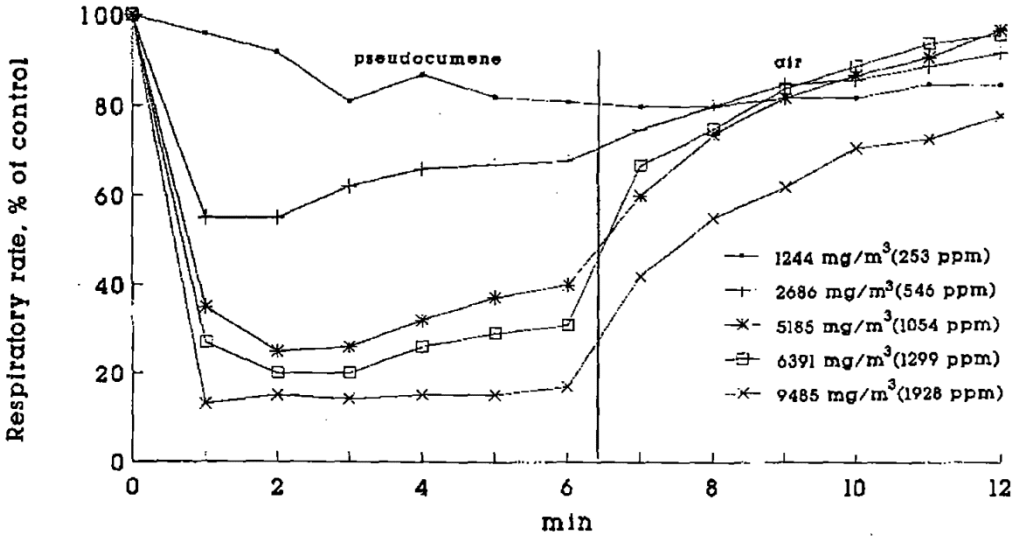
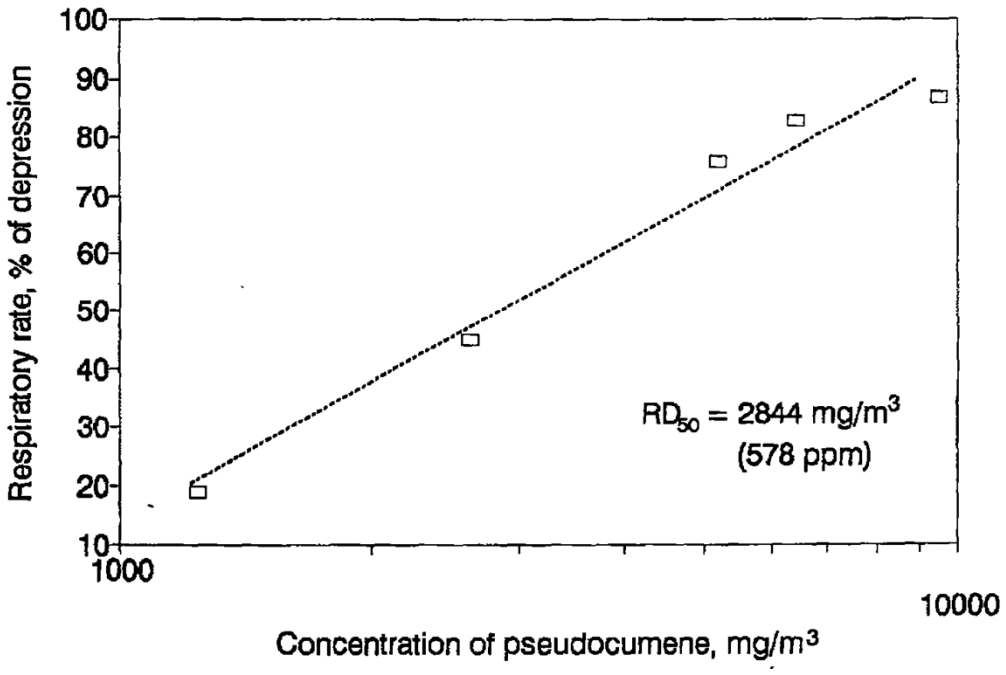


Figure 4. Respiratory rate of mice exposed to 1,2,4-TMB (i.e., pseudocumene) in 8–10 mice. The decrease of respiratory rate observed in the 1st minute of exposure was taken for consideration. The regression line was determined by the least squares procedure.



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.

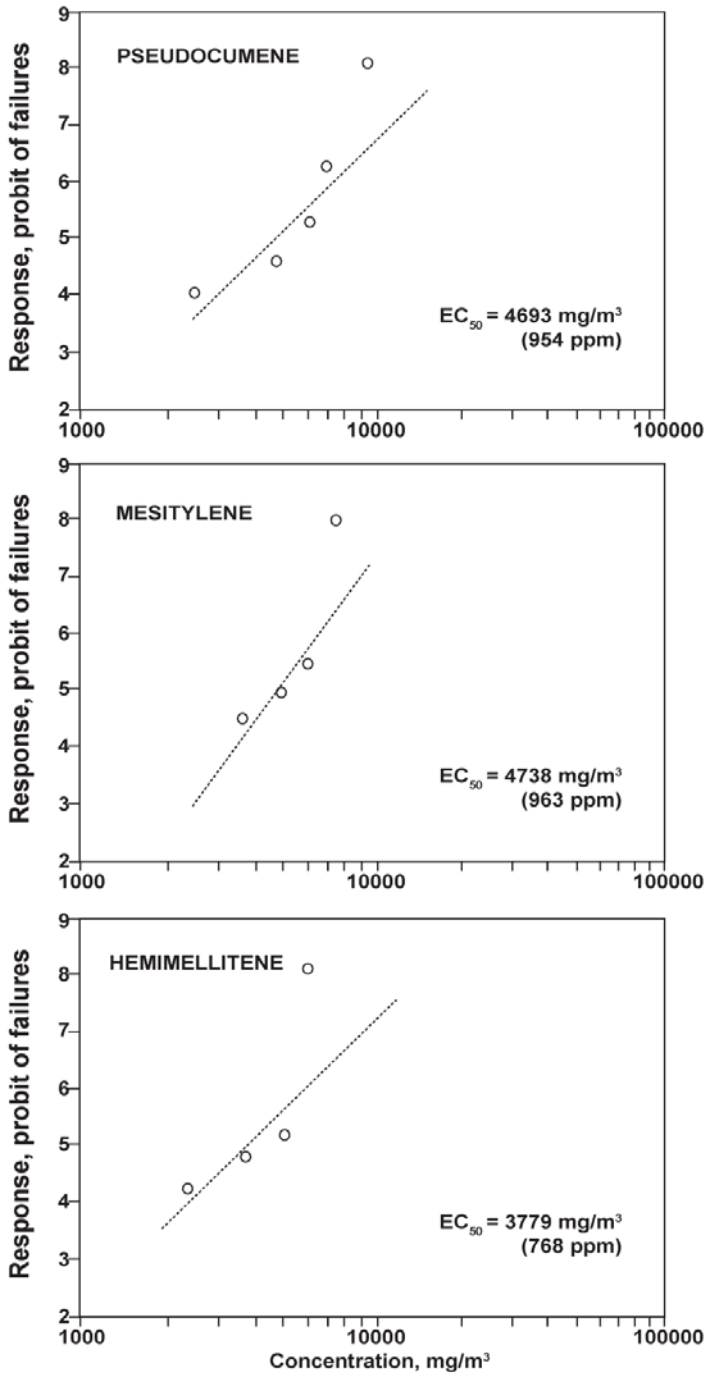
Table B-29. Characteristics and quantitative results for Korsak and Rydzyński (1996)

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 6h/d, 5 d/wk, 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

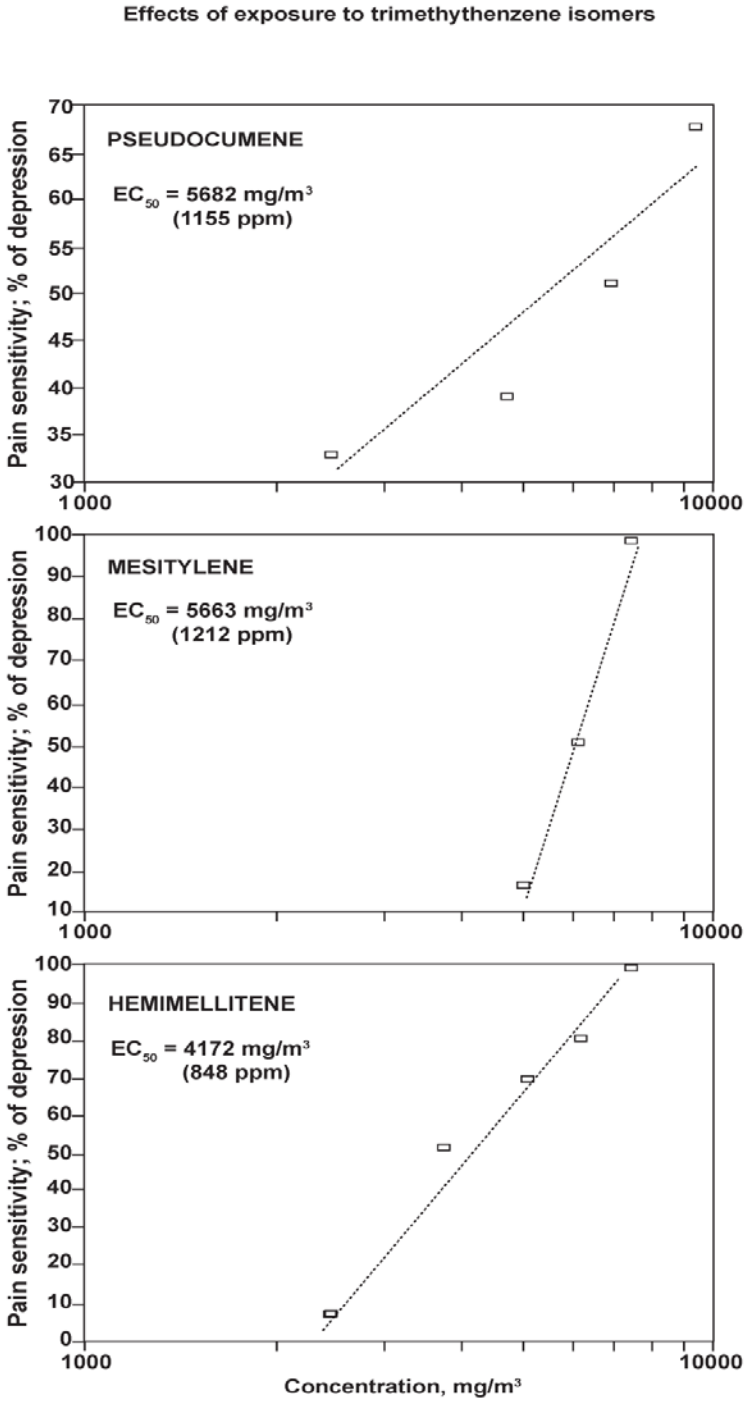
- Animals were exposed to either 1,2,3-, 1,2,4-, or 1,3,5-TMB in a dynamic inhalation chamber (1.3 m^3 volume) with 16 air changes/hr.
- Mean initial body weights were 250–300 g; rats were housed in wire mesh stainless steel cages, with food and water provided ad libitum.
- Animals were randomized and 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 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.

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)

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)

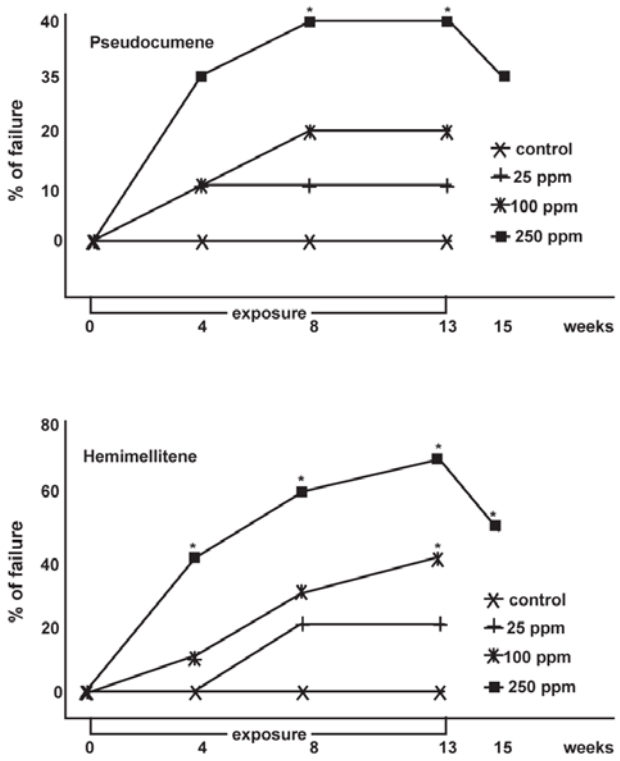


Figure 3. Rotarod performance of rats exposed to 1,2,3-TMB (hemimellitene) or 1,2,4-TMB (pseudocumene) at concentrations of 25, 100, and 250 ppm (123, 492, 1,230 mg/m^3) . Rats were exposed to vapors of solvents for 6 hr/d, 5 ds/wk, 3 mos. Statistical significance marked by asterisks, p<0.005.

Source: Reproduced from Korsak and Rydzyński (1996)

Observation	Latency of the paw-lick response, sec	
	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 wks 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-30. 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/d, 5 d/wk	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 d

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*

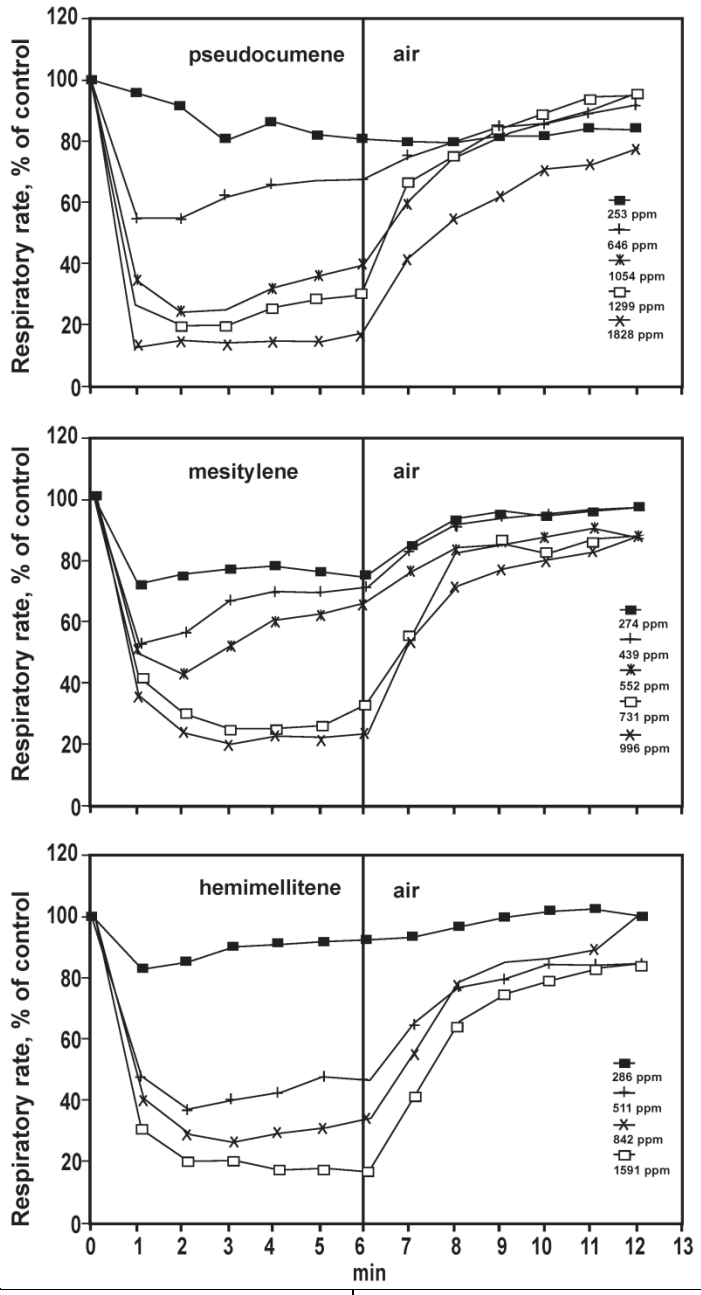


Figure 1. Time-response relationship for the effect of 1,2,4-TMB (i.e., pseudocumene) on respiratory rate in mice. Each point represents the mean value in 8–10 mice. After termination of 6 min exposure recovery of respiratory rate was observed.

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.

Table B-31. 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/d, 5 d/wk)	0, 123, 492, 1,230 mg/m^3	90 d

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 wk 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 ± 0.0050	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
	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	0.0028 ± 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		
Observation	**Exposure concentration (mg/m^3)**					
	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 (× 10^3/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	0.0589
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	76.8 ± 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
	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 (× 10^3/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	16 ± 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
Observation	**Exposure concentration (mg/m^3)**					
	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 ± 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

	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

Observation	**Exposure concentration (mg/m^3) [Dose Group ID]**					
	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

	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 wks after termination of exposure.
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.

Table B-32. 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 1,230 mg/m^3 group	Inhalation (6 hr/d, 5 d/wk)	0, 123, 492, 1,230 mg/m^3 1,2,3-TMB	90 d

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 wk 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 effects were graded using an arbitrary scale: 0 = normal status, 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)	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

	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

Observation	**Exposure concentration (mg/m^3)**					
	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 ($\times 10^3/mm^3$)[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 ($\times 10^3/mm^3$)[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 ($\times 10^3/mm^3$)[d] Males	10.09 ± 2.23	9.38 ± 3.29	7.71 ± 3.45	9.03 ± 275	6.3 ± 4.6	0.1661
WBCs ($\times 10^3/mm^3$)[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
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

Observation	**Exposure concentration (mg/m^3)**				
	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
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

Observation	**Exposure concentration (mg/m^3) [Dose group ID]**					
	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	24 (22.8)	1.3 (12.1)	1.5 (16.4)	L3 (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
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 wks after termination of exposure.
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.

Table B-33. 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 h/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 d

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 d. 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**

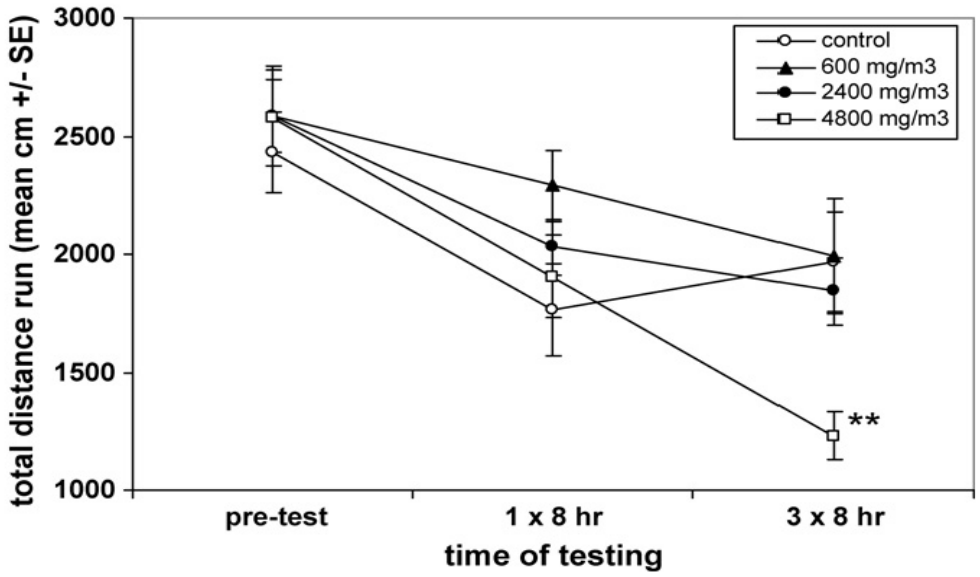


Figure 1. Effects of WS on total distance run during motor activity assessment in rats.

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**
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.				

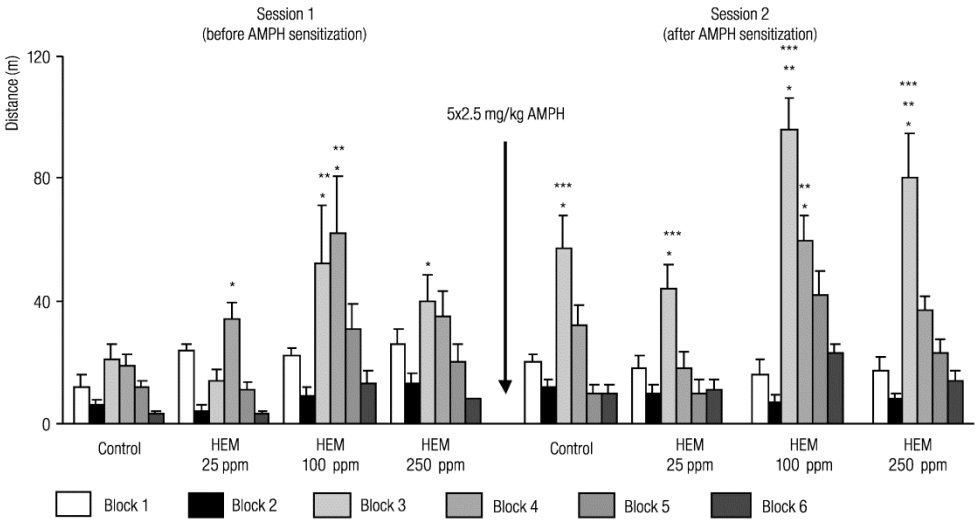
[a]Gait score indicates the severity of gait changes and is scored as 1 (normal) to 4 (severely abnormal).
[b]Click response was scored as 0 (no reaction) to 5 (exaggerated reaction).
[c]Data for parameters that did not show statistically significant group differences are not shown; statistical analysis: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

Table B-34. 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/d, 5 d/wk)	0, 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,3- or 1,2,4-TMB	4 wks

Additional study details

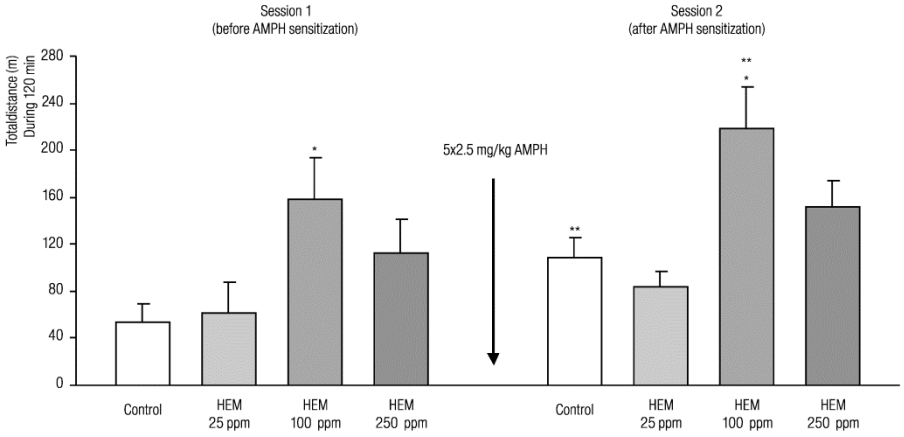
- Animals were exposed to 1,2,3- or 1,2,4-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/d, 5 days/wk for 4 wks. Food and water was provided ad libitum.
- Animals were randomized and assigned to the experimental groups.
- Behavioral sensitivity to amphetamine was measured via test of open-field 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.



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.

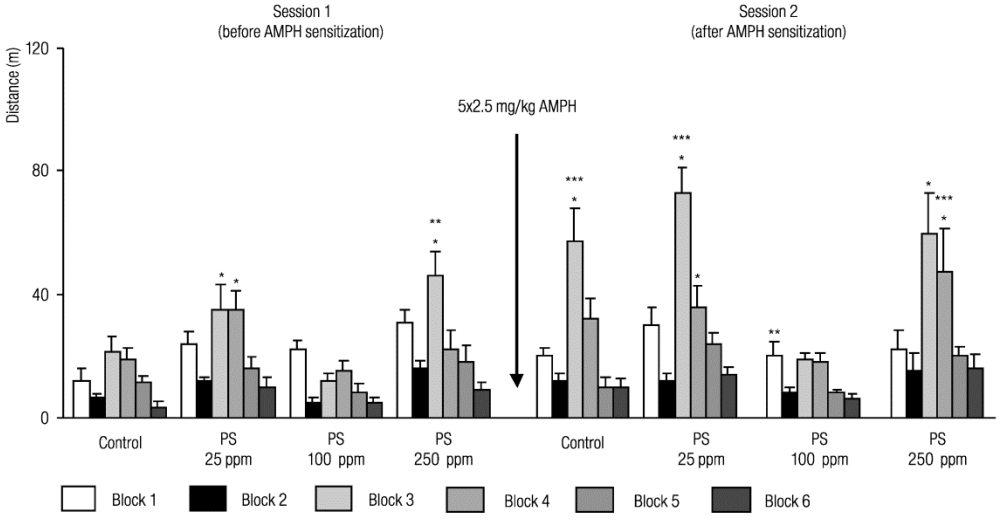
Figure 1. Diagram illustrating the effect of prior exposure to 1,2,3-TMB on the locomotor response (all measurements) to the amphetamine challenge before (session 1) and 14 days after (session 2) a repeated (2.5 mg/kg, 1/day × 5 day) amphetamine treatment.

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/d × 5 d).



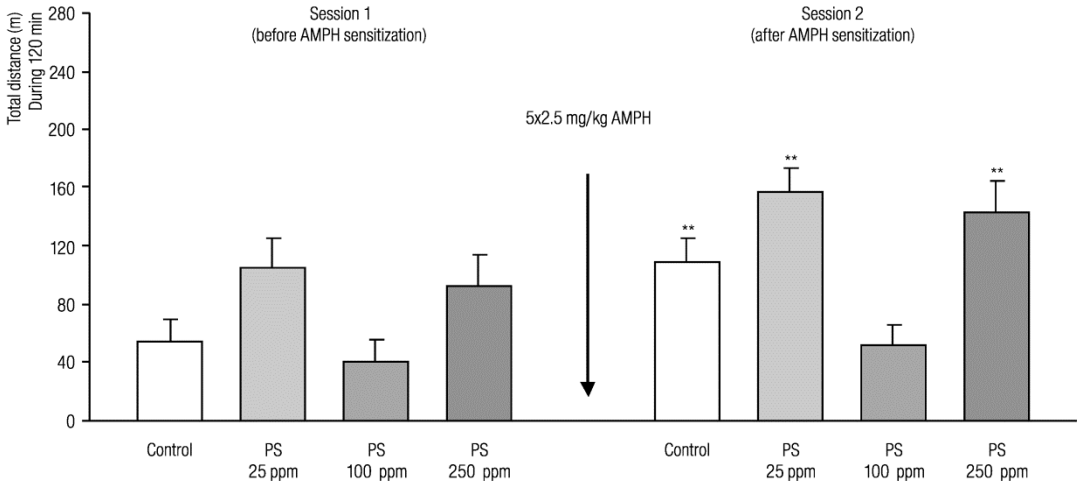
* P < 0.05 — compared to control. ** P < 0.05 — compared to corresponding measure before sensitization.
Bars represent mean values and SEM of the cumulated locomotor activity (distance in metres) during the 2-hour measurement following AMPH (0.5 mg/kg) challenge.

Figure 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.

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/d × 5 d).



* 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.

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.

Table B-35. 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 d/wk for 104 wks

Additional study details

- Rats were exposed to 1,2,4-TMB for 2 years via stomach tube administration 4 d/wk.
- Animals were 7 wks 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.

Observation	**Long-term carcinogenicity of 1,2,4-TMB**	
	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

1

	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.		

Table B-36. 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/d for 3 consecutive days

Additional study details

- Animals were exposed to 1,2,4-TMB for 8 hrs/d for 3 d 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.

Observation	**Exposure concentration 1,2,4-TMB (mg/m^3)**			
	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*
Observation	**Exposure concentration 1,2,4-TMB (mg/m^3)**			
	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
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
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 $p < 0.05$.

Table B-37. 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 h/d GD 6–20)	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 6–20

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/d starting on GD 6 and ending on GD 20.
- Animals were randomized and assigned to the experimental groups.
- After GD 20, dams were sacrificed and weighed, as were their uteri and any fetuses.
- Decreases in maternal body weight and fetal toxicity were observed.

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)**
	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**

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)
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)
Observation	**Exposure concentration to 1,2,4-TMB**				
	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**

Observation	Exposure concentration to 1,2,4-TMB				
	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
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**
Observation	**Exposure concentrations to 1,2,4-TMB**				
	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)
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 GD 6–21 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.

Table B-38. 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.

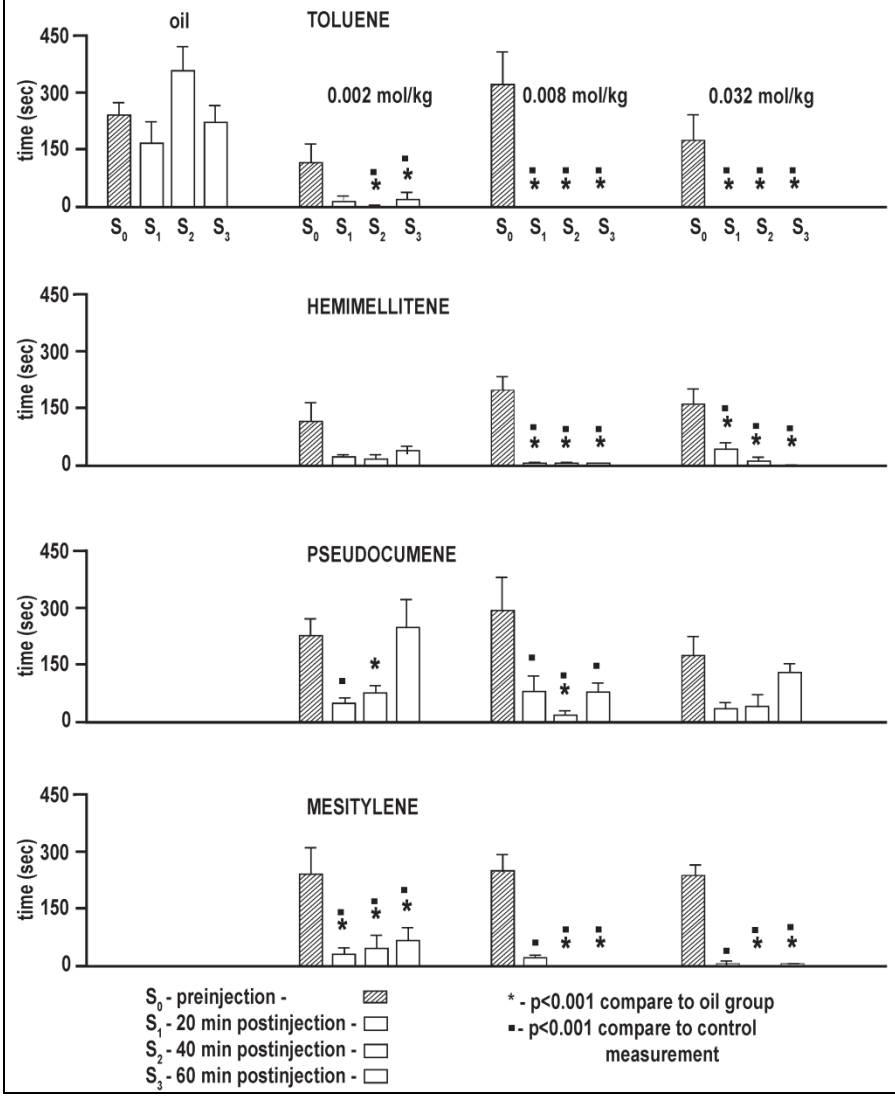


Figure 1. Changes in total duration of high-voltage spindle episodes following acute exposure to toluene and 1,2,3-, 1,2,4-, or 1,3,5-TMB at doses of 0.002, 0.008, and 0.032 mol/kg.

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

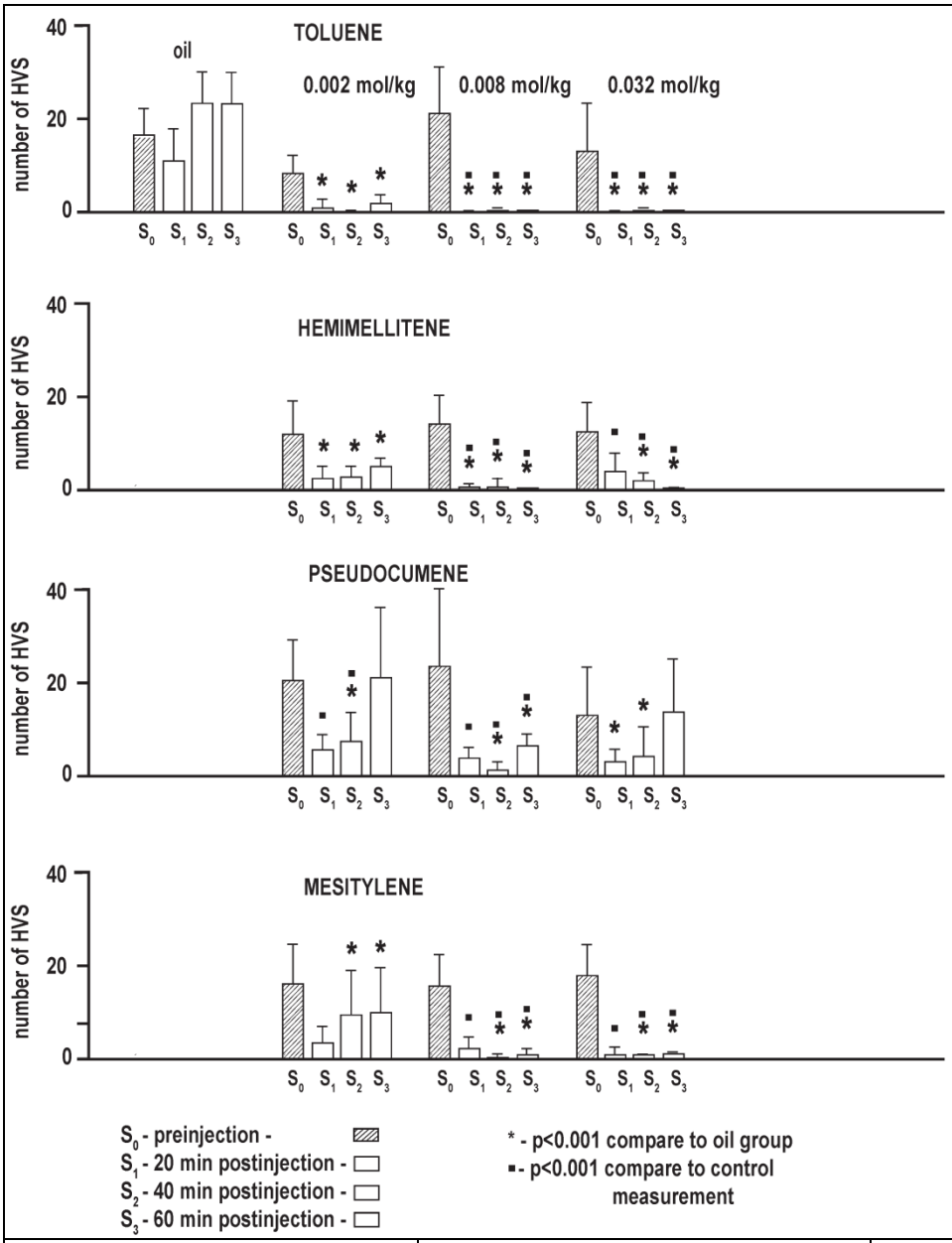


Figure 2. Changes in number of high-voltage spindle episodes following acute exposure to toluene and 1,2,3-, 1,2,4-, and 1,3,5-TMB at doses of 0.002, 0.008, and 0.032 mol/kg.

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.

Table B-39. 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.

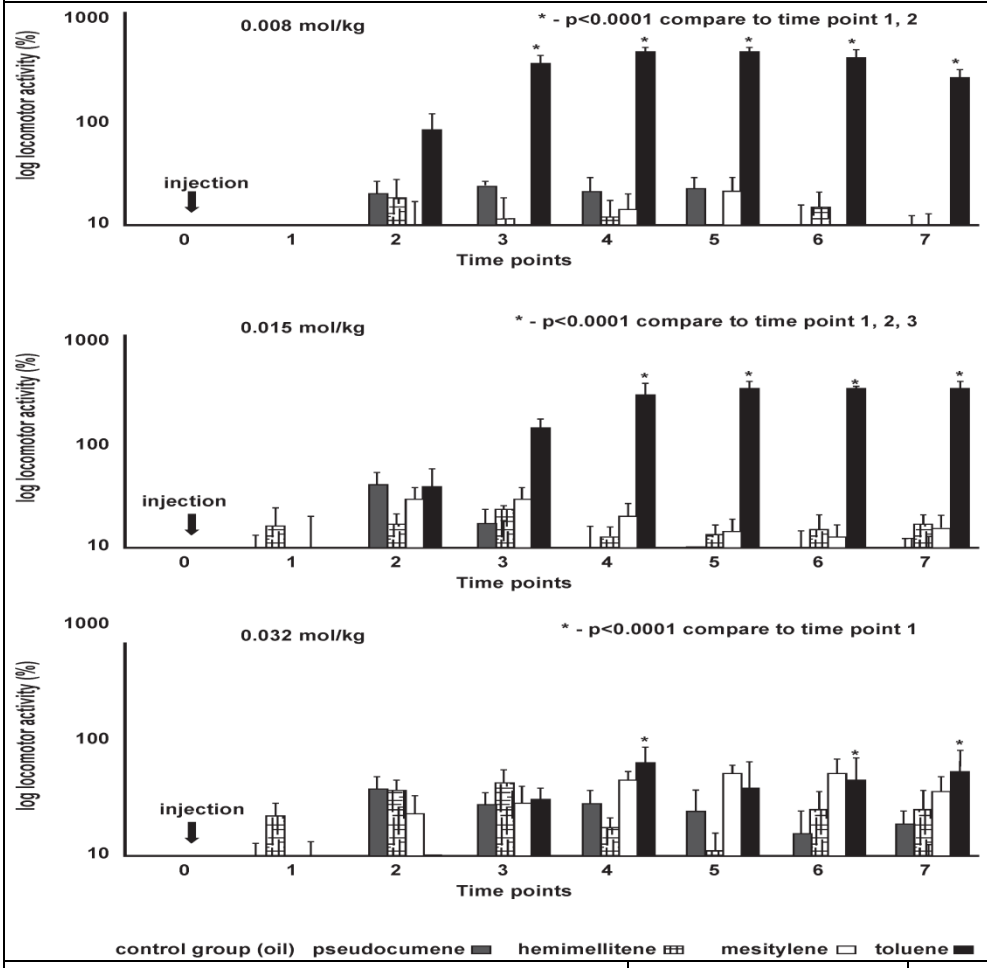


Figure 1. Locomotor activity following acute exposure to toluene and TMB isomers at doses of 0.008 mol/kg, 0.016 mol/kg, and 0.032 mol/kg.

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.

Table B-40. 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.

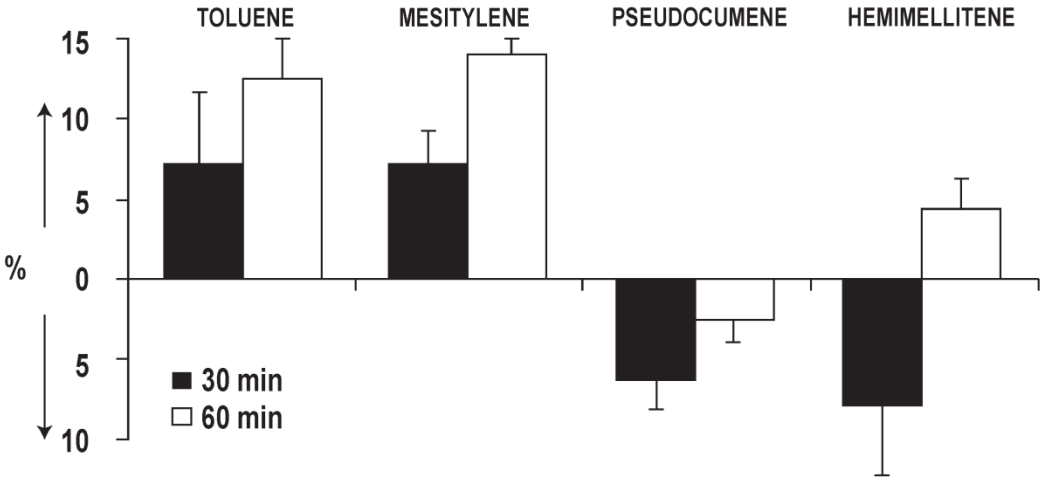


Figure 1. Amplitude abnormalities of the cortical N1 wave 30 and 60 min after i.p. solvent injection.

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

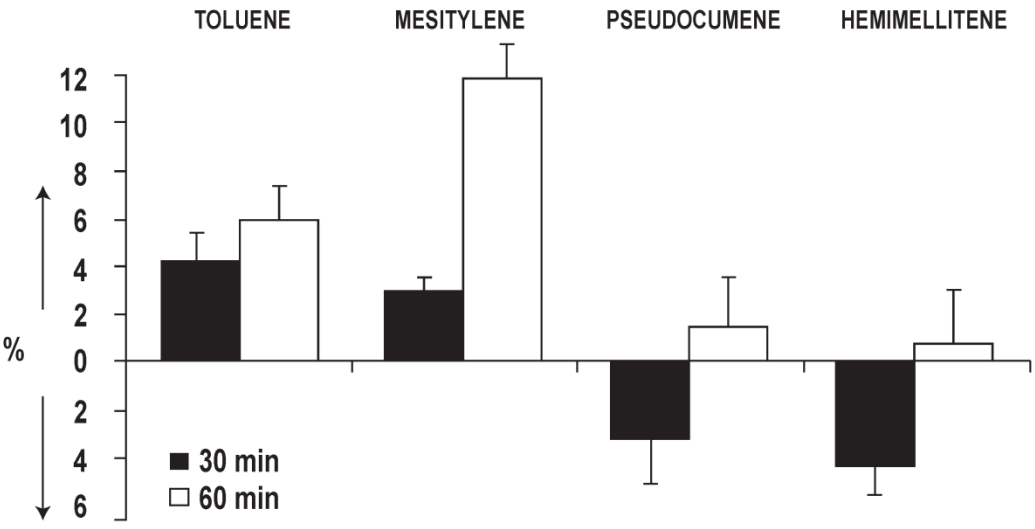


Figure 2. Amplitude abnormalities of the cortical P1–N1 wave 30 and 60 minutes after i.p. solvent injection.

Source: Reproduced (Tomas et al. 1999)

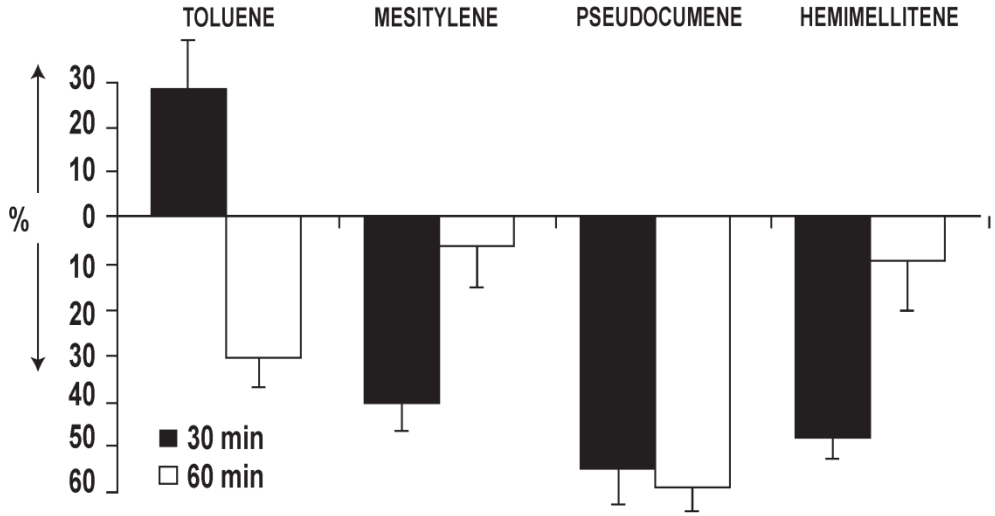


Figure 3. Amplitude abnormalities of the hippocampal N1 wave 30 and 60 min after i.p. solvent injection.

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

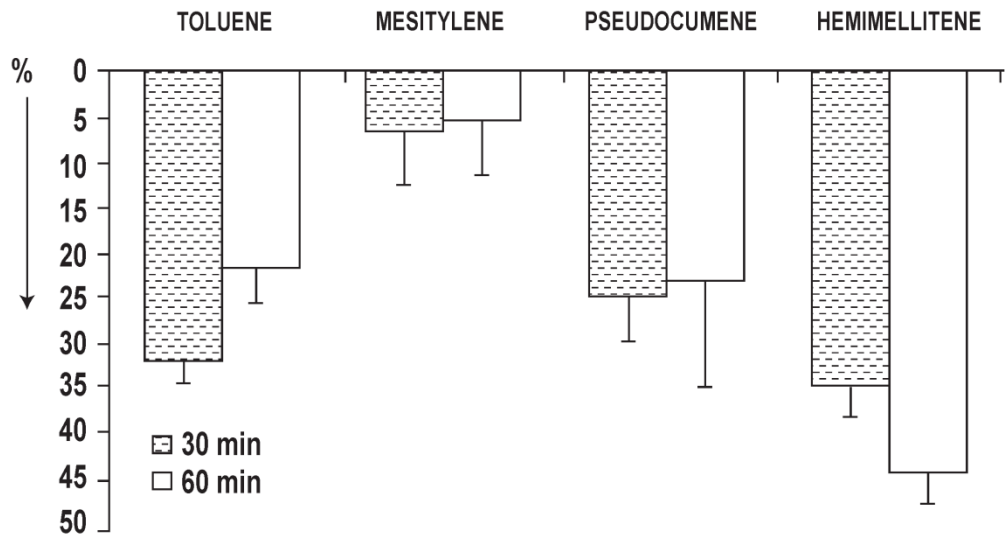


Figure 4. The effect of i.p. solvent injection on the cortical EEG in the 13–20.75 Hz frequency band.

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

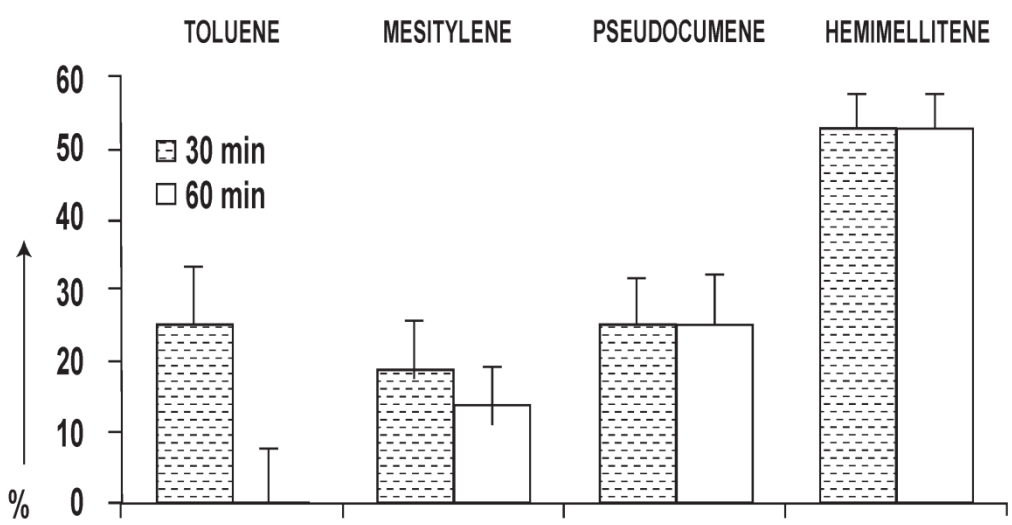


Figure 5. The effect of i.p. solvent injection on the hippocampal EEG in the 1–3.75 Hz frequency band.

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

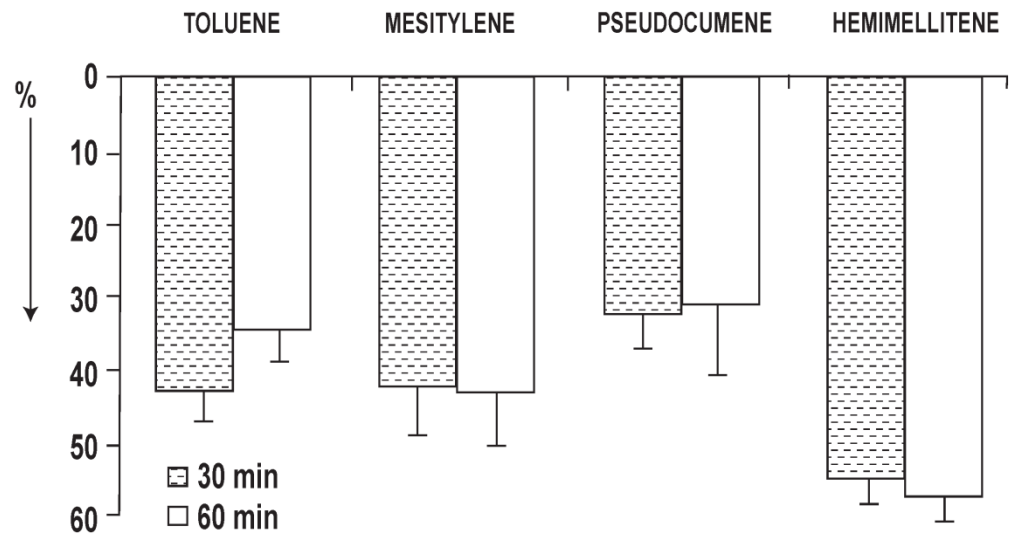


Figure 6. The effect of i.p. solvent injection on the hippocampal EEG in the 7–9.75 Hz frequency band.

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.

Table B-41. 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 h/d, 5 d/wk)	0 or 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,3-TMB	4 wks

Additional study details

- Animals were exposed to 1,2,3-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/d, 5 d/wk for 4 wks. 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).

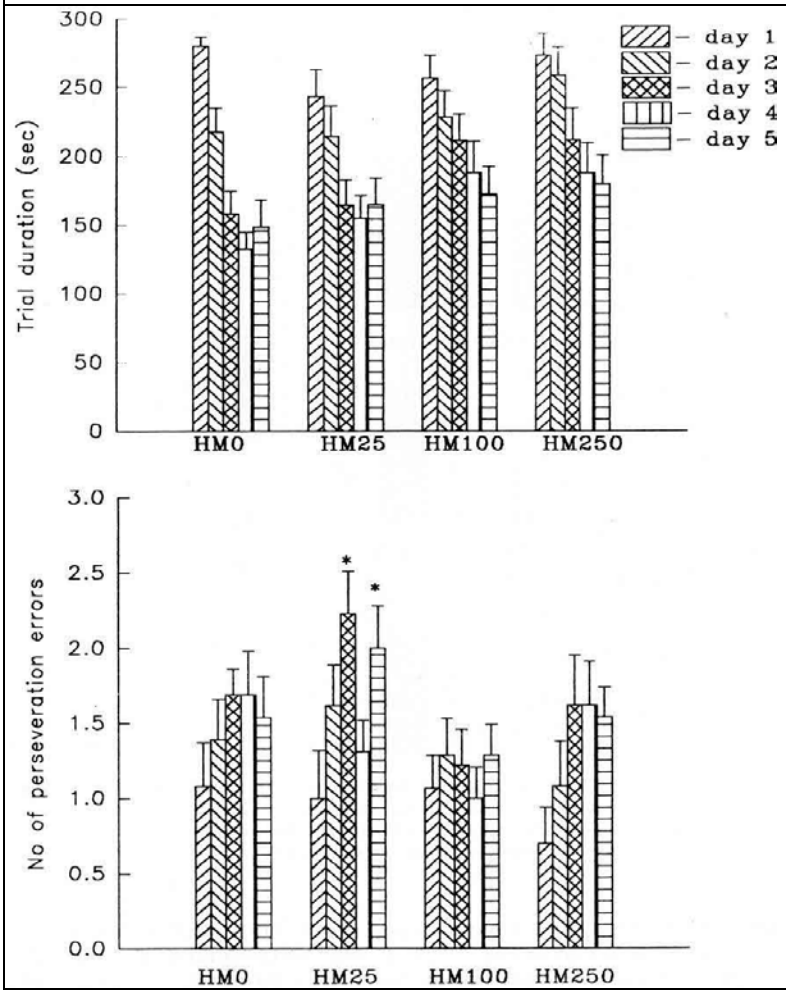


Figure 1. Radial maze performance of rats exposed for 4 weeks to 1,2,3-TMB. The test (one trial a day) was performed on days 14–18 after exposure. Upper diagram: changes in trial duration, i.e., the time of successive eight arm entries, during successive days of training. Lower diagram: number of perseveration 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.

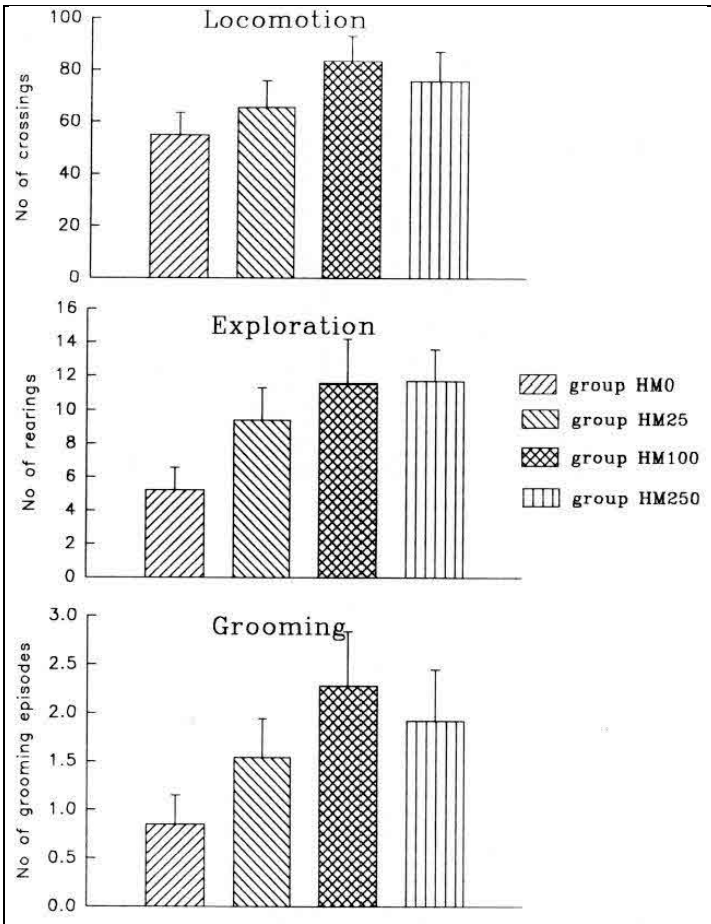


Figure 2. A comparison of spontaneous locomotor (upper diagram), exploratory (middle diagram), and grooming (lower diagram) activity of rats in an open field during a 5-min observation period. The test was performed 25 days after a 4-week exposure to 1,2,3-TMB. Denotation of groups as in Figure 1 (above). The bars represent group means and SE.

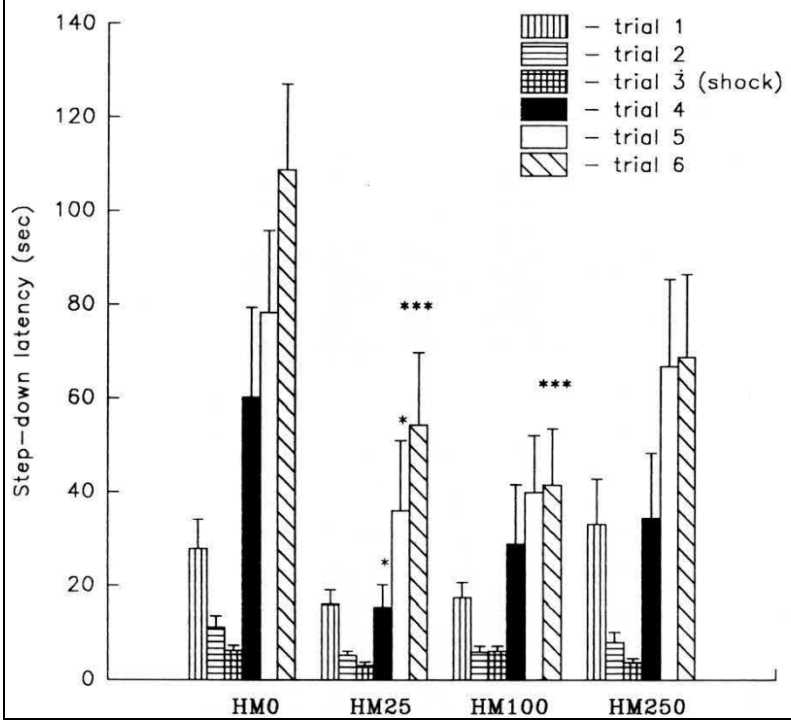


Figure 3. Diagrams illustrating the effect of a 4-week exposure to 1,2,3-TMB on the step-down passive avoidance learning in rats. The test was performed on days 39–48 after exposure. Trials 1, 2, and 3 were performed at 24 hr intervals. The step-down response was punished by a 10 sec footshock in trial 3 only. Trials 4, 5, and 6 were performed 24 hr, 3 d, and 7 d 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.

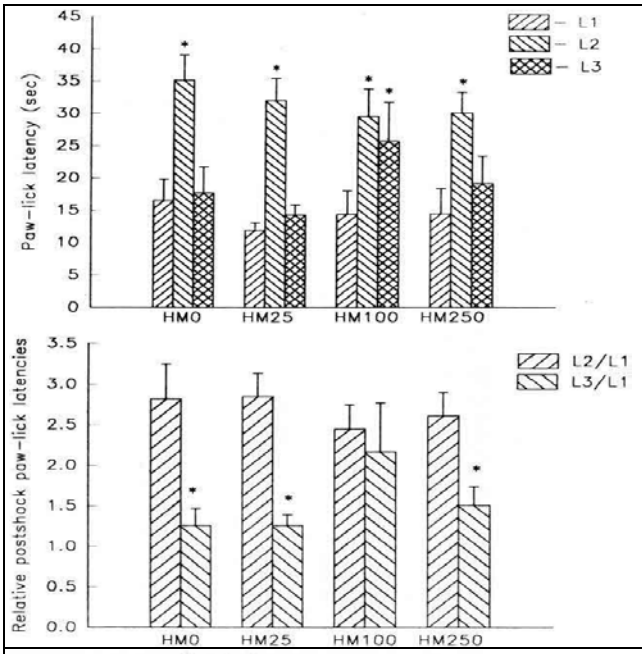


Figure 4. Hot-plate behavior tested in rats on day 50 (trials 1 and 2) and day 51 (trial 3) after a 4-week exposure to 1,2,3-TMB. Denotation of groups as in Figure 1 (above). The bars represent group means and SE. Upper diagram: A comparison of the latency of the paw-lick response to a thermal stimulus (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.
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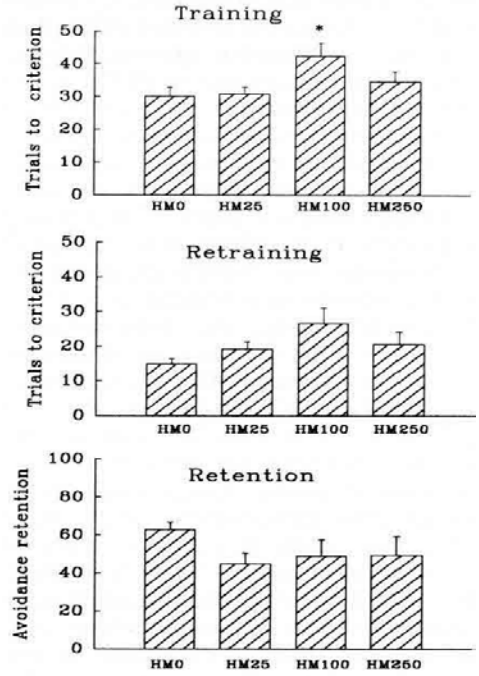


Figure 5. Active avoidance learning and retention in rats after a 4-week exposure to 1,2,3-TMB. Upper and middle diagrams: comparisons of the number of trials to attain an avoidance criterion (four avoidance responses during five successive trials) during the training (upper diagram and retraining (middle 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.

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.

Table B-42. 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/d, 5 d/wk)	0 or 25, 100, or 250 ppm (0, 123, 492, or 1,230 mg/m^3) 1,2,3-TMB	4 wks

Additional study details

- Animals were exposed to 1,3,5-TMB in 1.3 m^3 dynamic inhalation exposure chambers for 6 hrs/d, 5 d/wk for 4 wks. 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.

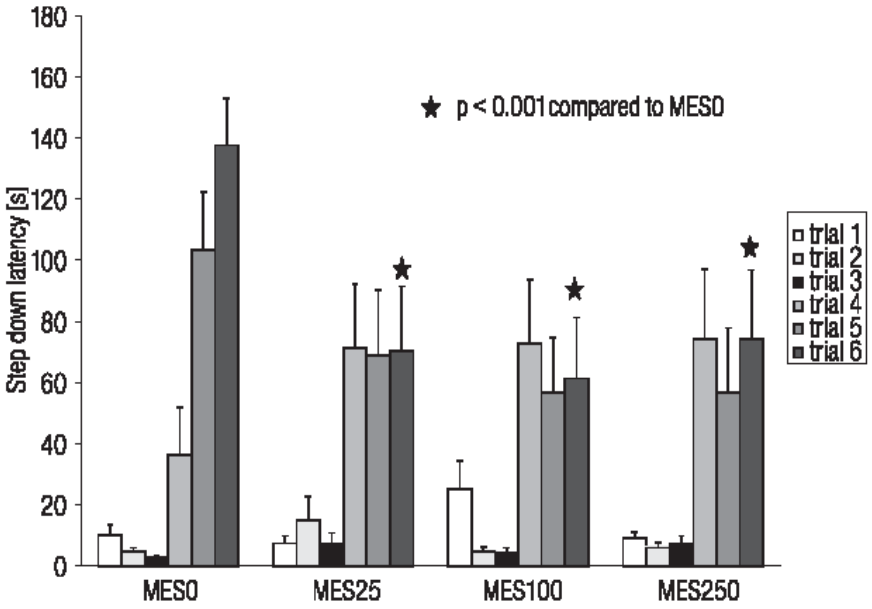


Figure 1. Passive avoidance. The comparison of the time of staying on the platform in the consecutive test trials. The test was performed between days 35 and 45 after the exposure to 1,3,5-TMB. Leaving the platform in trial 3 was punished by an electric shock. Trials 1, 2, 3, and 4 were performed at 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.

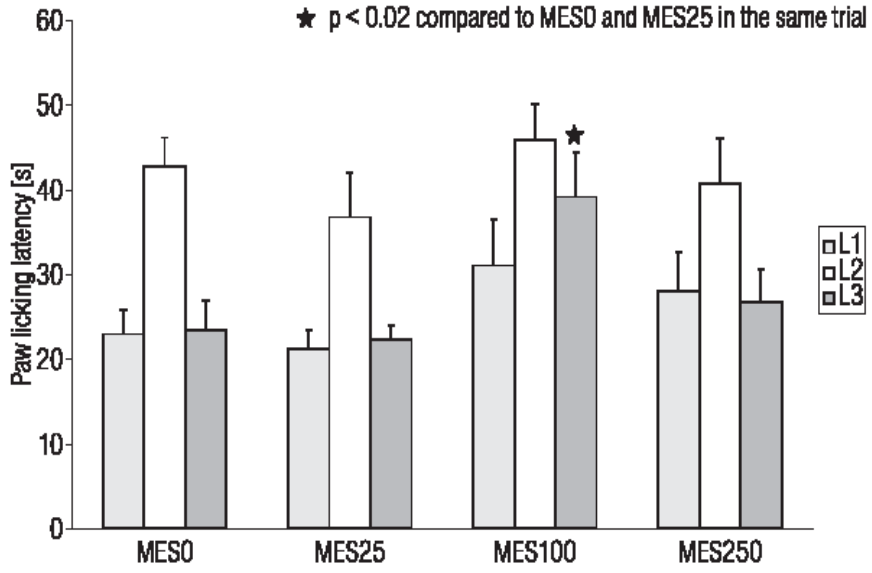


Figure 2. Hot plate. The comparison of latency of the reaction (paw-lick) to the thermal stimulus before (L1), immediately after (L2) and 24 hr after (L3) intermittent 2 min electric shock in rats exposed to 1,3,5-TMB. The test was performed on days 50 and 51 after the exposure.
The bars represent group means and SE.

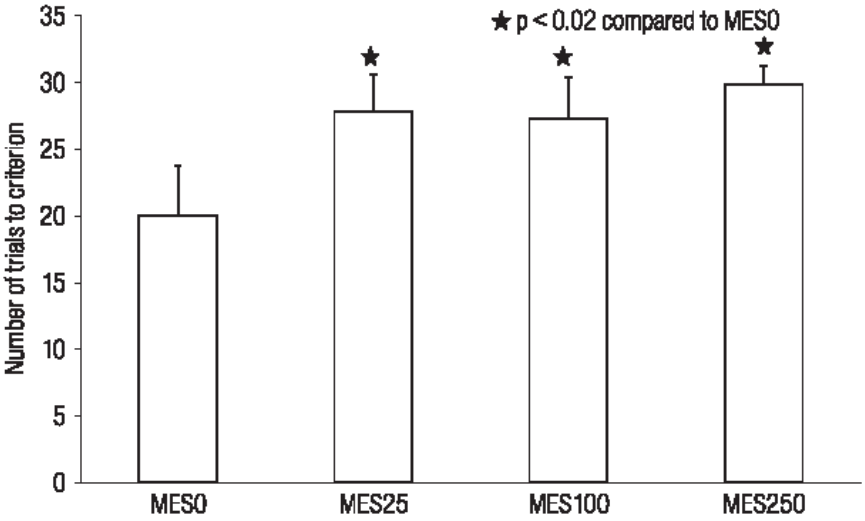


Figure 3. Active avoidance. The comparison of the rat groups exposed to 1,3,5-TMB for the number of trials (attempts) required to reach the avoidance criterion (4 shock avoidances) in 5 consecutive trials (attempts) during the training session. The test was performed on day 54 (training) and day 60 (retraining) after the exposure.
The bars represent group means and SE.

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.

Table B-43. Characteristics and quantitative results for Wiglusz et al. (1975a)

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/d, 6 d/wk for 5 wks

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/d, 6 d/wk, for 5 wks.
- 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.

Observation	**1,3,5-TMB exposure concentration (mg/L)—hematological parameters following single 6 hour exposure**			
	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

	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 ± 4.13	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	0.5 ± 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

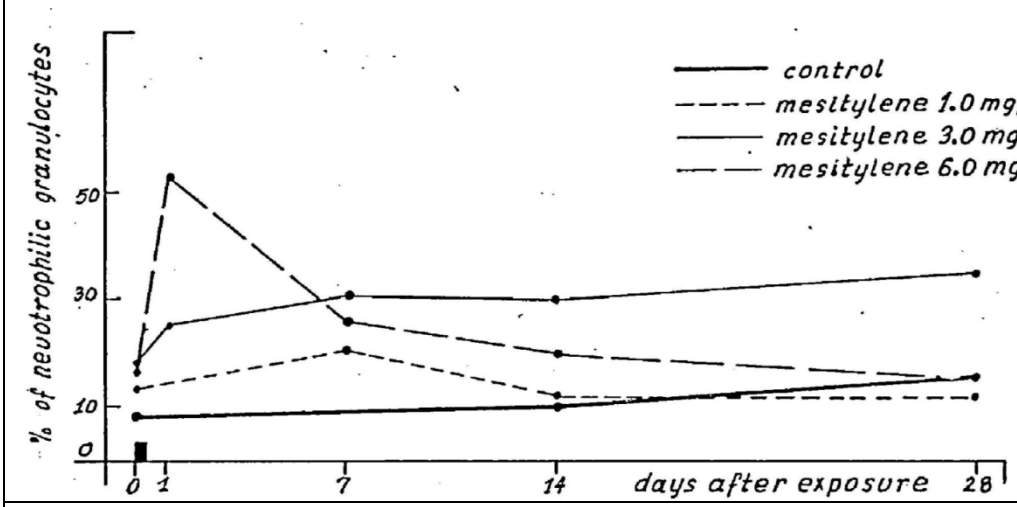


Figure 1. Percentage of segmented neutrophilic granulocytes after 6 hrs exposure to 1,3,5-TMB.

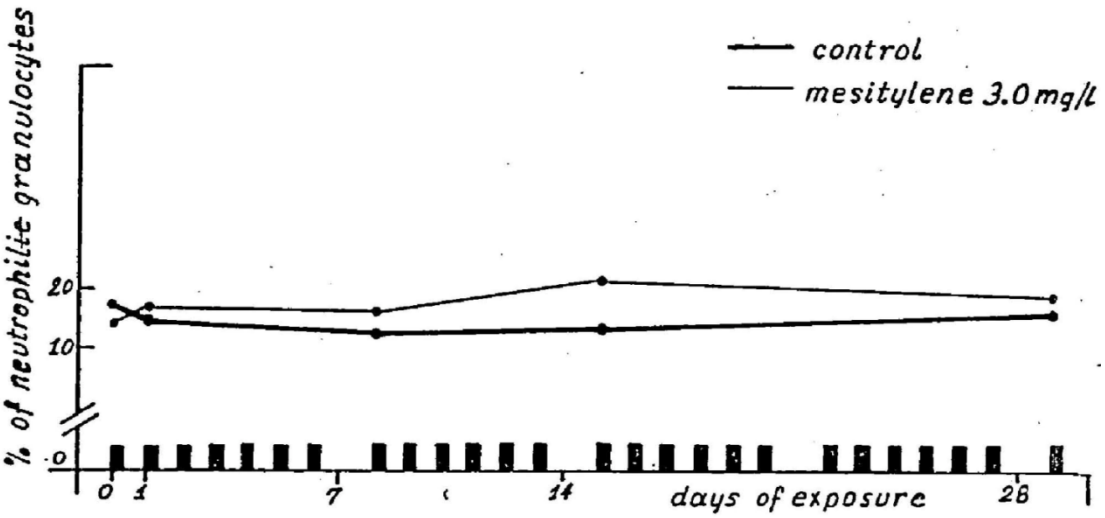


Figure 2. Percentage of segmented neutrophilic granulocytes during exposure to 1,3,5-TMB 3.0 mg/L for 6 hrs/d, 6 d/wk, for 5 wks.

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
	% 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.

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	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-erm study: 6 hrs/d, 6 d/wk for 5 wks

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/d, 6 d/wk, for 5 wks.
- 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.

Observation	**1,3,5-TMB exposure concentration (mg/L)—hematological parameters following single 6 hour exposure (means ± SE)**			
	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	308 ± 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	--

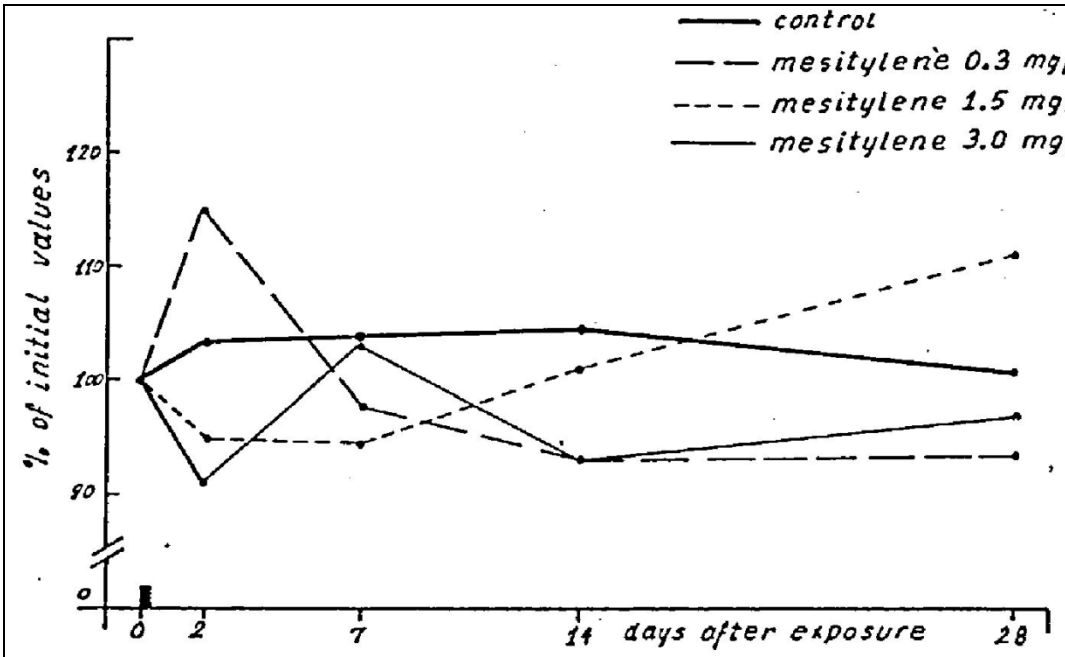


Figure 1. Serum activity of aspartate amino transferase after 6 hrs exposure to 1,3,5-TMB; values are expressed in % of initial values.

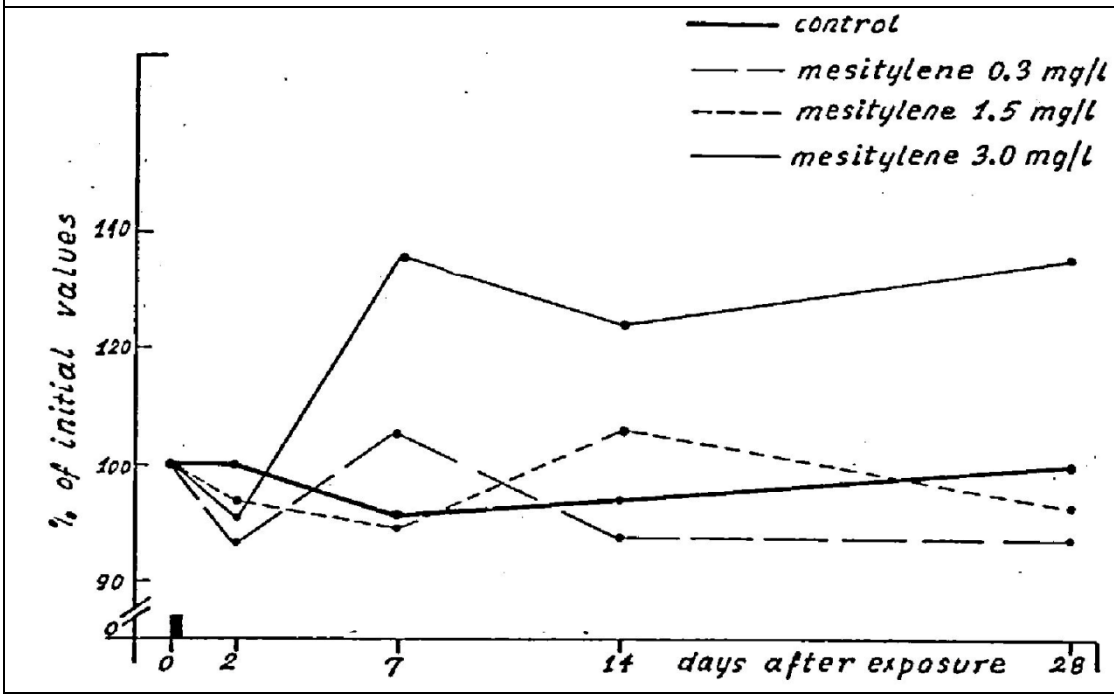


Figure 2. Serum activity of alanine amino transferase after 6 hrs exposure to 1,3,5-TMB; values are expressed in % of initial values.

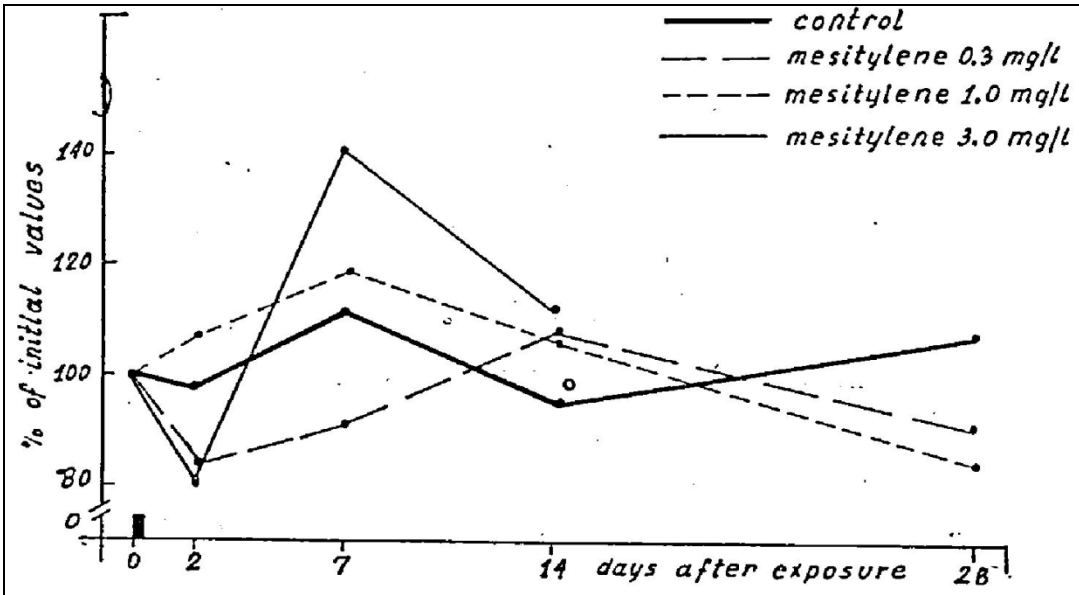


Figure 3. Serum activity of alkaline phosphatase after 6 hrs exposure to 1,3,5-TMB; values are expressed in % of initial values.

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

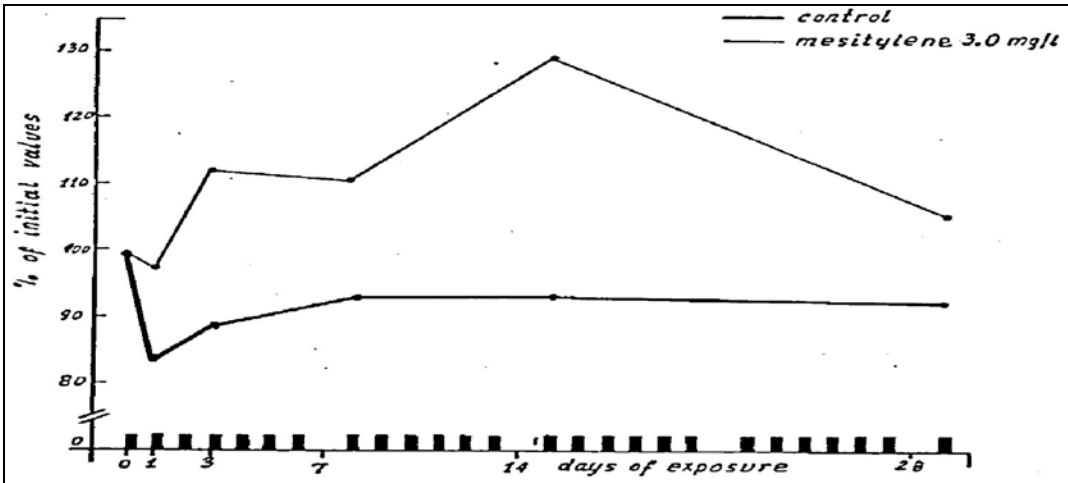


Figure 4. Serum activity of aspartate amino transferase during exposure to 1,3,5-TMB at 3.0 mg/L for 6 hrs/d, 6 d/wk, for 5 wks; values are expressed in % of initial values.

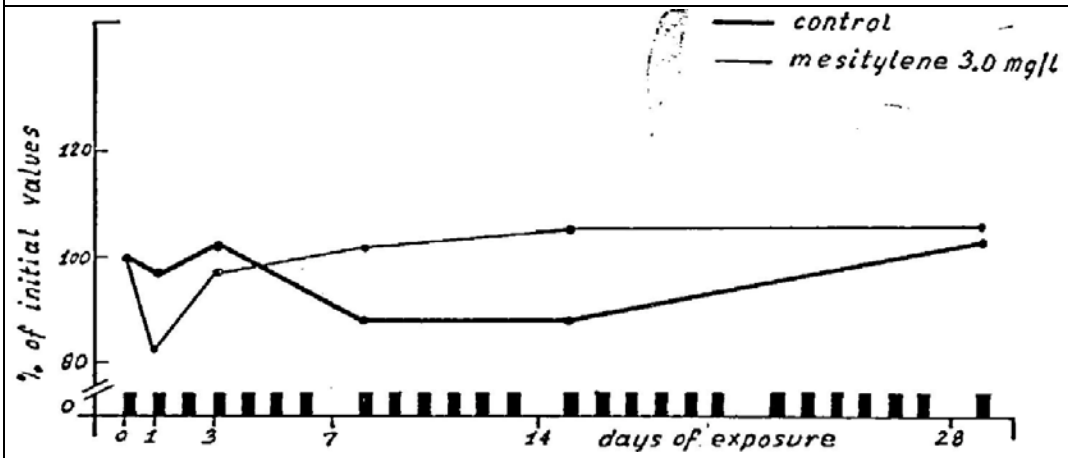


Figure 5. Serum activity of alanine amino transferase during exposure to 1,3,5-TMB at 3.0 mg/L for 6 hrs/d, 6 d per wk, for 5 wks; values are expressed in % of initial values.

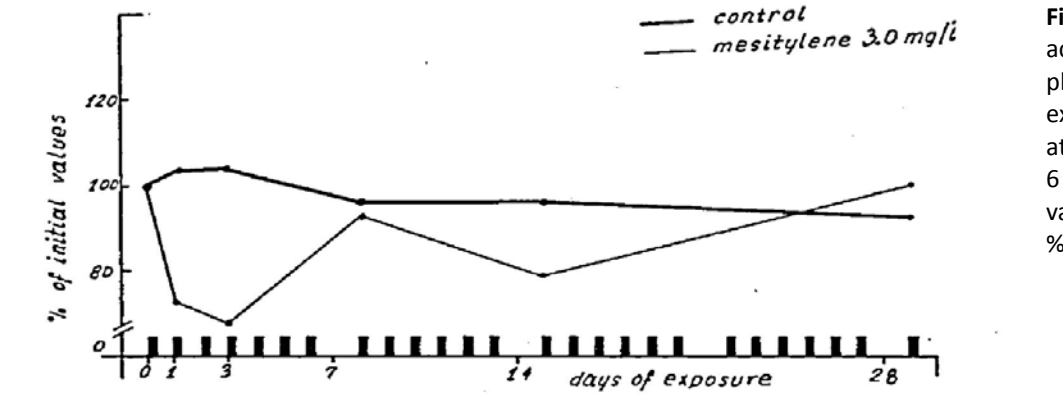


Figure 6. Serum activity of alkaline phosphatase during exposure to 1,3,5-TMB at 3.0 mg/L for 6 hrs/d, 6 d/wk, for 5 wks; values are expressed in % of initial values.

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 ($p < 0.05$).

B.6. HUMAN TOXICOKINETIC STUDIES

Table B-45. 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

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).

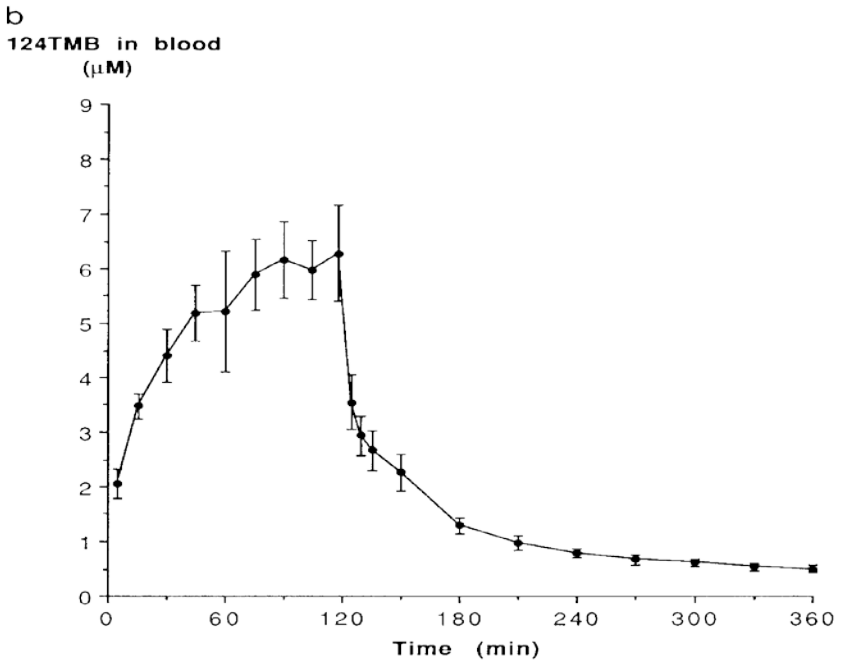


Figure 2. Concentration of 1,3,5-TMB in capillary blood during and after 2 hr exposure to 25 ppm **(**123 mg/m^3) 1,3,5-TMB (mean values ± 95% CI).

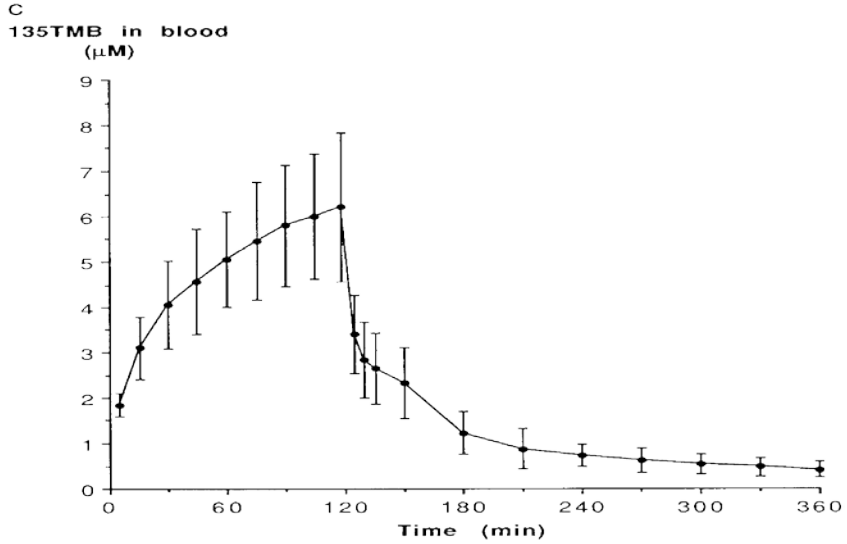


Figure 3. Concentration of 1,2,3-TMB in capillary blood during and after 2 hr exposure to 25 ppm **(**123 mg/m^3) 1,2,3-TMB (mean values ± 95% CI).

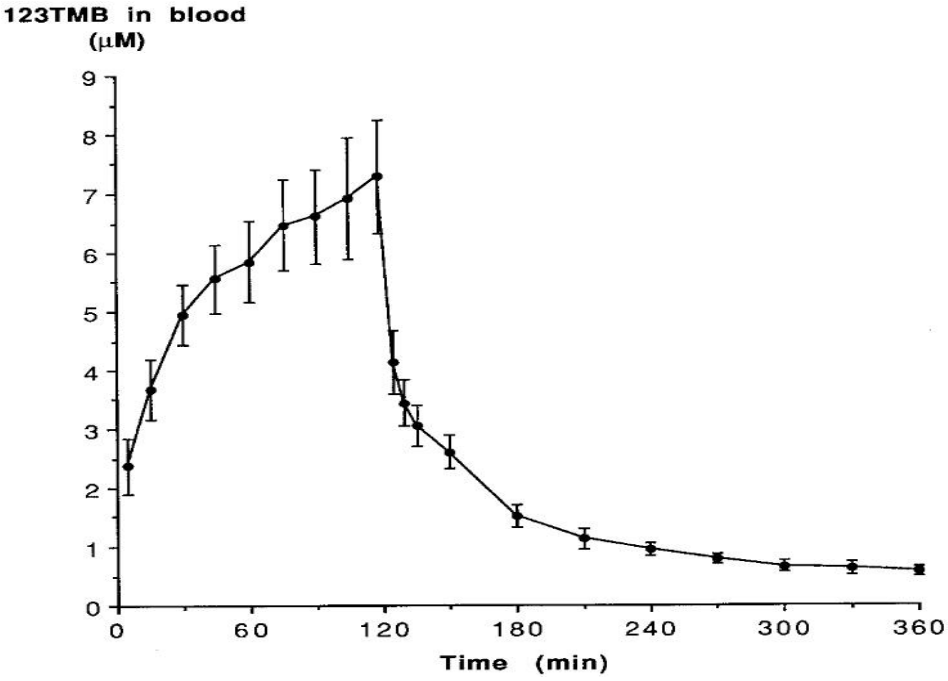
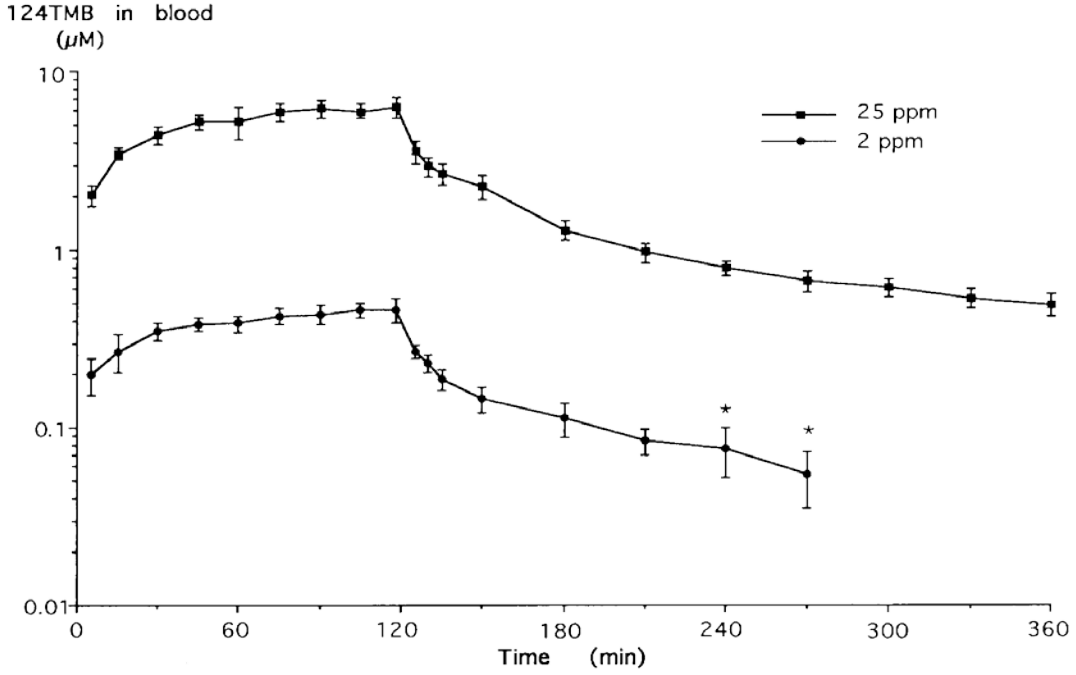


Figure 4. Concentration of 1,2,4-TMB in capillary blood from 10 subjects exposed to 2 and 25 ppm (~10 and 123 mg/m^3) of 1,2,4-TMB (mean values ± 95% CI)



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.

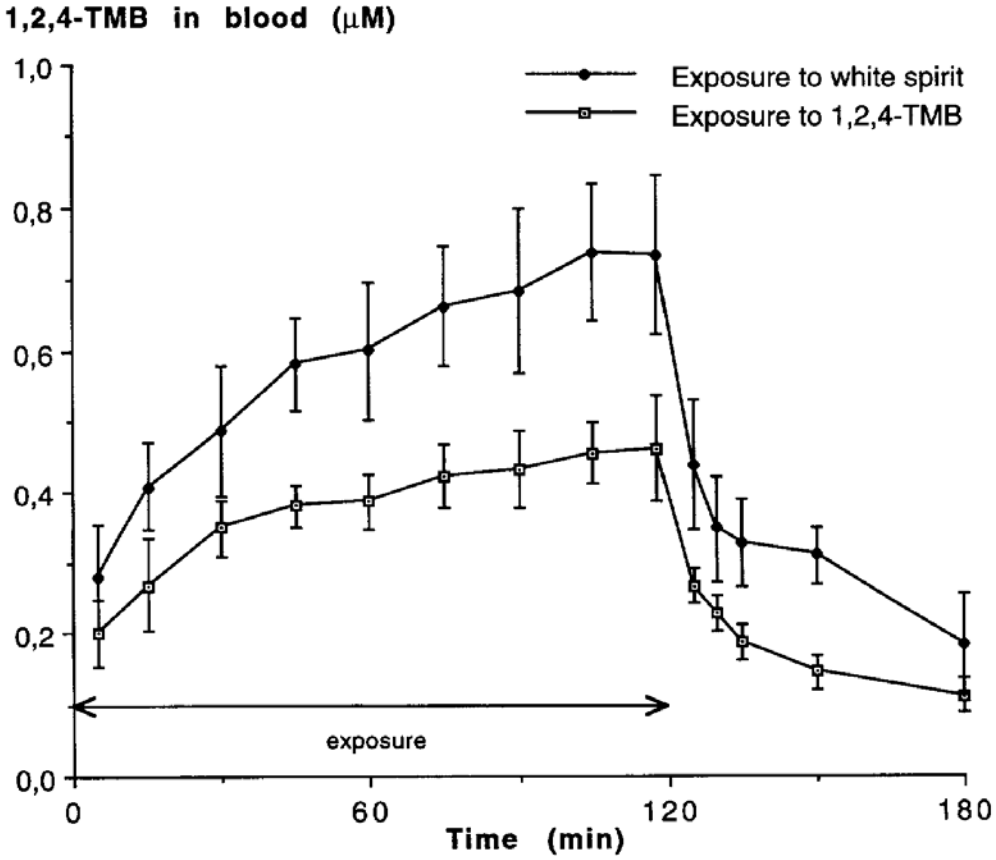
Table B-46. Characteristics and quantitative results for Järnberg et al. (1997a)

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

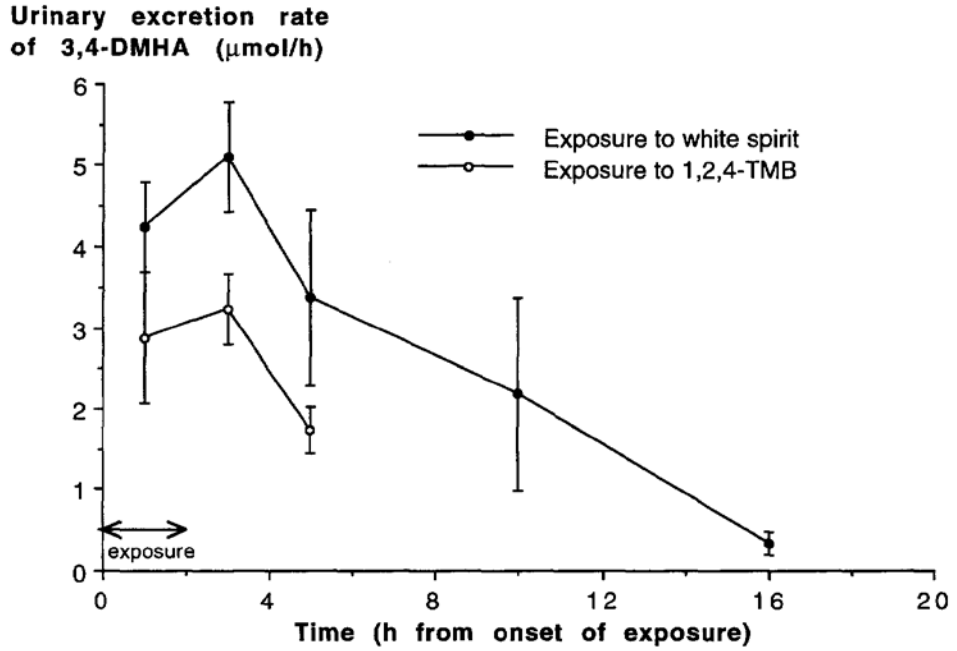
- Nine Caucasian males were exposed to 11 mg/m^3 1,2,4-TMB alone or 11 mg/m^3 1,2,4-TMB as a component of 300 mg/m^3 WS.
- Exposure lasted 2 hrs, during which study subjects were required to cycle producing 50 W continuously to simulate a work environment.
- Gas chromatography was used to measure 1,2,4-TMB 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.



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).



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

Table B-47. 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.

Table B-48. 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-–.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)
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

Table B-49. 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.

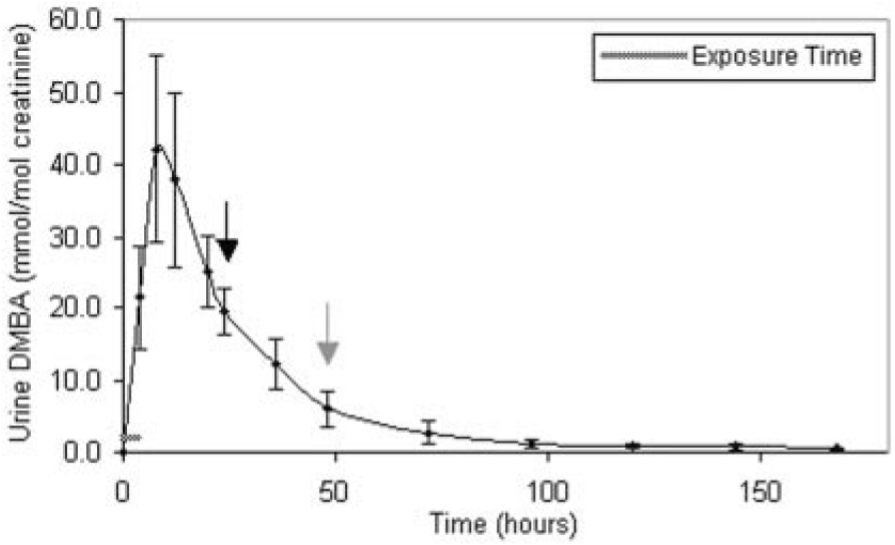


Figure 2. Mean ± SD blood levels of 1,3,5-TMB during and after 4 hr exposure to 25 ppm (1,2,3-TMB mg/m^3) 1,3,5-TMB.

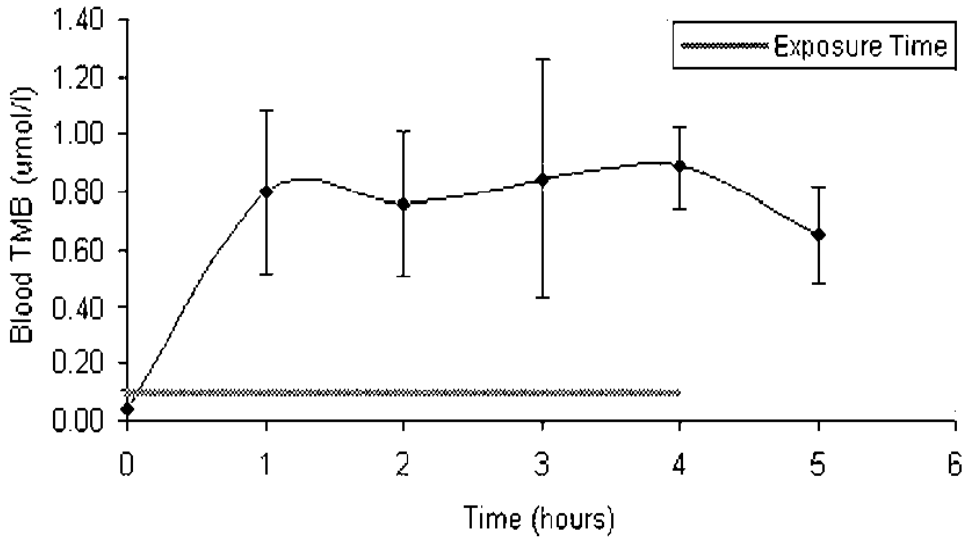
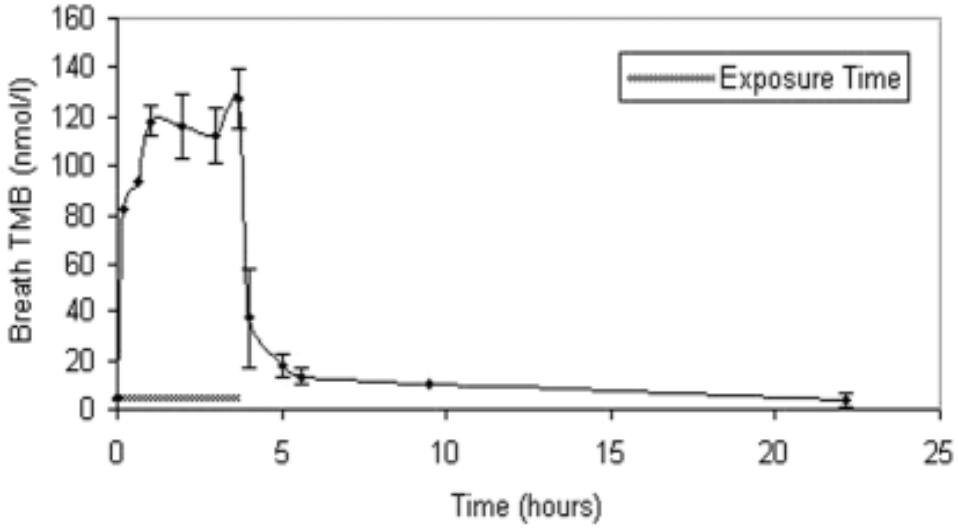


Figure 3. Mean ± SD breath levels of 1,3,5-TMB during and after 4 hr exposure to 1,3,5-TMB.



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.

Table B-50. 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 urinevia 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

Sampling time (hrs)	**1,2,3-TMB**		**1,2,4-TMB**		**1,3,5-TMB**	
	Blood concentr-ation (µg/dm^3)	**SD**	**Blood concentr-ation (µg/dm^3)**	**SD**	**Blood concentr-ation (µg/dm^3)**	**SD**
0	0	0	0	0.00	0	0.00
0.25	259	94.5	194	19.80	181	25.01
.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
.10	277	57.89	496	85.03	388	64.16
.15	287	38.18	447	106.69	309	38.78
.25	277	35.47	387	65.83	298	65.48
.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	--	--

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

Sampling time (hr)	**1,2,3-TMB exposure**			
	2,3-DMBA		**2,6-DMBA**	
	***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
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	--	--	--	--

Sampling time (hr)	**1,3,5-TMB exposure**	
	3,5-DMBA	
	***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.

B.7. ANIMAL TOXICOKINETIC STUDIES

Table B-51. 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	1-5000ppm 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.

Table B-52. 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	3806
300 ppm (1,476 mg/m^3)	115.5	220.9	256.3	282.4	12930
450 ppm (2,214 mg/m^3)	221.3	400.2	468.6	492.5	19270

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.

Table B-53. 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

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)

Metabolite	%Dose (0.08 mmol/kg) in urine			%Dose (0.8 mmol/kg) in urine					
	Free	Conjugated	Total	Free		Conjugated		Total	
	all rats	all rats	all rats	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat2
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.

Table B-54. 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 1.2g/kg body weight.
- In one experiment, urine was collected every 4 hrs over a period of 3 d.
- 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

Not treated	**% of dose (mean ± SD)**			
	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.

Table B-55. 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 timepoints 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

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.			

Table B-56. 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 wks

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 wks.
- 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 wks	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 wks	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 wk 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

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 wks (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 wks (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 wks (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 wks (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 wks)	0.38 ± 0.23	2.33 ± 1.24	21.95 ± 3.81
Temporal cortex (4 wks)	0.25 ± 0.07	2.03 ± 0.66	15.71 ± 3.54
Hippocampus (4 wks)	0.41 ± 0.27	3.03 ± 0.48	12.44 ± 2.63*
Cerebellum (4 wks)	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

Table B-57. 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 wks

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 wks.
- 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 wk 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 wk 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 wk 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

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			
Time	**1,3,5-TMB (µg/mL)**		
	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

Venous blood 1,3,5-TMB concentration following 4 wk 1,3,5-TMB inhalation exposure			
Time	**1,3,5-TMB (μg/mL)**		
	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 in comparison to brainstem

Table B-58. 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 d

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 d.
- HPLC used to quantify amount of dimethylbenzyl mercapturic acid in urine.

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

Dose (mmol/kg)	**% of dose ± SD**		
	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.

Table B-59. 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 d

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

2,4,6-Trimethylphenol

Dose (mmol/kg)	**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

Table B-60. 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

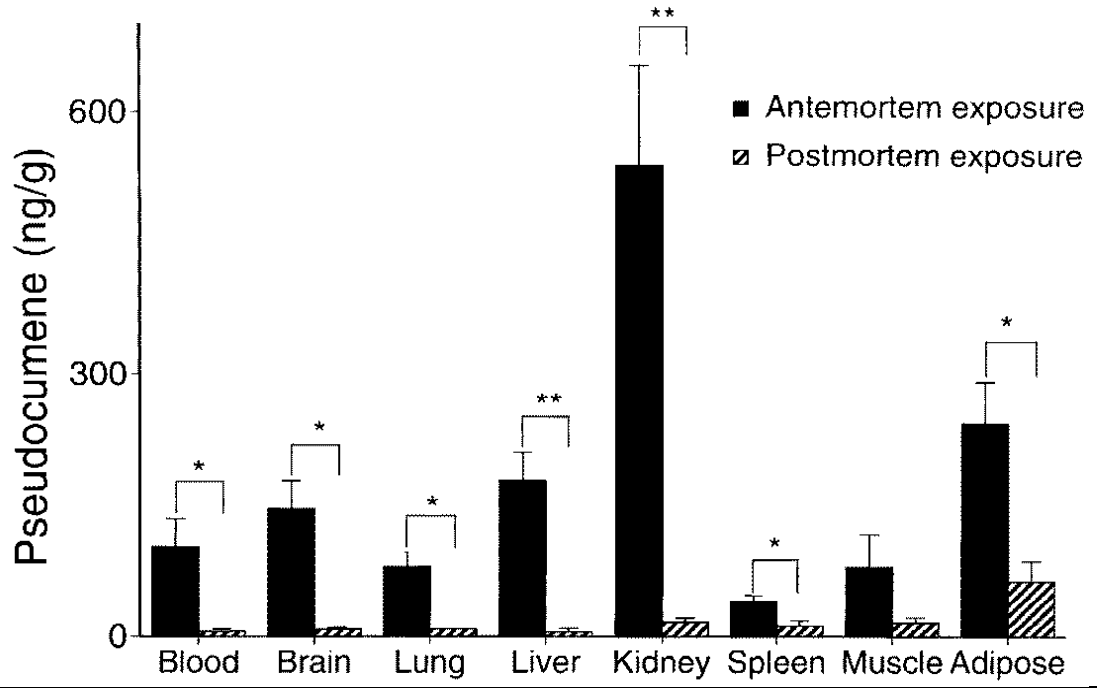
- In first experiment, rats were dermally exposed to kerosene on a saturated, sealed piece of cotton for 1 hr to analyze TMB and aliphatic hydrocarbon (AHC) dermal absorption.
- In second experiment, 44 rats were divided into four groups which varied by exposure duration, post-exposure time, and/or exposure 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	3.6 ± 1.6	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*

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

Figure 1. 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 (* $p < 0.05$, ** $p < 0.01$)



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).

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

Table B-61. 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.

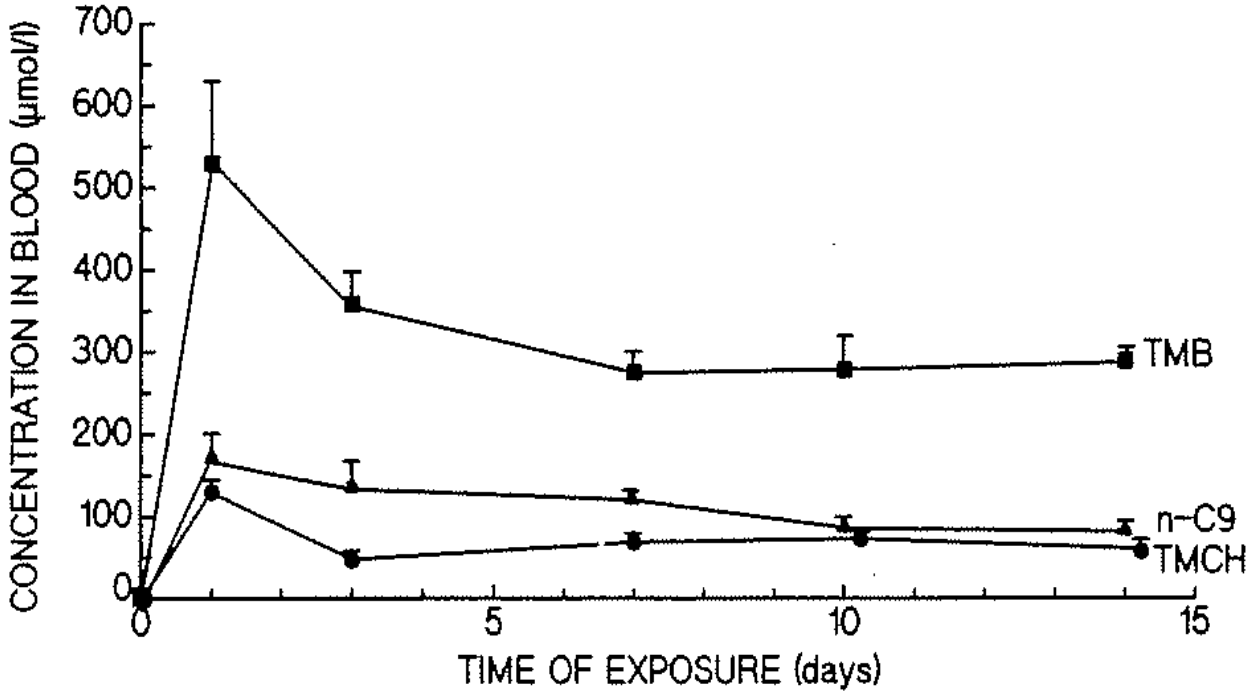


Figure 2. Brain concentrations (+SD) of n-nonane, 1,2,4-TMB, and 1,2,4-trimethylcyclohexane following 12 hr exposures on days 1, 3, 7, 10, and 14.

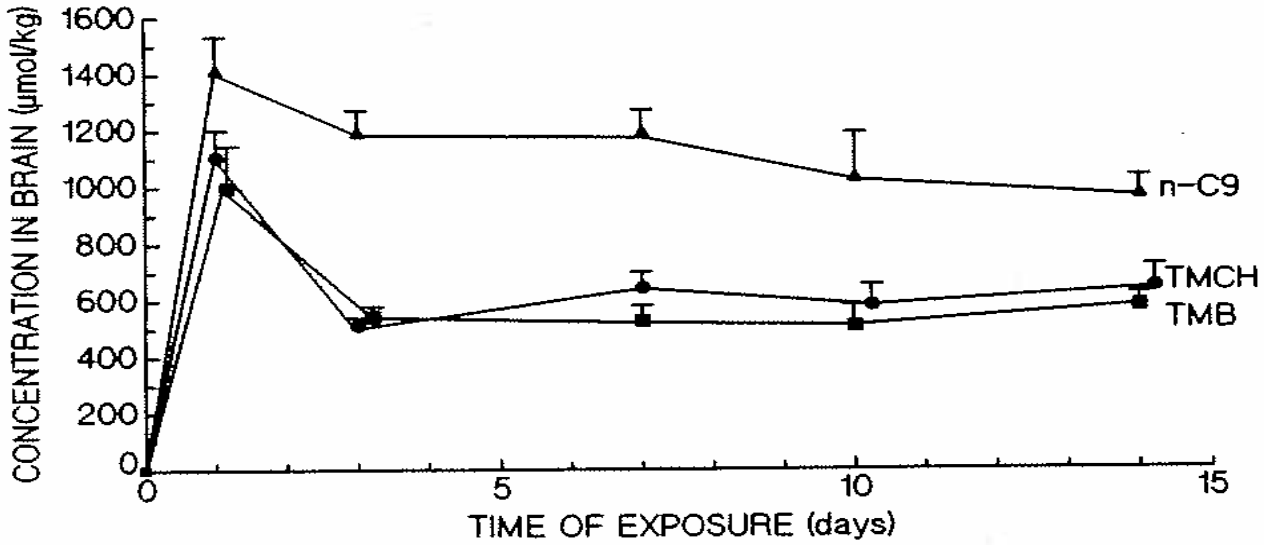
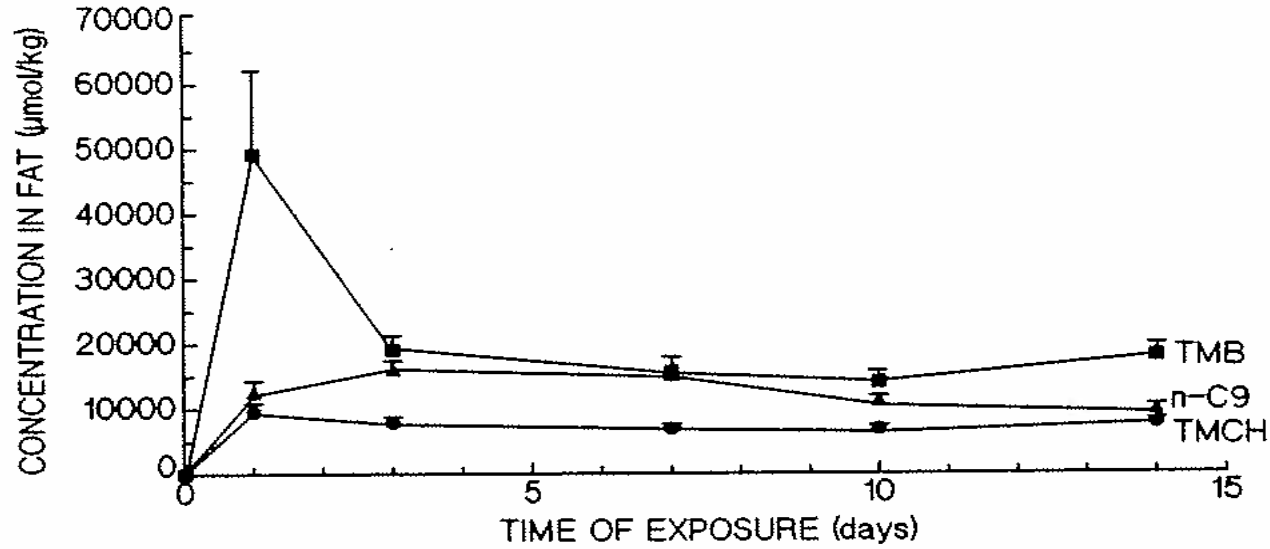


Figure 3. Perirenal fat concentrations (+SD) of n-nonane, 1,2,4-TMB, and 1,2,4-trimethylcyclohexane following 12 hr exposures on days 1, 3, 7, 10, and 14.



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.

Table B-62. 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 armoate	12 hours/day for 3 days

Additional study details

- Food and water was given *ad libitum*, except during exposure.
- Rats weighed between 150-200g 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

Observation	**C9 Aromate Concentration in Rat Tissues at Various Time Points (Mean±S.D)**
	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

B.8. ANIMAL AND HUMAN TOXICOKINETIC STUDIES

Table B-63. 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 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, respiratory, hematological, and developmental toxicity endpoints. The endpoints were modeled using the U.S. EPA's Benchmark Dose Software (BMDS). For every continuous endpoint, BMDS continuous models were fitted to the data. Model parameters were estimated using the maximum likelihood method. Model fit was assessed following the draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000) as follows. For each model, first the homogeneity of the variances (the "constant variance" case) 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 under the constant variance case. If Test 2 was rejected (χ^2 p-value < 0.10), the variance was tested as a power function of the mean (the "modeled variance" case) using a likelihood ratio test (BMDS Test 3). If Test 3 was not rejected (χ^2 p-value ≥ 0.10), the model was fitted to the data under the modeled variance case. For fitting models in either the constant variance or modeled variance case, models were tested for adequacy of fit to the means using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit).

Other factors were used to assess the model fit, such as scaled residuals, graphical fit, and adequacy of fit in the low-dose region and near the benchmark response (BMR). For the continuous endpoints (latency to paw-lick, decreased RBC, decreased reticulocytes, decreased clotting time, decreased fetal weight, and decreased maternal weight change), a BMR equal to a change in the meal response equivalent to 1 standard deviation of the estimated mean was chosen as the response level. A BMR equal to a change in the meal response equivalent to 1 standard deviation is recommended as the response level for endpoints for which no data exist as to what level of response to consider adverse (U.S. EPA, 2000). In addition to this a BMR of 5% relative deviance was also used as a response level for the decreased fetal weight endpoints. As a decrease of 10% body weight is often

used as a biologically significantly response level for adult animals, a 5% decrease in body weight was determined as biologically significant for prenatal rats.

For each endpoint, the best-fit model was selected from among the models exhibiting adequate fit. For each model, the BMDL was calculated using the profile likelihood method, where the BMDL refers to the 95% lower confidence limit on the benchmark does (BMD). If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, the model selected was the one that yielded the lowest Akaike Information Criterion (AIC) value. If more than one model had the lowest AIC, BMDL values from these models were averaged to obtain a POD. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD. When two models are displayed on the same row, this indicates that these models returned the same modeling results. This happens when a more complex model reverts to a simpler form. For example, a polynomial 3° model can revert to a polynomial 2° form if the beta3 coefficient is not estimated. When models in this case are selected as the best-fit model, the most simple form (i.e., poly 2° instead of poly 3°) is selected as the best-fit model.

Below are tables summarizing the modeling results for the modeled endpoints. The following parameter restrictions were applied:

- for multistage models, beta restricted to ≥ 0;
- for the polynomial models, betarestricted to ≥ 0; and
- for the Hill and continuous power models, power restricted to ≥ 1.

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).

Table C-1. Model predictions (constant variance, high dose dropped) for increased latency to paw-lick in male Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.5045	122.2153	0.42102	0.328286	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential 4 model was selected based on lowest BMDL.
Exponential 4[b]	**n/a**	**123.7699**	**0.233402**	**0.0864608**	
Linear **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]Although a goodness-of-fit *p*-value was not calculated for the Exponential 4 model (due to estimated model parameters = dose groups), inspection of scaled residuals and visual fit indicated appropriate model fit.

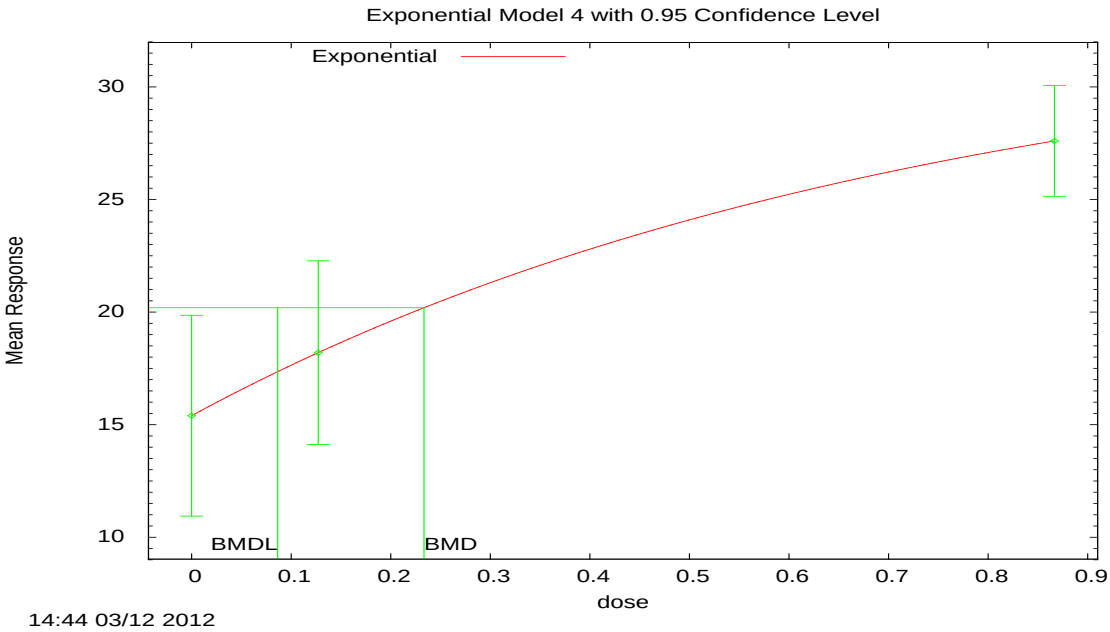


Figure C-1. Plot of mean response by dose (mg/L 1,2,4-TMB) for increased latency to paw-lick in male Wistar rats, with fitted curve for Exponential 4 model (BMR = 1 SD, constant variance, high dose dropped). (Korsak and Rydzyński, 1996)

Table C-2. Model predictions (constant variance, high dose dropped) for decreased red blood cells in male Wistar rats, 1,2,4-TMB (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 2	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 3[b]	n/a	61.79073	0.870338	0.469066	
Exponential 4	0.8653	59.81949	0.847227	0.184658	
Linear	**0.8864**	**59.811121**	**0.851043**	**0.499419**	
Polynomial 2°[b] **Polynomial 3° Power**	n/a	61.790726	0.869761	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]Although a goodness-of-fit *p*-value was not calculated for the Exponential 3, polynomial, or power models (due to estimated model parameters = dose groups), inspection of scaled residuals and visual fit indicated appropriate model fit.

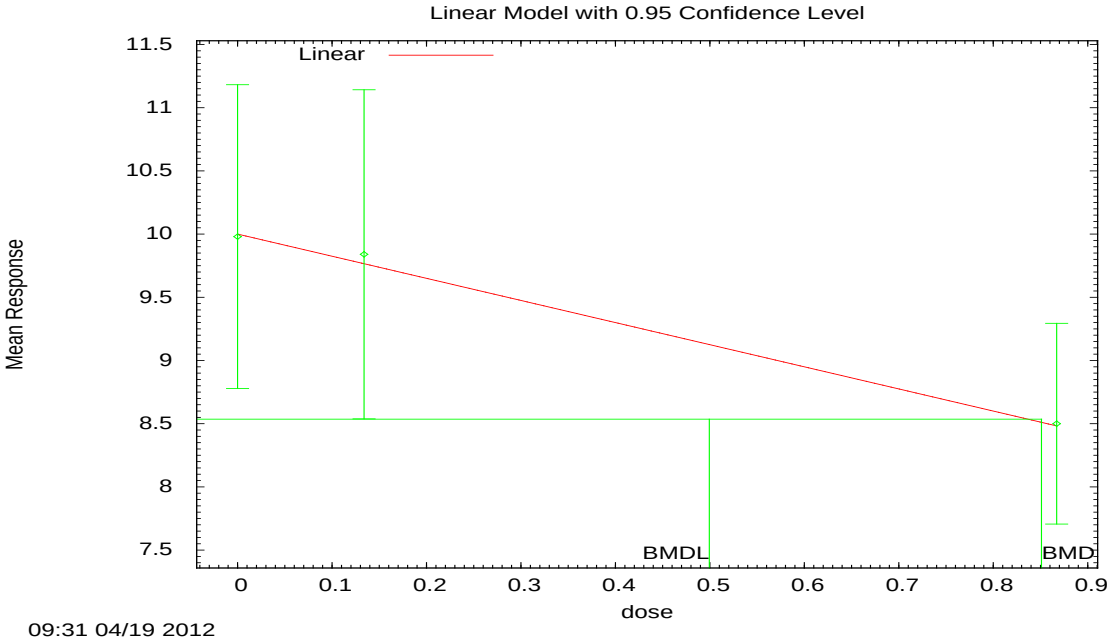


Figure C-2. Plot of mean response by dose (mg/L 1,2,4-TMB) for decreased red blood cells in male Wistar rats, with fitted curve for Linear model (BMR = 1 SD, constant variance, high dose dropped). (Korsak et al., 2000a)

Table C-3. Model predictions (constant variance, high dose dropped) for decreased clotting time in female Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.0676	151.6841	0.624689	0.35101	No model selected as Test 2 *p*-value was < 0.10
Exponential 4[b]	n/a	150.3436	0.118085	0.0006662	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.05648	151.99019	0.69465	0.441274	

[a]Constant variance case presented (Test 2 *p*-value = 0.008489). This *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.

[b]*p*-value not reported due to estimated model parameters = dose groups

Table C-4. Model predictions (modeled variance, high dose dropped) for decreased clotting time in female Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.00949	150.0056	0.829105	0.456483	No model selected as the only appropriate fitting model (Exponential4) returned an implausibly low BMDL estimate.
Exponential 4[b]	n/a	145.2775	0.154524	0.000850437	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.007771	150.362869	0.866447	0.533906	

[a]Modeled variance case presented (Test 3 *p*-value = 0.1159).

[b]A goodness-of-fit *p*-value was not calculated for the Exponential 4 model (due to estimated model parameters = dose groups), 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.

Table C-5. Model predictions (constant variance) for decreased fetal weight in male Sprague-Dawley rats, 1,2,4-TMB (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 2	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 3	0.8333	-83.91341	3,440.45	2,348.58	
Exponential 4	0.5714	-84.27301	2,803.48	2,052.08	
Exponential 5	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	

[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.

Table C-6. Model predictions (constant variance) for decreased fetal weight in male Sprague-Dawley rats, 1,2,4-TMB (Saillenfait et al., 2005)

Model[a]	Goodness-of-fit		$BMD_{5\%}$ (mg/m^3)	$BMDL_{5\%}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential 2	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 3	0.8333	-83.91341	2,861.09	1,716	
Exponential 4	0.5714	-84.27301	2,009.49	1,427.9	
Exponential 5	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.

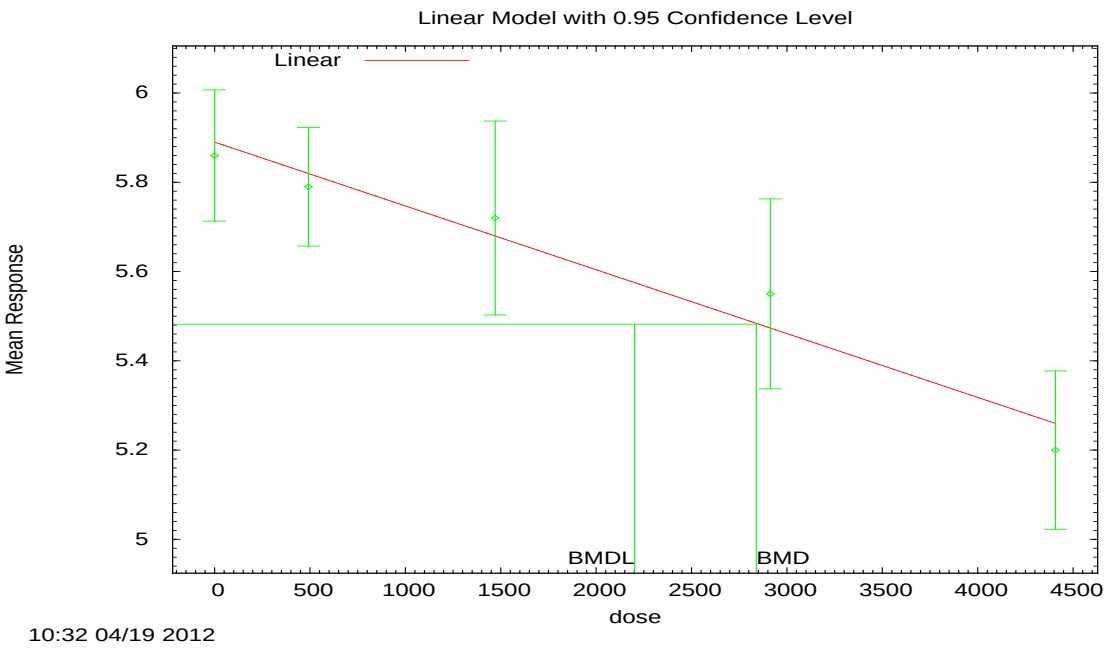


Figure C-3. Plot of mean response by dose (mg/m^3 1,2,4-TMB) for decreased fetal weight in male Sprague-Dawley rats, with fitted curve for Linear model (BMR = 1 SD, constant variance). (Saillenfait et al., 2005)

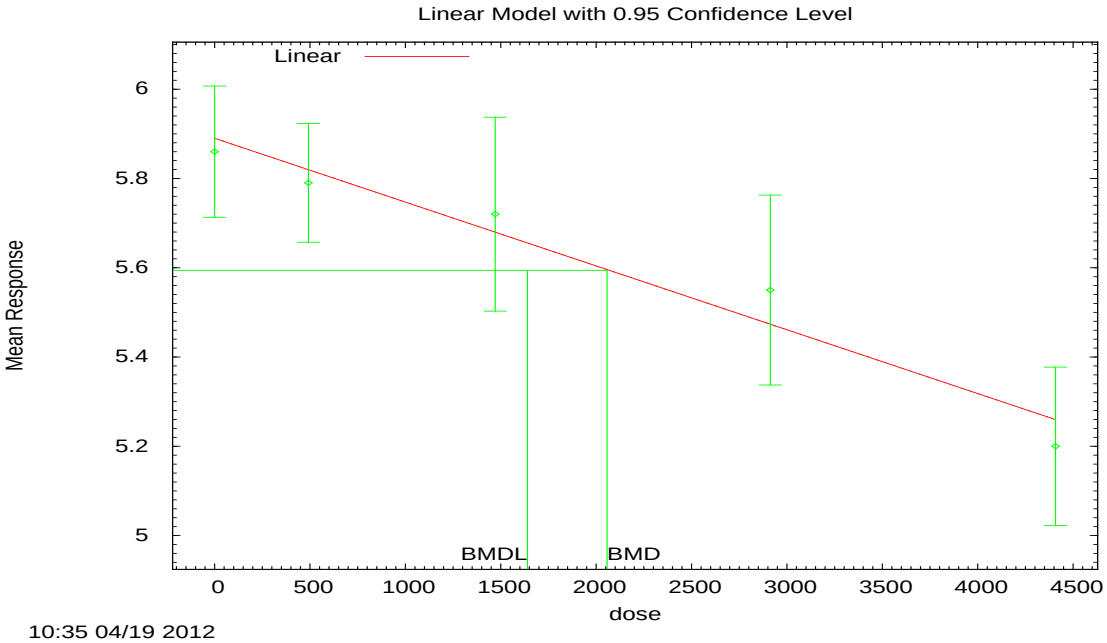


Figure C-4. Plot of mean response by dose (mg/m^3 1,2,4-TMB) for decreased fetal weight in male Sprague-Dawley rats, with fitted curve for Linear model (BMR = 5% RD, constant variance). (Saillenfait et al., 2005)

Table C-7. Model predictions (constant variance) for decreased fetal weight in female Sprague-Dawley rats, 1,2,4-TMB (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 2	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 3	0.654	-101.1358	3,312.88	2,212.4	
Exponential 4	0.5056	-101.6488	2,650.97	1,947.94	
Exponential 5	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	

[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.

Table C-8. Model predictions (constant variance) for decreased fetal weight in female Sprague-Dawley rats. 1,2,4-TMB (Saillenfait et al., 2005)

Model[a]	Goodness-of-fit		$BMD_{5\%}$ (mg/m^3)	$BMDL_{5\%}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential 2	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 3	0.654	-101.1358	2,778.64	1,662.76	
Exponential 4	0.5056	-101.6488	1,951.39	1,398.32	
Exponential 5	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, 1,471, 2,913, and 4,408 mg/m^3 were 0.39, -0.187, -0.566, 0.519, -0.158, respectively.

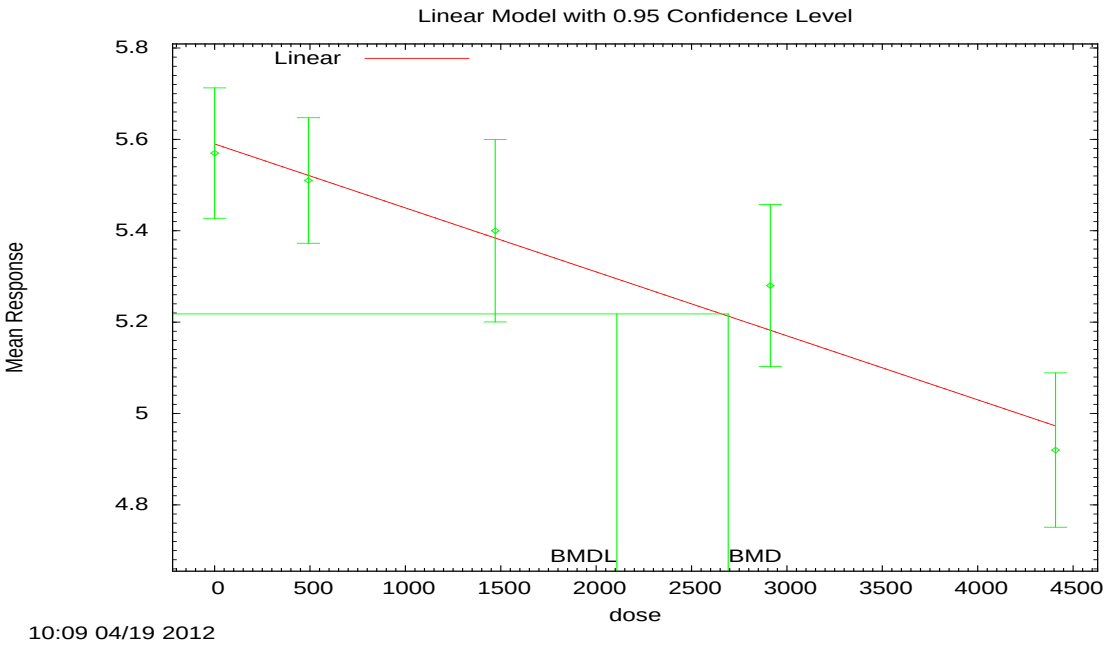


Figure C-5. Plot of mean response by dose (mg/m³ 1,2,4-TMB) for decreased fetal weight in female Sprague-Dawley rats, with fitted curve for Linear model (BMR = 1 SD, constant variance). (Saillenfait et al., 2005)

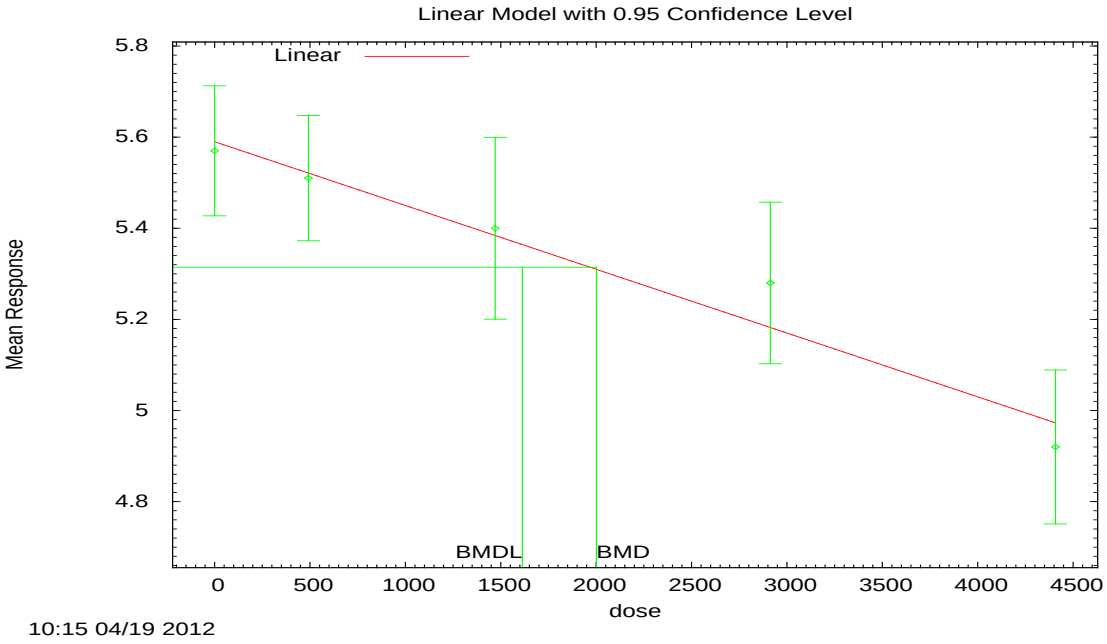


Figure C-6. Plot of mean response by dose (mg/m³ 1,2,4-TMB) for decreased fetal weight in female Sprague-Dawley rats, with fitted curve for Linear model (BMR = 5% RD, constant variance). (Saillenfait et al., 2005)

Table C-9. Model predictions (constant variance) for decreased maternal weight gain in female Sprague-Dawley rats, 1,2,4-TMB (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 2[b]	< 0.0001	1025.385	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 3	**0.7552**	**717.3518**	**2,821.1**	**2,247.99**	
Exponential 4[b]	< 0.0001	773.2296	Not Computed	0	
Exponential 5	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° **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 2 and 4 models did not return BMD and/or BMDL values and were excluded from further consideration.

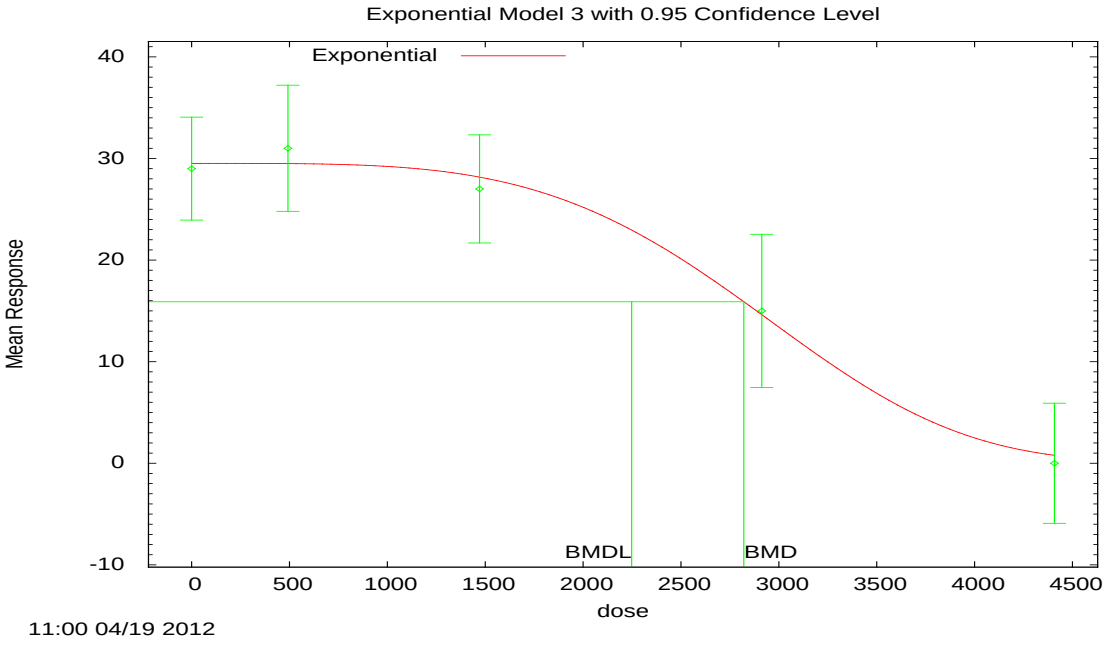


Figure C-7. Plot of mean response by dose (mg/m^3 1,2,4-TMB) for decreased maternal weight gain in female Sprague-Dawley rats, with fitted curve for Exponential 3 model (BMR = 1 SD, constant variance). (Saillenfait et al., 2005)

Table C-10. Model predictions (constant variance) for increased latency to paw-lick in male Wistar rats, 1,2,3-TMB (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 2 **Exponential 3**	0.005704	262.2082	700.938	566.333	No model selected as Test 2 *p*-value was < 0.1..
Exponential 4	0.5461	254.2393	192.288	107.132	
Exponential 5[b]	n/a	255.8749	201.187	111.315	
Hill[b]	n/a	255.874906	185.863	110.398	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.01728	259.991214	577.555	442.59	

[a]Constant variance case presented (Test 2 *p*-value = 0.0.0001146). This *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.
[b]*p*-value not reported due to estimated model parameters = dose groups

Table C-11. Model predictions (modeled variance) for increased latency to paw-lick in male Wistar rats, 1,2,3-TMB (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 2 **Exponential 3**	<0.0001	259.5324	496.844	329.318	No model selected as Test 3 *p*-value was < 0.1.
Exponential 4	0.301	241.4193	86.2091	46.7265	
Exponential 5[b]	n/a	242.5858	113.028	51.9836	
Hill[b]	n/a	265.438765	334.7333	Not calculated	
Linear **Polynomial 2°** **Polynomial 3°**[b] **Power**	0.0003247	254.414778	319.651	195.989	

[a]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.
[b]*p*-value not reported due to estimated model parameters = dose groups
[c]The 3rd degree polynomial model failed to converge.

Table C-12. Model predictions (modeled variance, high dose dropped) for increased latency to paw-lick in male Wistar rats, 1,2,3-TMB (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 2 **Exponential 3**	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 4[b]	n/a	202.0839	104.546	52.5736	
Linear **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]Although a goodness-of-fit *p*-value was not calculated for the Exponential 4 model (due to estimated model parameters = dose groups), inspection of scaled residuals and visual fit indicated appropriate model fit.

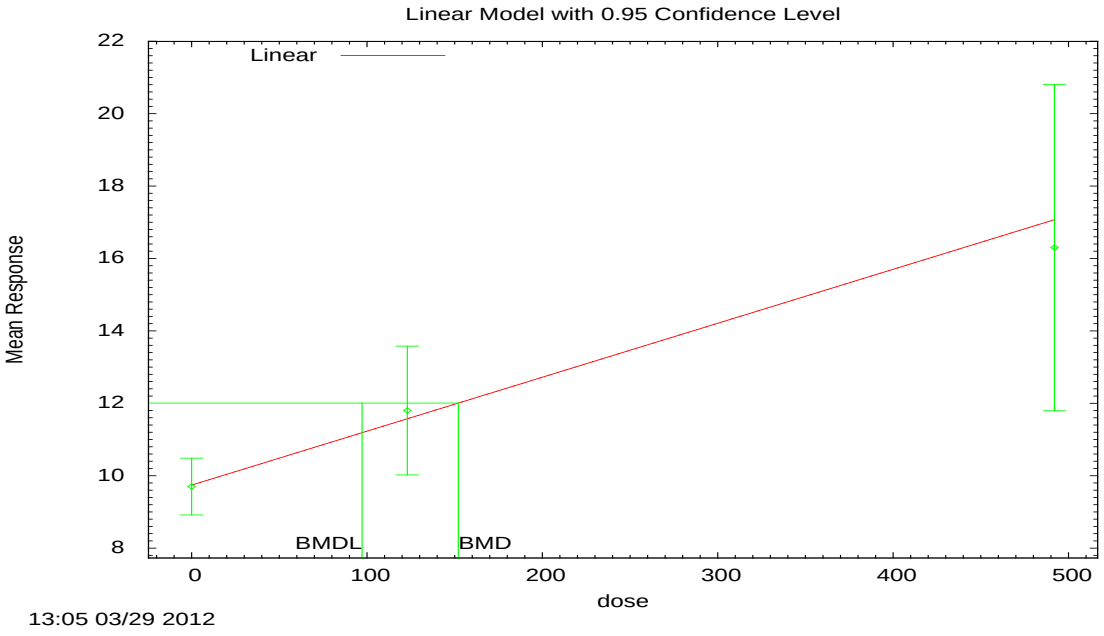


Figure C-8. Plot of mean response by dose (mg/m^3 1,2,3-TMB) for increased latency to paw-lick in male Wistar rats, with fitted curve for Linear model (BMR = 1 SD, modeled variance, high dose dropped). (Korsak and Rydzyński, 1996)

Table C-13. Model predictions (constant variance) for decreased segmented neutrophils in male Wistar rats, 1,2,3-TMB (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 2 Exponential 3	**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 4	0.4482	191.0108	814.879	261.734	
Exponential 5[b]	n/a	192.4867	547.805	137.551	
Hill[b]	n/a	192.486705	564.348	Not calculated	
Linear 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 -0.16, 0.16, -0.-1.94 × 10^{-07}, and 0.-4.06 × 10^{-08}, respectively.

[b]A goodness-of-fit *p*-value was not calculated for the Exponential 5 or Hill models, 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.

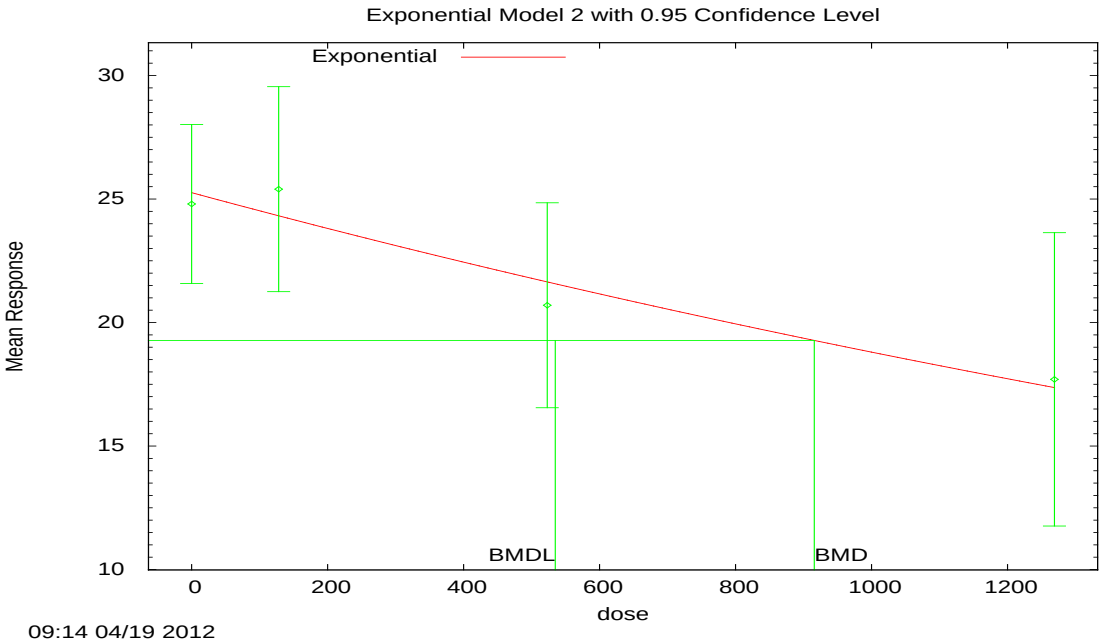


Figure C-9. Plot of mean response by dose (mg/m^3 1,2,3-TMB) for decreased segmented neutrophils in male Wistar rats, with fitted curve for Exponential 2 model (BMR = 1 SD, constant variance). (Korsak et al., 2000b)

Table C-14. Model predictions (constant variance) for decreased segmented neutrophils in female Wistar rats, 1,2,3-TMB (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 2 **Exponential 3**	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 4 **Exponential 5**	0.5208	179.1714	365.397	134.354	
Hill	**0.5692**	**179.083138**	**337.442**	**99.2111**	
Linear **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.

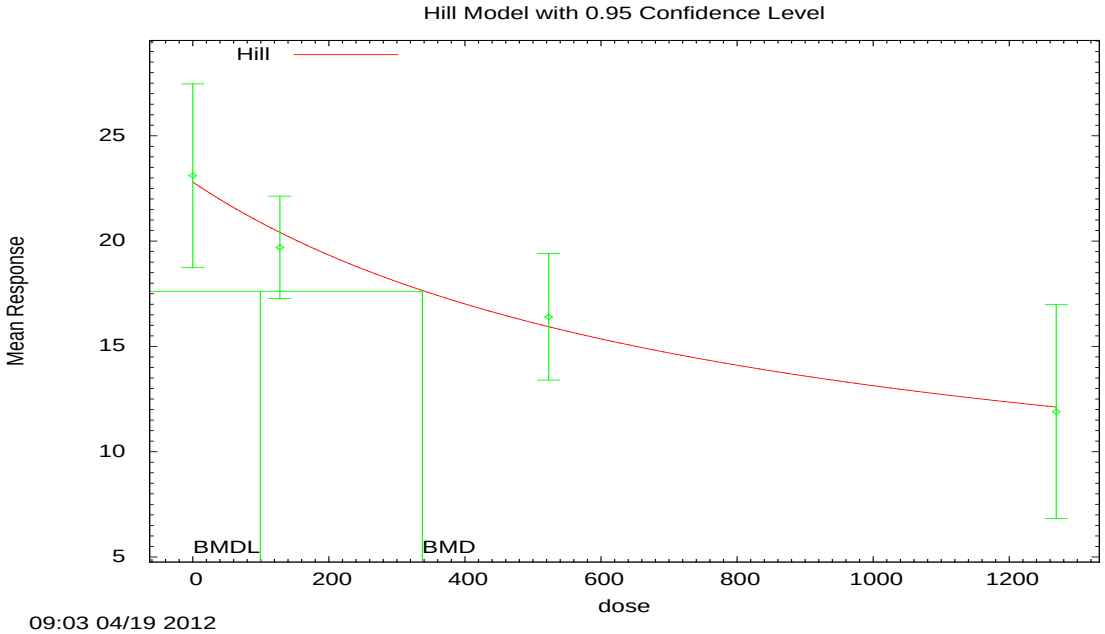


Figure C-10. Plot of mean response by dose (mg/m^3 1,2,3-TMB) for decreased segmented neutrophils in female Wistar rats, with fitted curve for Hill model (BMR = 1 SD, constant variance). (Korsak et al., 2000b)

Table C-15. Model predictions (constant variance) for increased reticulocytes in male Wistar rats, 1,2,3-TMB (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 2 **Exponential 3**	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 4	0.1397	90.67033	900.404	308.017	
Exponential 5[b]	n/a	91.37006	540.186	140.925	
Hill[b]	n/a	91.370061	554.848	Not calculated	
Linear **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]A goodness-of-fit *p*-value was not calculated for the Exponential 5 or Hill models, 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.

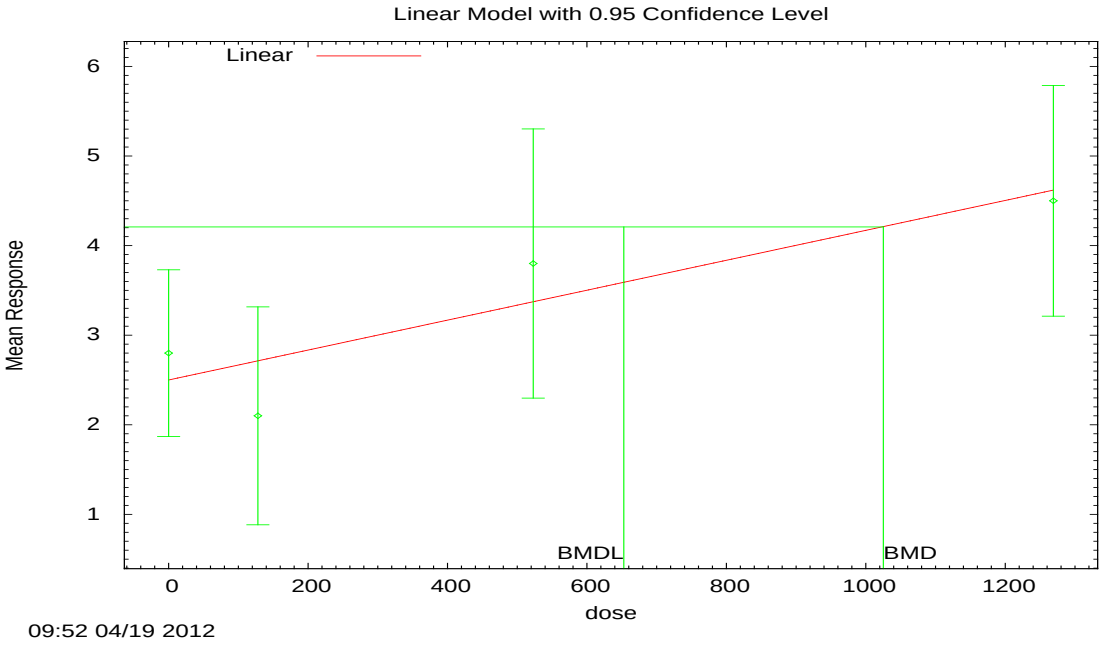


Figure C-11. Plot of mean response by dose (mg/m^3 1,2,3-TMB) for increased reticulocytes in male Wistar rats, with fitted curve for Linear model (BMR = 1 SD, constant variance). (Korsak et al., 2000b)

Table C-16. Model predictions (constant variance) for decreased fetal weight in male Sprague-Dawley rats, 1,3,5-TMB (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 2 **Exponential 3**	0.6927	-66.94125	3,396.62	2,560.01	No model selected as Test 2 *p*-value was < 0.10
Exponential 4	0.6981	-65.6776	2,604.81	1,341.07	
Exponential 5	0.397	-63.67902	2,603.37	1,341.3	
Hill	0.4094	-63.715888	2,572.4	1,274.69	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.6496	-66.753074	3,513.03	2,694.51	

[a]Constant variance case presented (Test 2 *p*-value = 0.002368), this *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.

Table C-17. Model predictions (modeled variance) for decreased fetal weight in male Sprague-Dawley rats, 1,3,5-TMB (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 2 **Exponential 3**	0.5214	-73.29149	2,523.27	1,779.29	No model selected as Test 3 *p*-value was < 0.10
Exponential 4	0.4304	-71.85947	2,041.7	1,125.34	
Exponential 5	0.3877	-70.79949	2,044.66	1,237.6	
Hill	0.4276	-65.644335	2,407.38	1,295.43	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.4791	-73.066751	2,636.36	1,890.46	

[a]Modeled variance case presented (Test 3 *p*-value = 0.06027, except the Hill model, for which Test 3 *p*-value = 0.00544). This *p*-value indicates that a modeled variance model does not adequately describe the observed variances. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

Table C-18. Model predictions (modeled variance) for decreased fetal weight in male Sprague-Dawley rats, 1,3,5-TMB (Saillenfait et al., 2005)

Model[a]	Goodness-of-fit		$BMD_{5\%}$ (mg/m^3)	$BMDL_{5\%}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential 2 **Exponential 3**	0.5214	-73.29149	2,187.66	1,645.82	No model selected as Test 3 *p*-value was < 0.10
Exponential 4	0.4304	-71.85947	1,781.91	1,025.78	
Exponential 5	0.3877	-70.79949	1,872.45	1,137.83	
Hill	0.4276	-65.644335	1,652.76	793.582	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.4791	-73.066751	2,282.12	1,744.39	

[a]Modeled variance case presented (Test 3 *p*-value = 0.06027, except the Hill model, for which Test 3 *p*-value = 0.00544). This *p*-value indicates that a modeled variance model does not adequately describe the observed variances. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

Table C-19. Model predictions (constant variance) for decreased fetal weight in female Sprague-Dawley rats, 1,3,5-TMB (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 2 **Exponential 3**	0.9112	-61.96218	3,581.71	2,669	No model selected as Test 2 *p*-value was < 0.10
Exponential 4 **Exponential 5**	0.7655	-59.96227	3,573.06	1,915.99	
Hill	0.7656	-59.962704	3,569.61	1,865.62	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.9085	-61.950195	3,676.95	2,794.36	

[a]Constant variance case presented (Test 2 *p*-value < 0.0001), this *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.

Table C-20. Model predictions (modeled variance) for decreased fetal weight in female Sprague-Dawley rats, 1,3,5-TMB (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 2 **Exponential 3**	0.01931	-67.53742	2692.79	1827.72	No model selected as Test 3 *p*-value was < 0.10
Exponential 4	0.05097	-69.49883	1481.66	798.275	
Exponential 5	0.5334	-73.06401	1469.46	1069.57	
Hill	0.4769	-59.505126	3161.1	1614.44	
Linear **Polynomial 2°** **Polynomial 3°**	0.0148	-67.061071	2841.13	1969.76	
Power	0.01552	-67.061071	2841.13	1969.76	

[a]Modeled variance case presented (Test 3 *p*-value = 0.01301), this *p*-value indicates that the modeled variance does not adequately describe the observed variances. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

Table C-21. Model predictions (modeled variance) for decreased fetal weight in female Sprague-Dawley rats, 1,3,5-TMB (Saillenfait et al., 2005)

Model[a]	Goodness-of-fit		$BMD_{5\%}$ (mg/m^3)	$BMDL_{5\%}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Exponential 2 **Exponential 3**	0.01931	-67.53742	2,244.13	1,633.96	No model selected as Test 3 *p*-value was < 0.10
Exponential 4	0.05097	-69.49883	1,447.04	850.802	
Exponential 5	0.5334	-73.06401	1,472.61	1,125.04	
Hill	0.4769	-59.505126	2,009.89	928.261	
Linear **Polynomial 2°** **Polynomial 3°**	0.0148	-67.061071	2,346.47	1,739.45	
Power	0.01552	-67.061071	2,346.47	1,739.45	

[a]Modeled variance case presented (Test 3 *p*-value = 0.01301), this *p*-value indicates that the modeled variance does not adequately describe the observed variances. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

Table C-22. Model predictions (constant variance) for decreased maternal weight gain in female Sprague-Dawley rats, 1,3,5-TMB (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 2	< 0.0001	805.8321	3.36×10^{-51}	Bad_Completion	No model selected as Test 2 *p*-value was < 0.10
Exponential 3	< 0.0001	807.8353	6.29281	Bad_Completion	
Exponential 4	< 0.0001	701.8275	Not_Computed	0	
Exponential 5	0.00262	649.4267	2,057.15	1,396.23	
Hill	0.5141	639.963339	2,035.36	1,353.4	
Linear **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	

[a]Constant variance case presented (Test 2 *p*-value = 0.003114), this *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.

Table C-23. Model predictions (modeled variance) for decreased maternal weight gain in female Sprague-Dawley rats, 1,3,5-TMB (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 2[b]	< 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 3[B]	< 0.0001	923.089	Not_Computed	0	
Exponential 4	< 0.0001	698.0766	3.76×10^{-46}	3.76×10^{-46}	
Exponential 5	< 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°	0.2014	631.886974	1,797.1	Not calculated	
Power	**0.1981**	**631.236865**	**1,826.86**	**1,302.02**	

[a]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.

[b]The Exponential 2 and 3 models did not return BMD and/or BMDL values and were excluded from further consideration.

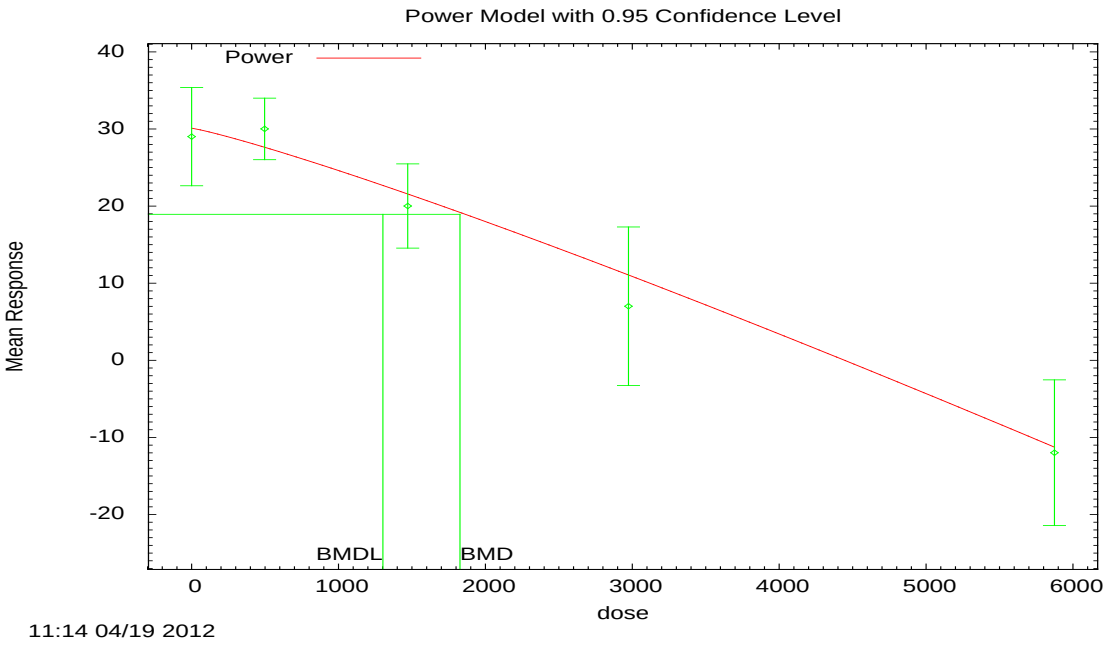


Figure C-12. Plot of mean response by dose (mg/m^3 1,3,5-TMB) for decreased maternal weight gain in female Sprague-Dawley rats, with fitted curve for Power model (BMR = 1 SD, modeled variance). (Saillenfait et al., 2005)

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-24. Model predictions (constant variance) for increased latency to paw-lick in male Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.00061	190.1611	3.62226	2.73586	No model selected as Test 2 *p*-value was < 0.10
Exponential 4	0.8239	177.4066	0.242222	0.104385	
Exponential 5[b]	n/a	179.3571	0.268238	0.105201	
Hill[b]	n/a	179.357065	0.237108	0.0889465	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.0009125	189.355645	3.15451	2.22737	

[a]Constant variance case presented (Test 2 *p*-value = 0.07651). BMDS recommends using a non-homogenous variance model.
[b] *p*-value not reported due to estimated model parameters = dose groups

Table C-25. Model predictions (modeled variance) for increased latency to paw-lick in male Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.000633	191.8156	3.38239	2.34048	No model selected as Test 2 *p*-value was < 0.10
Exponential 4	0.8604	179.1164	0.231414	0.09854	
Exponential 5[b]	n/a	181.0855	0.252014	0.0990336	
Hill[b]	n/a	181.982905	0.292816	Not calculated	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.001014	190.872265	2.8175	1.72529	

[a]Modeled variance case presented (Test 3 *p*-value = 0.0371). This *p*-value indicates that a modeled variance model does not adequately describe the observed variances. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.
[b b]A goodness-of-fit *p*-value was not calculated for the Exponential 5 or Hill models, inspection of scaled residuals and visual fit indicated appropriate model fit. However, the Hill model failed to calculate a BMDL and was excluded from consideration.

Table C-26. Model predictions (constant variance) for decreased red blood cells in male Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	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 4	0.7345	77.52579	0.795033	0.241565	
Exponential 5[b]	n/a	79.41075	0.842867	0.249166	
Hill[b]	**n/a**	**79.410749**	**0.835638**	**0.212686**	
Linear **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}, 4.83×10^{-08} and -6.99×10^{-08}, respectively.

[b]Although the Exponential 5 and Hill model returned no goodness-of-fit *p*-value, inspection of scaled residuals and visual fit indicated appropriate model fit.

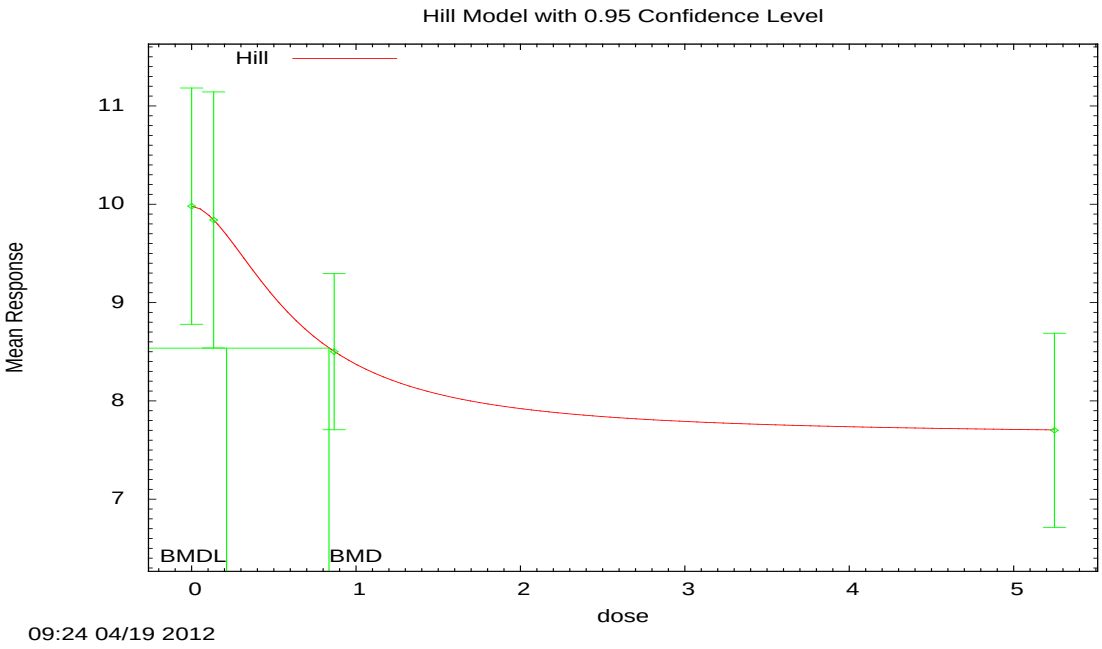


Figure C-13. Plot of mean response by dose (mg/L 1,2,4-TMB) for decreased red blood cells in male Wistar rats, with fitted curve for Hill model (BMR = 1 SD, constant variance). (Korsak et al., 2000a)

Table C-27. Model predictions (constant variance) for decreased clotting time in female Wistar rats (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 2 **Exponential 3**	0.00311	207.7609	13.2329	4.78502	No model selected as Test 2 *p*-value was < 0.10
Exponential 4	0.3078	199.2547	0.119261	0.000258705	
Exponential 5[b]	n/a	201.2538	0.12336	0.000534297	
Hill[b]	n/a	201.25379	0.129946	1.20×10^{-10}	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.003013	207.824506	12.5899	5.12676	

[a]Constant variance case presented (Test 2 *p*-value = 0.02286). This *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.

[b] *p*-value not reported due to estimated model parameters = dose groups

Table C-28. Model predictions (modeled variance) for decreased clotting time in female Wistar rats (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 2 **Exponential 3**	0.0001725	209.2185	16.2811	5.15229	No model selected as the only appropriate fitting models (Exponential 5 and Hill) either calculated no BMDL, or calculated an implausibly low BMDL.
Exponential 4	0.09227	196.7223	0.297031	0.000698259	
Exponential 5[b]	n/a	198.7223	0.235929	7.68×10^{-05}	
Hill[b]	n/a	204.758516	0.138361	Not calculated	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.0001675	209.276823	15.0257	5.46511	

[a]Modeled variance case presented (Test 3 *p*-value = 0.2001, except Hill model for which Test 3 *p*-value = < 0.0001).

[b]Although the Exponential 5 and Hill model returned no goodness-of-fit *p*-value, inspection of scaled residuals and visual fit indicated appropriate model fit. However, these models either failed to calculate a BMDL or calculated a BMDL that is biologically unreasonably low. Therefore, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

Table C-29. Model predictions (constant variance) for decreased reticulocytes in female Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.05738	91.21206	5.67056	0.775822	No model selected as Test 2 *p*-value was < 0.10
Exponential 4	0.2784	88.67076	0.107641	0.000190582	
Exponential 5[b]	n/a	90.67077	0.111117	0.000273446	
Hill	0.3149	88.506257	0.11386	6.85×10^{-15}	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.04654	91.631076	6.34191	3.62271	

[a]Constant variance case presented (Test 2 *p*-value = < 0.0001). This *p*-value indicates that a constant variance model does not adequately describe the observed variances. BMDS recommends using a non-homogenous variance model.

[b] *p*-value not reported due to estimated model parameters = dose groups

Table C-30. Model predictions (modeled variance) for decreased reticulocytes in female Wistar rats, 1,2,4-TMB (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 2 **Exponential 3**	0.01667	75.37239	12.0859	4.65557	No model selected as the only appropriate fitting model (Exponential4, 5, and Hill) calculated no BMDL
Exponential 4[b] **Exponential 5**[b]	0.3582	70.02825	Not_Computed	0	
Hill[c]	n/a	89.127269	Not_Computed	Not_Computed	
Linear **Polynomial 2°** **Polynomial 3°** **Power**	0.009093	76.584735	8.44761	5.29336	

[a]Modeled variance case presented (Test 3 *p*-value = 0.253).

[b]Although the Exponential 4 and 5 models display appropriate goodness-of-fit *p*-values, these models do not calculate BMD or BMDL values. As these are the only appropriately fitting models, this endpoint cannot be modeled in BMDS and the NOAEL/LOAEL approach is recommended.

[c] *p*-value not reported due to estimated model parameters = dose groups

APPENDIX D. DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

Documentation of the IRIS Program's Implementation of the 2011 NRC Recommendations in the External Peer Review Draft Toxicological Review of Trimethylbenzenes (June 2012)

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law[1]. The report language included direction to EPA for the IRIS Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde[2]. 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 their 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 table 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 focused

[1]Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.
[2]National Research Council, 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

on assessments near the end of the development process and close to final posting. The IRIS TMBs assessment is in Phase 2 of implementation, which addresses all of the short-term recommendations from Table 1. The 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 2, including the development of a standardized approach to describe the strength of evidence for noncancer effects . On May 16, 2012, EPA announced[3] 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.

[3]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

Table D-1. National Research Council recommendations that EPA is implementing in the short term	**Implementation status**
General recommendations for completing the IRIS formaldehyde assessment that EPA will adopt for all IRIS assessments (p. 152 of the NRC report)	
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.
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

Table D-1. National Research Council recommendations that EPA is implementing in the short term	Implementation status
	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.	**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 and 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.
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

Table D-1. National Research Council recommendations that EPA is implementing in the short term	**Implementation status**
	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.
Other specific recommendations (p. # in NRC report)	
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. National Research Council recommendations that EPA is implementing in the short term	**Implementation status**
Evidence Identification: Literature Collection and Collation Phase (p. 164) 10. Select outcomes on the basis of available evidence and understanding of mode of action.	**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 ammonia, the available evidence is informed by the mode of action information as discussed in Section 1.1.
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.	**Implemented.** General principles for reviewing epidemiologic and experimental animal studies are described in Section 4 of the Preamble.

Table D-1. National Research Council recommendations that EPA is implementing in the short term	Implementation status
Selection of Studies for Derivation of Reference Values and Unit Risks (p. 165) 17. Establish clear guidelines for study selection.	**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.
a. Balance strengths and weaknesses.	
b. Weigh human vs. experimental evidence.	
c. Determine whether combining estimates among studies is warranted.	
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.
20. Provide adequate documentation for conclusions and estimation of	**Implemented.** The new template structure that has been developed in response

Table D-1. National Research Council recommendations that EPA is implementing in the short term	Implementation status
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.	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 EPA is implementing in the long-term (p. # in NRC report)	**Implementation status**
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[4] 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 may hold additional workshops 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.

[4] EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

APPENDIX E. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND EPA'S DISPOSITION

To be added

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