Interim Ground Water Criterion Recommendation for 1,2,4-Trimethylbenzene

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Background

The New Jersey Department of Environmental Protection Site Remediation Program (NJDEP-SRP) requested that the NJDEP Office of Science (OS) develop an interim health-based ground water criterion for 1,2,4-trimethylbenzene (1,2,4-TMB) to assist in its site remediation work. OS conducted an online search of the published literature using the PubMed.gov database of the U.S. National Library of Medicine, of the National Institutes of Health, and the Hazardous Substances Data Bank of the Toxicology Data Network of the US National Library of Medicine. OS also obtained and reviewed additional relevant peer-reviewed publications and non-peer reviewed reports cited in the USEPA 1,2,4-Trimethylbenzene Provisional Peer Reviewed Toxicity Value document (USEPA, 20007a) and the USEPA IRIS Toxicological Review of Trimethylbenzenes External Review Draft (USEPA, 2012).

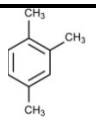
Summary

No oral data are available that can be used to derive a RfD for 1,2,4-TMB. An oral RfD for 1,2,4-TMB proposed in a draft IRIS assessment (USEPA, 2012) is based on route-to-route extrapolation from an inhalation study and depends on PBPK modeling that has not yet been peer reviewed. The available data do not indicate that 1,2,4-TMB is carcinogenic. It is concluded that data are not available to support an Interim Specific Ground Water Criterion for 1,2,4-TMB. The Interim Generic Ground Water Criterion for contaminants with no evidence of carcinogenicity of 100 µg/L is recommended.

Physical and Chemical Properties

1,3,5-Trimethylbenzene is a colorless, flammable liquid with a strong aromatic odor. The physical and chemical properties of 1,2,4-trimethylbenzene (Table 1; USEPA, 2012) are similar to those of the other TMB isomers (1,2,3-TMB and 1,3,5-TMB).

Table 1. *Physical and Chemical Properties of 1,2,4-Trimethylbenzene (USEPA, 2012)*

CAS Registry Number	**95-63-6**
Synonym(s)	pseudocumene, asymmetrical trimethylbenzene
Molecular formula	C_9H_{12}
Molecular weight	120.19
Chemical structure	
Melting point, °C	-43.8
Boiling point, °C @ 760 mm Hg	168.9
Vapor pressure, mm Hg @ 25°C	2.10
Density, g/mL at 20 °C	0.8758

Flashpoint, °C	44
Water solubility, mg/L at 25 °C	57
Other solubilities	ethanol, benzene, ethyl ether, acetone, petroleum ether
Henry's law constant, at mm^3/mol	6.16×10^{-3}
Log K_{OW}	3.78
Log K_{OC}	2.73
Bioconcentration factor	439
Odor Threshold (air)	0.4 ppm
Conversion factors	1 ppm = 4.92 mg/m^3 1 mg/m^3 = 0.2 ppm

Production and Use

1,2,4-Trimethylbenzene is a component of commercially available trimethylbenzene (TMB, CAS No. 25551-13-7) which consists of the three TMB isomers in various proportions. TMB is found in the C9 aromatic fraction (aromatic hydrocarbons with nine carbons) that is produced during petroleum refining. The C9 aromatic fraction contains about 40% 1,2,4-TMB (USEPA, 2012). In 1991, about 80 billion pounds (40 million tons) of the C9 fraction were produced in the U.S. (USEPA, 2012). Vehicle emissions are a source of TMBs in the environment, due to the widespread use of the C9 fraction in gasoline (USEPA, 2012). TMBs are also used as solvents, dyestuff intermediates, paint thinners, and as a UV oxidation stabilizer for plastics (USEPA, 2012).

Environmental Occurrence and Fate

In 2008, 5.8 million pounds (2,900 tons) of 1,2,4-TMB were released to the atmosphere and 265,000 pounds (132.5 tons) were released to surface waters, underground injection sites, or land (USEPA, 2012). Based on its physical and chemical properties, 1,2,4-TMB is expected to bind to soil and sediments and to volatize from soil and surface water. In the atmosphere, it exists in the vapor phase and degrades through reaction with photochemically-produced hydroxyl radicals and nitrate radicals, with half-lives for these reactions of about 12 hours and 6-30 days, respectively. Non-volatilized TMBs may biodegrade under aerobic conditions (HSDB, 2012). Based on its estimated bioconcentration factors (31-275), 1,2,4-TMB is not expected to be highly bioaccumulative.

Health Assessments, Guidelines, & Standards Developed by Other Agencies (USEPA, 2012)

USEPA (2007) Provisional Peer Reviewed Toxicity Values

1,2,4-TMB was assessed by the USEPA Provisional Peer Reviewed Toxicity Value (PPRTV) program in 2007. USEPA (2007) concluded that the available data were inadequate to derive an oral Reference Dose (RfD) for 1,2,4-TMB. Subchronic provisional Reference Concentrations (p-RfCs) of 0.07 mg/m^3 were developed based on the NOAEL for decreased clotting time in female rats and 0.1 mg/m^3 based on the NOAEL pulmonary toxicity in male rats in a 90 day inhalation study (Korsak et al., 2000, see below) . A chronic p-RfC of 0.007 mg/m^3 was developed by applying an uncertainty factor of 10 for subchronic-to-chronic extrapolation to the subchronic p-RfC for decreased clotting time. USEPA (2007) states that the confidence in the subchronic and chronic p-RfCs is low due to low confidence in the principal study and the overall database.

Draft USEPA (2012) IRIS Assessment

A draft IRIS assessment for 1,2,4-TMB and the other TMB isomers that was completed in June 2012 proposes an inhalation Reference Concentration (RfC) and an oral Reference Dose (RfD) for 1,2,4-TMB. According to information posted on the USEPA IRIS Track website as of 2/27/13, external peer review of this document will begin in the 3rd quarter of 2013. No schedule is provided for completion of external peer review or the subsequent steps in the IRIS process - final agency review/interagency science discussion and posting of final assessment.

The draft IRIS (USEPA, 2012) inhalation RfC for 1,2,4-TMB, 0.02 mg/m^3 is based on decreased pain sensitivity in a subchronic rat study (Korsak and Rydzyński, 1996). The draft IRIS document (USEPA, 2012) states that this effect was observed in multiple studies of acute, short-term, and subchronic durations. The RfC was derived through the following series of steps: First, a rat PBPK model (Hissink et al., 2007) was used to convert the external concentrations (in mg/m^3) from the animal study to an internal blood metric (weekly average venous 1,2,4-TMB concentration, in mg/L). Benchmark dose modeling was then performed on the dose-response data for decreased pain sensitivity in terms of the internal blood metric. A BMDL of 0.086 mg/L (in terms of weekly average venous 1,2,4-TMB concentration) was estimated based on a benchmark response (BMR) equal to a change in the mean of 1 standard deviation from the model estimated control mean. Next, the air concentration (human equivalent concentration, HEC), that would result in a human venous 1,2,4 TMB concentration at the BMDL (0.086 mg/L) was estimated using a human PBPK model (Hissink et al., 2007). This HEC, 15.8 mg/m^3, was then used as the point of departure (POD) for derivation of the RfC. A total uncertainty factor (UF) of 1,000 was applied to the HEC of 15.8 mg/m^3, including UFs of 3 for interspecies variability, 10 for interindividual variability, 10 for subchronic-to-chronic extrapolation, and 3 for database deficiencies (no two-generation reproductive/developmental toxicity or developmental neurotoxicity studies), resulting in a chronic RfC of 2×10^{-2} mg/m^3. The draft IRIS assessment (USEPA, 2012) states that confidence in the study which is the basis for the RfC (Korsak and Rydzyński, 1996) is medium, confidence in the database is low-to-medium because of the lack of chronic, multi-generation reproductive/developmental, and developmental neurotoxicity studies, and because the studies supporting the critical effect mostly are from the same research institute, and that the overall confidence in the RfC is low-to-medium.

The draft IRIS assessment (USEPA, 2012) states that, because only single dose (acute) oral studies of 1,2,4-TMB were located, the proposed RfD, 6×10^{-3} mg/kg/day, is based on route-to-route extrapolation from the inhalation RfC. A human PBPK model (Hissink et al., 2007) was modified by USEPA to include an oral compartment; this oral component of the PBPK model has not yet been peer reviewed. Constant oral ingestion and 100% absorption of 1,2,4-TMB via constant infusion rate into the liver were assumed, and the reduction of the blood concentration from oral exposure as compared to an equivalent inhalation dose due to hepatic first-pass metabolism was considered. The PBPK model was used to estimate the oral dose, 6.3 mg/kg/day, which would result in the blood concentration at the POD used in the RfC, 0.086 mg/L (above). The same composite UF of 1,000 used for derivation of the RfC (above) was applied to derive the RfD of 6×10^{-3} mg/kg/day. The draft IRIS document (USEPA, 2012) states that, in addition to the medium confidence in the critical study and the low-to-medium confidence in the database discussed above for the RfC, there is additional uncertainty regarding the RfD related to

the use of the PBPK model for route-to-route extrapolation. It is stated that the overall confidence in the RfD for 1,2,4-TMB is low.

The draft IRIS assessment (USEPA, 2012) states that there is "inadequate information to assess carcinogenic potential" of 1,2,4-TMB. As discussed in more detail below, there is no information about the carcinogenicity of 1,2,4-TMB in humans, and there were no statitistically significant carcinogenic effects in the only chronic study of 1,2,4-TMB (Maltoni et al., 1997). Additionally, based on the only study of genotoxicity of 1,2,4-TMB (Janik-Spiechowicz et al.,1998), there is inadequate evidence to conclude that this compound is genotoxic (USEPA, 2012).

Other Air Guidelines

The NIOSH Recommended Exposure Limit (REL) for TMBs is a time weighted average of 25 ppm (123 mg/m^3) 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 (NIOSH, 1992). Similarly, the ACGIH (2002) Threshold Limit Value (TLV) for VOC mixtures containing 1,2,4-TMB and 1,3,5-TMB is a time weighted average of 25 ppm (123 mg/m^3) time weighted average for an 8 hour work day and a 40-hour work week, based on the risk of irritation and central nervous system effects. Both of these levels are based on Battig et al. (1956).

Acute Exposure Guideline Levels (AEGLs) for emergency exposure situations have been developed for 1,2,4-TMB (USEPA, 2007). The Level 1 AEGLs for nondisabling effects range from 180 ppm (890 mg/m^3) for 10 minute exposures to 45 ppm (220 mg/m^3) for 8 hour exposures, and the Level 2 AEGLs for disabling effects range from 460 ppm (2300 mg/m^3) for 10 minute exposures to 150 ppm (740 mg/m^3) for 8 hour exposures.

The Ontario Ministry of the Environment (MOE, 2006) has developed a 24 hour Ambient Air Quality Criterion (AAQC) for TMBs of 0.3 mg/m^3 and a 30 minute Point of Impingement (POI) level of 0.9 mg/m^3, both based on CNS effects.

Pharmacokinetics and Metabolism

Absorption and Distribution

A single oral dose of ^{14}C-labelled 1,2,4-TMB in rats was rapidly absorbed and widely distributed throughout the body (Huo et al., 1989). Levels of radioactivity were higher in adipose tissue than in other organs or tissues examined. Excretion was rapid, with > 99% of the administered radioactivity recovered in the urine during the first 24 hours. 1,2,4-TMB is also readily absorbed after inhalation exposure. The respiratory uptake is similar in humans and rats (60 ± 3% and 44– 50%, respectively) (Järnberg et al., 1996; Dahl et al., 1988).

After 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.

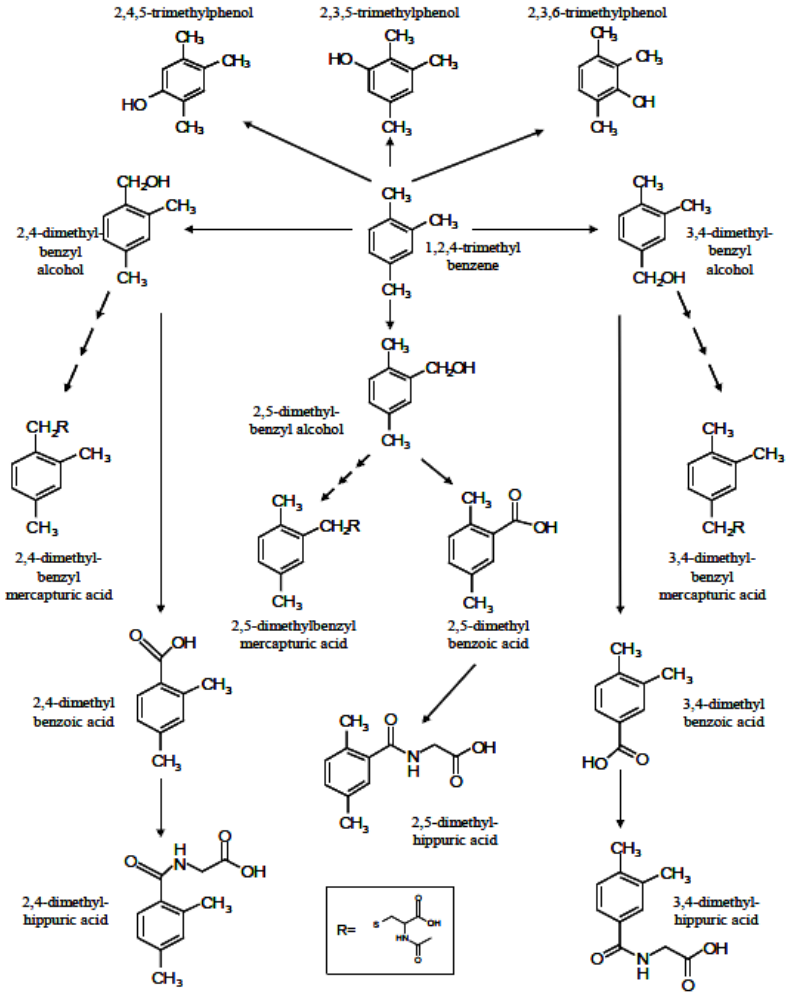
Metabolism

The major metabolic pathways of 1,2,4-TMB are similar in experimental animals and humans after inhalation or oral exposure, and are shown in Figure 1 (USEPA, 2012). 1,2,4-TMB is

oxidized to trimethyl phenols, and to dimethyl benzyl alcohols which can be further oxidized to dimethyl benzoic acids. The dimethyl benzoic acids are conjugated with glycine to form hippuric acids, and the dimethyl benzyl alcohols are conjugated with glutathione and further metabolized to mercapturic acids. Formation of glucuronide and sulfate conjugates has also been reported.

Internal concentrations of 1,2,4-TMB were significantly lower in repeatedly exposed animals than in animals exposed only once to higher concentrations (Swiercz et al., 2003; Swiercz et al., 2002; Zahlsen et al.,1990). Cytochrome P-450 enzymes that metabolize 1,3,5-TMB were induced in the livers, kidneys, and lungs of rats exposed to 1,200 mg/kg/day for 3 days (Pyykko, 1980), and it has been hypothesized that the lower levels of 1,2,4-TMB after repeated exposures are also due to induction of metabolic enzymes.

Figure 1. *Metabolic Pathways of 1,2,4-Trimethylbenzene (USEPA, 2012)*



Excretion

In humans (n=10) exposed to 1,2,4-TMB at a concentration of 25 ppm (123 mg/m^3) by inhalation for 2 hours, there were four phases of elimination from the blood, with half-lives of 1.3 ± 0.8 min, 21 ± 5 min, 3.6 ± 1.1 hr, and 87 ± 27 hr, respectively (Jarnberg et al, 1996). A

considerable portion of the absorbed dose (20–37%) was exhaled, while very little was excreted unchanged in the urine (<0.002%) (Janasik et al., 2008; Järnberg et al., 1997). In rats exposed to 25 ppm (123 mg/m^3) 1,2,4-TMB for 6 hours, the terminal half-life of elimination was 3.6 hours (Swiercz et al., 2006; Swiercz et al., 2002). As dose increased, the half-life increased to 17.3 hours.

Toxicity

Human Studies

Neurological effects and other health effects have been reported in workers exposed to mixtures of 1,2,4-trimethylbenzene, other trimethylbenzene isomers, and other volatile organic compounds (reviewed in USEPA, 2012). These effects include self-reported vertigo, dizziness, and drowsiness during work, asthmatic bronchitis, anemia, and increased clotting time/tendency to hemorrhage (Baettig et al., 1958); increased prevalence of neuropsychological symptoms correlating with exposure duration (Chen et al., 1999); decreased performance in computer-based tests of neurobehavioral function (Lee et al., 2005); symptoms including abnormal fatigue, reduced appetite, and eye and throat irritation (Norseth et al., 1991). In a study of residential exposure, air concentration to 1,2,4-TMB was associated with asthma (Billionett et al., 2011). Some deficits in performance in tests designed to assess attention were observed in human volunteers during 4 hours exposures to 57 or 570 mg/m^3 white spirit, a complex mixture containing 1,2,4-TMB and other compounds (Lammers et al., 2007). However, no effects on measures of overt CNS depression (heart rate and pulmonary ventilation) or subjective rating of CNS symptoms (i.e., headache, fatigue, nausea, dizziness, and intoxication) were found in volunteers exposed to 11 or 123 mg/m^3 for 2 hours (Järnberg et al., 1996). Because these observations reflect mixed exposures to other trimethyl benzenes as well as other volatile chemicals, they cannot be used as the basis for quantitative risk assessment for 1,2,4-TMB.

Animal Studies

Acute

Oral

Effects of a single oral gavage dose of 1,2,4-TMB in olive oil (0, 240, 960, or 3840 mg/kg) on electrocortical arousal in WAG/Rij rats (6 per group) were evaluated by measuring the number and duration of high voltage spindle episodes seen in electrocardiograms 20, 40, and 60 minutes after dosing (Tomas et al., 2000). Changes in these parameters were observed at all dose levels.

In a study of the acute effects of 1,2,4-TMB on locomotor activity, WAG/Rij rats (10 per group) were dosed by oral gavage with a single dose of 0, 960, 1920, or 3850 mg/kg in olive oil (Tomas et al., 1999). Significant effects on locomotor activity as assessed by an open field test were seen at the highest dose, but not the two lower doses.

Inhalation

In an acute neurotoxicity study, male Wistar rats (10/group) were exposed to concentrations of 250-2000 ppm (1227-9816 mg/m^3) 1,2,4-TMB (>97% pure) for 4 hours (Korsak et al., 1995; Korsak and Rydzyński,1996). 1,2,4-TMB caused concentration-related impairment in a rotarod

performance test (EC50 = 4693 mg/m^3) and concentration-related decreased pain sensitivity (as measured by increased paw-lick response latency; EC_{50} = 5682 mg/m^3).

Korsak et al. (1997) exposed Balb/C male mice (8-10/group) to 1,2,4-TMB (97% pure) concentrations of 253 to 1591 ppm (1926-9453 mg/m^3) by inhalation for 6 minutes and evaluated respiratory effects. 1,2,4-TMB caused respiratory irritation and concentration-dependent decreases in respiratory rate. The concentration that reduced the respiratory rate by 50% was 519 ppm (2547 mg/m^3).

Subacute/Subchronic

Oral

Limited data are available on subchronic oral effects of 1,2,4-TMB. Borriston Laboratories (1984) studied the potential for 1,2,4-trimethylbenzene to cause nephrotoxicity. This study was not considered in the draft EPA (2012) IRIS assessment of 1,2,4-TMB because it was not peer-reviewed. Male Fischer-344 rats (10 per group) were dosed by gavage with 0.5 or 2.0 g/kg neat 1,2,4-trimethylbenzene, 5 days/week for 4 weeks; the duration-adjusted doses were 357 and 1429 mg/kg-day, respectively. Controls were gavaged with saline. Gross necropsy was performed on all rats, but histopathology was examined only in the kidneys. Mortality during the treatment period was 0/10, 1/10, and 10/10, in the control, low dose, and high dose groups, respectively, with deaths in the high-dose group beginning on the third day of treatment. Final body weights and absolute kidney weights of low-dose rats were not significantly different than controls. In low-dose rats, speckled cortical surfaces in the kidneys and white gelatinous material inside the urinary bladders were found. In the high dose rats, mottled and red thymus, spotty kidney and liver surfaces, enlarged adrenals, gas filled and yellow intestines and lung congestion were observed. Notwithstanding histopathological changes in both low and high-dose rats, 1,2,4-Trimethylbenzene did not significantly increase the incidence or severity of nephropathy *per se*, as assessed by the incidence of hyaline droplet changes, regenerative epithelium, and tubular dilation with granular materials relative to controls. However, the authors state that it is possible that high-dose rats died before nephropathy could develop.

Inhalation

In an unpublished study by IBT (1981), groups of 5 male and 5 female COBS rats were exposed by inhalation to 49 or 480 mg/m^3 MCS-1809 6 hours/day, 5 days/week for 4 weeks. MCS-1809 is a mixture containing 75% 1,2,4-trimethylbenzene and 25% C9 aromatics (Monsanto, 1992). This study was not considered by EPA IRIS because it was not peer-reviewed.

The following parameters were assessed: daily observations, weekly body-weight measurements, organ weights (adrenal glands, brain, gonads, heart, kidneys, liver, lungs, spleen and thyroid gland), gross necropsy and histopathological examination of adrenal glands, brain, bronchi, gonads, heart, kidneys, liver, lungs, pancreas, pituitary glands, lymph nodes, spleen, trachea and thyroid gland of the control and high dose groups. Tissues from the low dose group were examined if significant findings were found in the high dose group.

Exposure to MCS-1809 did not result in deaths in this study. Clinical signs of toxicity in the low dose group included hypoactivity and ruffed fur were observed. More severe clinical signs were observed in the high dose group including ataxia and hypoactivity that persisted between exposures, ptosis (drooping of the upper eyelid), red ocular discharge, and ruffed fur. Body weight gain was not affected in the low dose group, and was significantly decreased by 35% in

males only in the high dose group. Relative liver weight was significantly increased in females in both dose groups, and relative spleen weight was increased in females in the high dose group. The only histological changes found were focal or diffuse testicular atrophy in 3/5 high dose males; testis weight was not significantly changed. Testicular effects were not observed in low dose or control groups.

Korsak et al. (1997) evaluated the effects of 1,2,4-TMB on parameters related to respiratory irritation in male Wistar rats (10/group) exposed by inhalation to 0, 25, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) for 90 days (6 hours/day, 5 days/week). 1,2,4-TMB exposure did not cause mortality or significant effects on final body weight. Cells were isolated by centrifugation from bronchoalveolar lavage (BAL) fluid collected 24 hours post-exposure. Total cell and macrophage numbers in BAL were significantly increased in all treated groups compared to controls. Significant increases were also observed in total protein, lactate dehydrogenase (LDH) and acid phosphatase (AP) in the BAL fluid supernatant from all treated groups. These effects were either at or near their highest observed response in the low-dose group, and further concentration-related increases were not observed. It should be noted that these respiratory system effects resulting from inhalation exposure are not relevant endpoints for risk assessment of oral exposure to 1,2,4-TMB.

In a subchronic inhalation study of 1,2,4-TMB (Korsak et al., 2000), male and female outbred Imp:WIST rats (10/sex/group; 20/sex/group in the high dose group) were exposed to target concentrations of 0, 25, 100 or 250 ppm for 6 hours/day, 5 days/week for 3 months. Blood was drawn for hematological examination prior to initiation of exposures and 1 week prior to exposure termination. Clinical chemistry testing was performed at the end of the 3-month exposure period. Histopathological examinations were performed on tissues from brain, nose, larynx, trachea, thymus, lungs, heart, liver, spleen, kidney, adrenals, thyroid, pancreas, gonads, urinary bladder, stomach, duodenum, small and large intestines and salivary glands.
There were no significant clinical or effects on food consumption or body weights. The absolute and relative weights of lungs, liver, spleen, kidneys, adrenals, heart and gonads were evaluated. A few differences in organ weights occurred in a single dose group and did not appear to be treatment-related. In blood samples taken 1 week before exposure ended, there were concentration-related trends ($p<0.01$) for decreased numbers of red blood cells and increased numbers of white blood cells in males only, and red and white blood cell counts were significantly different ($p<0.01$) compared to controls in the high dose males. There was a significant trend ($p<0.01$) for concentration-related decreases in reticulocyte count and clotting time in females. Reticuloctye counts and clotting time significantly differed from controls ($p<0.05$) in the high dose and mid- and high dose females, respectively. The only notable clinical chemistry change was a significant increase in serum sorbitol dehydrogenase in all exposed groups of male rats; this effect was not concentration related. Histopathological changes were seen only in the lungs. These included increased severity of pulmonary lesions, including increased proliferation of peribronchial lymphatic tissue only in mid-dose males, increased alveolar macrophages in high dose-males, and increased interstitial lymphocytic infiltrations in mid-dose males and high-dose females. As above, these respiratory system effects resulting from inhalation exposure are not relevant endpoints for risk assessment of oral exposure to 1,2,4-TMB.

Neurobehavioral

In a subchronic study that assessed neurobehavioral effects by rotarod performance and hot-plate behavior (a measure of pain sensitivity), male Wistar rats (10 per group) were exposed to 0, 25, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) 1,2,4-TMB for 6 hours/day, 5 days/week for 3 months (Korsak and Rydzyński, 1996). Body weight was not significantly affected by 1,2,4-TMB, and there were no clinical signs of toxicity. Neurotoxicity was assessed. Rotarod performance was tested prior to start of the study, weekly during exposure, and 2 weeks after the termination of the exposure. The rotarod performance failure rate in control rats was 0% throughout the study period. Rotarod performance failure increased with 1,2,4-TMB concentration, and also increased over time in the two highest dose groups. However, this effects was statistically significant only in the highest exposure group after 8 or 13 weeks of exposure (40% failure; $p<0.05$). The failure rate in the highest exposure group remained at 30% after a 2-week recovery period; data for the other groups are not shown. Hot-plate behavior was tested immediately after the 3 month exposure ended. Pain sensitivity (as assessed by increased latency of the paw-lick response) was also decreased in all dose groups in a concentration dependent manner and was statistically significant in the two highest dose groups After a 2-week recovery period, pain sensitivity in the high dose group was no longer significantly different than in controls..

In a study of persistent behavioral effects of 1,2,4-TMB, Gralewicz et al. (1997a) exposed male Wistar rats (15/group) to 0, 50, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks. Animals were subjected to the following sequence of behavioral testing:

1. radial maze (test of short-term working memory): 2 weeks before exposure and on days 14-18 after exposure,
2. open field activity (test of spontaneous activity): day 25 after exposure,
3. passive avoidance days 35-45 after exposure,
4. hot-plate test (pain avoidance after foot shock): days 50 and 51 after exposure,
5. active avoidance: (test of long-term memory and learning ability): day 54 after exposure.

1,2,4-TMB did not affect body weight or cause clinical signs of toxicity. In post-exposure testing, 1,2,4-TMB did not cause significant effects in the active avoidance or radial maze tests. Passive-avoidance learning 35-45 days post-exposure was significantly ($p<0.001$) decreased in the mid- and high-dose, with a greater effect in the mid-dose group. In the hot-plate test following foot shock 50 days post-exposure, paw-lick latency time was significantly increased in the mid- and high-dose groups. In the open field test 25 days post-exposure, grooming behavior was increased in all 3 dose groups, and this effect was significant in the mid-dose group, but there was no significant effect on spontaneous movement or on rearing behavior. The results of this study suggest that 4-week exposures to 1,2,4-TMB at concentrations that did not cause overt clinical signs of toxicity can produce long-term effects on the functional state of the rat central nervous system. In this study, the NOAEL for persistent behavioral effects was 123 mg/m^3 and the LOAEL was 491 mg/m^3.

Gralewicz and Wiaderna (2001) studied the persistent behavioral effects of trimethylbenzene isomers and *m*-xylene using a protocol similar to that of Gralewicz et al. (1997a). For 1,2,4-TMB, 5 month old male Wistar rats (n=11) were exposed to 100 ppm (491 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks. The control group consisted of 10 rats exposed to air only. The sequence of behavioral testing was similar to that of Gralewicz et al. (1997a), as follows:

1. radial maze: 1 week before exposure and on days 14-18 after exposure,

2. open field activity: day 8 before exposure and day 25 after exposure,
3. passive avoidance: days 39-48 after exposure,
4. hot-plate test: days 50 and 51 after exposure,
5. active avoidance: days 54 and 60 after exposure.

There was no significant effect on body weight. As in Gralewicz et al. (1997a), 1,2,4-TMB did not affect short-term working memory (radial arm maze test), but caused the following effects: significantly increased spontaneous locomotor activity in the open field test, impaired passive avoidance learning, significantly longer paw-lick latencies in the hot-plate test 24 hours after foot shock, and impaired acquisition, but not retention, of the two-way active avoidance response. In general, these results are consistent with the findings of Gralewicz et al. (1997a).

Gralewicz et al. (1997b) studied the effects of 1,2,4-TMB on the occurrence of spike-wave discharges (SWD) in the neurocortex. The authors hypothesized that exposure to neurotoxic solvents such as 1,2,4-TMB could accelerate the aging process in the brain. They investigated the effects of 1,2,4-TMB on SWD, since these brain discharges increase in number and/or duration with age. Male Wistar rats (9-10/group) were exposed by inhalation to 0, 25, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) 1,2,4-TMB (purity not stated) for 6 hours/day, 5 days /week for 4 weeks. Electrodes were implanted into the fronto-parietal cortex and the dorsal hippocampus, and 1 hour EEG recordings were performed immediately prior to exposure, at the end of the exposure period, and 1 month and 3 months post-exposure. 1,2,4-TMB did not cause statistically significant effects on body weight. A similar pattern of change in the duration of SWD over time was observed in the control and low dose groups, with duration of SWD increasing over time, with a significantly greater duration compared to the pre-exposure level 3 months after exposure. In contrast, the duration of SWD tended to decrease over time in the mid- and high-exposure groups, and this effect was statistically significant compared to pre-exposure levels in the mid-exposure group 1 month post-exposure. A similar trend was seen when the number of SWD bursts per hour was determined. As was the case for duration of SWD, the frequency of SWD bursts increased similarly with age in the control and low exposure groups and tended to decline with time in the mid- and high-exposure groups. However, this effect was significantly different from the pre-exposure measure only in the high dose group 3 months after exposure. The results suggest that 1,2,4-TMB can cause persistent effects on brain activity.

Finally, Lutz et al. (2010) evaluated persistent effects of 1,2,4-TMB on spontaneous locomotor behavior in rats, as well as the persistent effects of 1,2,4-TMB exposure on changes in spontaneous locomotor behavior in response to an amphetamine challenge before and after amphetamine sensitization. Male Wistar rats (6-8 per group) were exposed to 0, 15, 100, or 150 ppm 1,2,4-TMB by inhalation, 6 hours/day, 5 days/week, for 4 weeks. The tests of locomotor behavior began two weeks post-exposure, long after 1,2,4-TMB had been eliminated from the body. Spontaneous locomotor behavior was assessed under baseline conditions, after a saline injection, and after an amphetamine challenge (0.5 mg/kg). Starting one day after the behavioral testing, the rats were sensitized to amphetamine by injection of 2.5 mg/kg/day amphetamine for 5 days. The behavioral testing protocol (baseline, after saline injection, and after 0.5 mg/kg amphetamine challenge) was repeated 3 weeks after the amphetamine sensitization was completed.

Two weeks post-exposure to 1,2,4-TMB, spontaneous locomotor behavior was increased in all three previously exposed groups compared to controls. This increase was significant only in the

high dose (250 ppm) group. The effects of 1,2,4-TMB on spontaneous locomotor behavior after the amphetamine challenge was non-monotonic. After the challenge, the increased response in locomotor behavior in response to amphetamine was greater in the low and high dose (25 and 250 ppm) 1,2,4-TMB groups than in the control group, while the response to amphetamine was decreased in the mid-dose (100 ppm) group compared to the control group. After sensitization to amphetamine, the amphetamine challenge caused a similar increase in locomotor behavior in the control, low, and high dose groups, but had no effect on locomotor behavior in the mid dose group.

In summary, 1,2,4-TMB alone caused persistent increases in spontaneous locomotor behavior in rats. 1,2,4-TMB also caused persistent effects on behavioral response to amphetamine challenge and amphetamine sensitization in rats; these effects followed a non-montonic dose-response within the dose range tested.

Reproductive and Developmental

In a study of the developmental toxicity of 1,2,4-TMB, pregnant female Sprague-Dawley rats (24/group) were exposed by inhalation to 0, 100, 300, 600 or 900 ppm (0, 491, 1475, 2950 or 4425 mg/m^3) for 6 hours/day on gestation days 6 - 20. The dams were sacrificed for necropsy on gestation day 21 (Saillenfait et al., 2005). During necropsy, the uterus was weighed and numbers of corpora lutea, implantation sites, resorptions and dead and live fetuses were recorded. Live fetuses were weighed, sexed and examined for external anomalies. Visceral examination was performed on half of the live fetuses from each litter and skeletal examination was conducted on the other half. No deaths occurred prior to necropsy and no clinical signs of toxicity were observed. Maternal food consumption was significantly decreased in the two highest treatment groups, by approximately 12-14% and 15-19%, respectively, relative to controls. Body weight gain was significantly reduced in the 600 ppm group only during the first week of exposure, but was significantly decreased in the highest (900 ppm) dose group (22-52% lower than controls) throughout the exposure period. At necropsy on gestation day 21, mean body weight gain (corrected for gravid uterine weight) was significantly depressed in both 600- and 900-ppm dams (approximately 50% lower than controls). Mean fetal body weight was significantly lower in both 600- and 900-ppm exposure groups (approximately 5 and 11% lower, respectively, than controls). These fetal effects occurred only at doses which significantly decreased maternal body weight gain and were likely secondary to maternal toxicity.

Chronic/Carcinogenicity

Only one chronic study has been conducted on 1,2,4-TMB. In a chronic oral study, Sprague-Dawley rats (50/sex/group) (Maltoni et al., 1997) were dosed with olive oil (controls) or 800 mg/kg-day of 1,2,4-TMB in olive oil starting at 7 weeks of age for 4 days/week for 104 weeks, until the animals were 111 weeks old. The animals were then kept under observation until natural death. Food and water consumption and body weight data are not reported. It was reported that there was "intermediate" reduction of survival in male rats and a "slight" reduction in females dosed with 1,2,4-TMB, but quantitative information on survival was not reported. It was also reported that the percent of animals with malignant tumors, and benign plus malignant tumors, was similar in control and treated groups, but the number of malignant tumors per 100 animals was slightly higher in the 1,2,4-TMB treated animals (33 tumors per 100 animals) than in controls (24 tumors per 100 animals), with similar data for males and females. The number of head cancers (total of zymbal gland, ear duct, nasal cavity, and oral cavity tumors) was higher in the 50 treated males (10) than in the 50 controls (2).

Neuroesthesioepitheliomas occurred in 1 treated male and 2 treated females, while none were seen in controls. This tumor type, which arises from the olfactory neuroepithelium, was reported to be "quite rare" in the rat colony used in this experiment. No tests of statistical significance were reported by Maltoni et al. (1997) for these data. However, EPA (2007a) reported the Fisher's exact test showed that the increase in incidence of neuroesthesioepitheliomas was not statistically significant at the $p<0.05$ level. As discussed above, the draft IRIS assessment (USEPA, 2012) concludes that there is "inadequate information to assess carcinogenic potential" of 1,2,4-TMB.

Mutagenicity and Genotoxicity

Limited genotoxicity data suggest that 1,2,4-trimethylbenzene is not mutagenic. 1,2,4-trimethylbenzene gave negative results in the Ames test in *Salmonella typhimurium* strains TA97a, TA98, TA100 and TA102 both in the presence and absence of rat liver S9 metabolic activation (Janik-Spiechowicz et al., 1998). 1,2,4-Trimethylbenzene was negative in the mouse micronucleus test, but was positive in sister chromatid exchange (SCE) tests of bone marrow cells of Imp:Balb/c mice treated *in vivo* (Janik-Spiechowicz et al., 1998). According to the draft IRIS assessment (USEPA, 2012), " increased frequency of SCEs indicates that DNA damage has occurred as a result of exposure to these isomers, but it does not provide a specific indication of mutagenic potential, as there is no known mechanistic association between SCE induction and a transmissible genotoxic effect."

Recommendation of Interim Generic Ground Water Criterion

As discussed above, there are no oral data that can be used to derive a RfD for 1,2,4-TMB. Available data suggest that systemic toxicity of TMBs is similar for oral and inhalation exposures. The oral RfD for 1,2,4-TMB proposed in the draft IRIS assessment (USEPA, 2012) is based on route-to-route extrapolation from an inhalation study that is the basis for the proposed RfC for 1,2,4-TMB. A published PBPK model for extrapolation of rat-to-human inhalation exposures of 1,2,4-TMB was used in the development of the proposed RfC. This model was modified by USEPA to include an oral component which has not yet been peer reviewed; the oral component was used in the development of the proposed RfD. For this and other reasons, USEPA (2012) states that the confidence in the proposed RfD is low.

The available data do not indicate that 1,2,4-TMB is carcinogenic, and USEPA (2012) does not propose an IRIS assessment based on carcinogenicity.

If an Interim Specific Ground Water Criterion were to be developed for 1,2,4-TMB, it would need to be based on route-to-route extrapolation from inhalation studies. The oral component of the PBPK model used by USEPA (2012) for this extrapolation has not yet been peer reviewed, and USEPA states that its confidence in its proposed RfD is low. As discussed above, the USEPA (2012) draft IRIS assessment must undergo several more steps of internal and external review, and it could be significantly revised before it is finalized.
For the reasons discussed above, it is concluded that data are not available to support an Interim Specific Ground Water Criterion for 1,2,4-TMB. The Interim Generic Ground Water Criterion for contaminants with no evidence of carcinogenicity of 100 µg/L is recommended.

For purposes of comparison, the health-based ground water concentration based on the RfD of 6×10^{-3} mg/kg/day that is proposed in the draft IRIS assessment (USEPA, 2012), using default exposure assumptions of 70 kg body weight, 2 L/day water consumptions, and 0.2 Relative

Source Contribution factor, would be 40 µg/L. This is quite close to the Interim Generic Ground Water Criterion of 100 µg/L when considered in the context of the high level of uncertainty associated with its derivation. The fact that the RfD-based (40 µg/L) value is within 3-fold of the generic value (100 µg/L) supports the conclusion that the Interim Generic Criterion is sufficiently health protective.

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