United States
Environmental Protection
Agency

EPA/690/R-07/037F
Final
6-11-2007

Provisional Peer Reviewed Toxicity Values for

1,2,4-Trimethylbenzene
(CASRN 95-63-6)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 1,2,4-TRIMETHYLBENZENE (CASRN 95-63-6)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and

circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Neither a reference dose (RfD) nor a reference concentration (RfC) are available for 1,2,4-trimethylbenzene in the Integrated Risk Information System (IRIS) database (U.S. EPA, 2007) or the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997). There is no Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile on 1,2,4-trimethylbenzene, other trimethylbenzene isomers, or mixtures of trimethylbenzene isomers (ATSDR, 2006). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994a) and the HEAST (U.S. EPA, 1997) list a Health and Environmental Assessment (HEA) for trimethylbenzenes (U.S. EPA, 1987a); however, the available toxicity data were considered inadequate for quantitative risk assessment (U.S. EPA, 1997). The CARA (U.S. EPA, 1991, 1994a) lists a Drinking Water Health Advisory for 1,2,4-trimethylbenzene (U.S. EPA, 1987b). Because available human and animal toxicity data were considered inadequate for longer-term and lifetime quantitative risk assessment, the U.S. EPA (1987b) derived an RfD of 0.64 mg/kg-day for 1,2,4-trimethylbenzene based on assumptions that the Threshold Limit Value (TLV) of 25 ppm (125 mg/cu.m) for mixed trimethylbenzenes recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 2001, 2005) represents a NOAEL for 1,2,4-trimethylbenzene and that exposure results in 50% absorption. The National Institute of Occupational Safety and Health (NIOSH) adopted a recommended exposure limit (REL) time-weighted average (TWA) of 25 ppm (123 mg/m^3) for 1,2,4-trimethylbenzene (NIOSH, 2006). The Occupational Safety and Health Administration (OSHA) has not adopted a permissible exposure limit (PEL) for 1,2,4-trimethylbenzene (OSHA, 2006). Health assessments for 1,2,4-trimethylbenzene are not available from other major sources, including CalEPA (2006), the

National Toxicology Program (NTP, 2006), the World Health Organization (WHO, 2006), and the International Agency for Research on Cancer (IARC, 2006).

A Group D (not classifiable as to human carcinogenicity) cancer classification is included in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). A cancer assessment for 1,2,4-trimethylbenzene is not available on IRIS (U.S. EPA, 2007) or the HEAST (U.S. EPA, 1997). A cancer assessment for 1,2,4-trimethylbenzene is not available from CalEPA (2006), the National Toxicology Program (NTP, 2006), the World Health Organization (WHO, 2006), or the International Agency for Research on Cancer (IARC, 2006). Occupational exposure limits for 1,2,4-trimethylbenzene listed by NIOSH (2006) include no cancer notation.

Literature searches were performed to identify relevant information for 1,2,4-trimethylbenzene for the years 1986-1998 in the databases HSDB, RTECS, TSCATS, MEDLINE, and TOXLINE. Update literature searches were conducted in TOXLINE, MEDLINE (plus PubMed cancer subset), and DART/ETICBACK for the time period including January, 1998 to December, 2005. Update search of the TOXCENTER database was performed for the time period of August, 2000 to December, 2005. Databases searched without date limitations in December, 2005, included TSCATS, RTECS, GENETOX, HSDB and CCRIS. Search of Current Contents encompassed July to December, 2005.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure

No data were located regarding the oral toxicity or carcinogenicity of 1,2,4-trimethylbenzene in humans.

Inhalation Exposure

Data regarding the inhalation toxicity of 1,2,4-trimethylbenzene in humans come from an occupational exposure study in which workers were exposed to a mixture of trimethylbenzene isomers. Bättig et al. (1958) examined 27 workers exposed to Fleet-X DV 99 solvent in the painting shop of a Swiss transportation plant. The solvent was analyzed spectrographically and was found to consist primarily of aromatic hydrocarbons (97.5%) and paraffinic and naphthenic hydrocarbons (2.5%). The aromatic hydrocarbon portion was composed of 1,2,4-trimethylbenzene (>50%), 1,3,5-trimethylbenzene (>30%), and possibly included 1,2,3-trimethylbenzene, 1-methyl-2-ethyl benzene, 1-methyl-3-ethyl benzene, and 1-methyl-4-ethyl benzene. Based on analysis of air samples collected from the plant, the concentration of the solvent was roughly estimated at 10-60 ppm (49-295 mg/m^3). The control group consisted of 10 unskilled workers employed in a different section of the plant. Although the authors stated that the Fleet-X DV 99 solvent was used for "a period of some ten years," the average exposure duration of the workers was not reported. The workers reported CNS symptoms (vertigo, headaches and drowsiness) more often than the control group (70% versus 30% in the controls).

Chronic asthma-like bronchitis (30% of workers versus 10% of controls), anemia [defined as < 4.5 million erythrocytes/mm^3 and usually combined with normal hemoglobin] (52% versus 20%) and alterations in blood clotting (30% versus 10%) were also observed in the exposed workers. The incidence of CNS symptoms was statistically significantly higher in the exposed workers than in the control group (Fisher's exact test conducted for this assessment; $p<0.05$). For the other effects, the incidences did not significantly differ between the groups. A higher incidence of vitamin C deficiency was observed in the control group, suggesting that the two groups may not have been matched for socioeconomic status. If the assumption is made that the solvent exclusively contained trimethylbenzene isomers, then this study identifies a LOAEL in the range of 10-60 ppm (49-295 mg/m^3) for signs of neurotoxicity.

Animal Studies

Oral Exposure

The database of repeated oral exposure studies in animals for 1,2,4-trimethylbenzene is limited to a 4-week study (Borriston Laboratories, 1984) and a chronic exposure carcinogenicity study (Maltoni et al., 1997). No oral developmental or reproductive toxicity studies were located for 1,2,4-trimethylbenzene.

The primary focus of the Borriston Laboratories (1984) study was the ability of 1,2,4-trimethylbenzene to induce nephrotoxicity. In this study, groups of 10 male Fischer-344 rats were administered doses of 0.5 or 2.0 g/kg neat 1,2,4-trimethylbenzene by gavage 5 days/week for 4 weeks; the duration-adjusted doses were 357 and 1429 mg/kg-day, respectively. A group of rats serving as controls were gavaged with saline. Gross necropsy was conducted in all rats, but only the kidneys underwent histopathologic examination. Mortality rates during treatment in the control, low-, and high-dose groups were 0/10, 1/10, and 10/10, respectively. Deaths in the high-dose group occurred as early as the third day of treatment. Final body weight and absolute kidney weight of low-dose rats were not significantly different than controls. Gross necropsy findings in low-dose animals included speckled cortical surfaces in the kidneys and white gelatinous material inside the urinary bladders. High-dose rats exhibited mottled and red thymus, spotty kidney and liver surfaces, enlarged adrenals, gas filled and yellow intestines and lung congestion. The presence or absence of hydrocarbon nephropathy was determined by examining the incidence of hyaline droplet changes, regenerative epithelium and tubular dilation with granular material. Treatment with 1,2,4-trimethylbenzene did not significantly increase the incidence or severity of nephropathy relative to controls; however, according to the authors, it is possible that high-dose rats died before nephropathy could develop. A NOAEL or LOAEL could not be determined due to the limited scope of the study, although the high dose of 1429 mg/kg-day was clearly a frank effects level (FEL) for mortality.

Maltoni et al. (1997) investigated the carcinogenicity of 1,2,4-trimethylbenzene (99% pure) in a long-term oral exposure experiment. Male and female Sprague-Dawley rats (50/sex/group) received doses of either 0 or 800 mg/kg (4 days/week) of 1,2,4-trimethylbenzene by gavage in 1 ml olive oil for 104 weeks. Food and water consumption and body weights were recorded throughout the experiment. Upon death or terminal sacrifice at 123 weeks, the animals were subjected to systemic necropsy. Histopathology was performed on brain, pituitary gland,

Zymbal glands, salivary glands, Harderian glands, head, tongue, thymus, mediastinal lymph nodes, lung, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, esophagus, stomach, intestine (four levels), bladder, prostrate, uterus, vagina, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes, sternum, femur, spinal cord and any other organs and tissues with pathological lesions. No statistical analysis of the data was presented.

"Slight" reduction in the survival of the female Sprague-Dawley rats and an "intermediate" reduction in the survival of male rats were reported (Maltoni et al., 1997). However, quantitative survival data were not presented in the report and no statistical analysis of the decreases in survival were presented. Although the study report indicated that food and water consumption and body weight data were recorded, these data were not included in the report. There was no significant increase in the incidence of animals bearing either malignant or benign + malignant tumors (Table 1). Fisher's exact tests conducted for EPA indicated that the differences in total tumors between the exposed and treated animals were not statistically significant ($p<0.05$). Neuroesthesioepitheliomas were observed in the nasal cavity of 3/100 exposed animals (M + F). This tumor was not seen in concurrent controls, and a Fisher's exact test of the data showed that the increase in incidence of neuroesthesioepitheliomas was not statistically significant ($p<0.05$). The authors, however, indicated that these tumors are quite rare in the colony of Sprague-Dawley rats used for these experiments and suggested that this finding presents some evidence for carcinogenicity of 1,2,4-trimethylbenzene.

Table 1. Incidences of Benign and Malignant Tumors in Male and Female Sprague-Dawley Rats after a Long-term (104 week) Oral Exposure to 1,2,4-Trimethylbenzene.[a]

Dose (mg/kg bw)[b]	Animals		Percent of animals with tumors	
	Sex	Number	Benign + Malignant	Malignant
800	M	50	62	26
	F	50	66	24
	M + F	100	64	25
0	M	50	54	24
	F	50	70	22
	M + F	100	62	23

[a]Source: Maltoni et al., 1997
[b]Gavage dose administered 4 days/week for 104 weeks and animals were terminated after 123 weeks.

Inhalation Exposure

Korsak and Rydzyński (1996) examined the neurotoxic effects of acute exposure of male Wistar rats (10/group) to 1,2,4-trimethylbenzene (>97% pure) and other trimethylbenzene isomers, and also examined the neurotoxic effects of subchronic exposure to 1,2,4-trimethylbenzene and 1,2,3-trimethylbenzene. In the acute experiment, rats were exposed to concentrations of 250-2000 ppm (1227-9816 mg/m^3) for 4 hours. Acute exposure to 1,2,4-trimethylbenzene caused concentration-related impairment in a rotarod performance test (EC_{50} = 4693 mg/m^3) and concentration-related decreased pain sensitivity (as measured by increased paw-lick response latency; EC_{50} = 5682 mg/m^3).

In the subchronic experiment, rats were exposed to 1,2,4-trimethylbenzene at concentrations of 0, 25, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3), 6 hours/day, 5 days/week for 3 months and observed for exposure-related clinical signs and body weight effects (Korsak and Rydzyński, 1996). Rotarod performance and hot-plate behavior were measured as indices of the neurotoxicity of trimethylbenzene isomers. Rotarod performance was tested prior to start of the study, weekly during exposure, and 2 weeks after the termination of the exposure. Hot-plate behavior was tested immediately after termination of the exposure. Fisher's exact test was used for analysis of rotarod performance and the Kruskall-Wallis test used for changes in pain sensitivity (hot plate behavior). Exposures to 1,2,4-trimethylbenzene did not result in any apparent body weight effects or clinical signs of toxicity. However, exposure-related indicators of neurotoxicity were noted. Rotarod performance failure increased in a concentration-related manner in the groups exposed to 1,2,4-trimethylbenzene, but reached the level of statistical significance (40% failure; $p<0.05$) only in the highest (1227 mg/m^3) exposure group following 8 or 13 weeks of exposure. The incidence of rotarod performance failure in control rats was 0% throughout the study period. Although the mean rotarod performance failure rate in the highest exposure group remained at 30% after a 2-week recovery period, the rate was not significantly different from controls. Pain-sensitivity was also decreased in a concentration dependent manner (evidenced by increased latency of the paw-lick response). As shown in Table 2, the increased latency reached the level of statistical significance in the 491- and 1227-mg/m^3 groups. After a 2-week recovery period, the highest (1227 mg/m^3) exposure group no longer exhibited a significant difference in pain sensitivity, relative to controls. This study identified a NOAEL of 123 mg/m^3 and a LOAEL of 491 mg/m^3 (6 hours/day, 5 days/week) for significantly decreased pain sensitivity.

Table 2. Exposure-Related Effect on Latency of the Paw-Lick Response in Rats Exposed to 1,2,4-Trimethylbenzene Vapors 6 Hours/Day, 5 Days/Week for 3 Months.[a]

Number of rats	Exposure level (mg/m^3)	Mean latency of paw-lick response (seconds)
9	0	15.4 ± 5.8[b]
10	123	18.2 ± 5.7
9	491	27.6 ± 3.2[c]
10	1227	30.1 ± 7.9[c]

[a] Source: Korsak and Rydzyński, 1996
[b] The authors did not specify whether standard deviation or standard error of the mean is presented
[c] Statistically significantly different from controls ($p \leq 0.01$)

Gralewicz et al. (1997a) investigated 1,2,4-trimethylbenzene-induced behavioral effects on groups of male Wistar rats (15/group) exposed to vapor concentrations of 0, 50, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) for 6 hours/day, 5 days/week for 4 weeks. To assess the effect of exposure on short-term working memory, choice accuracy in a radial arm maze was tested. Effects on spontaneous activity were evaluated with an open field test. Effects on long-term memory and learning ability were assessed on the basis of conditioned passive and active avoidance tests. The hot-plate test was performed to compare the groups with respect to the decrease in responsiveness to a thermal stimulus following a brief intermittent foot shock. Animals were subjected to the following sequence of behavioral testing:

1. radial maze: 2 weeks before exposure and on days 14-18 after exposure,
2. open field activity: day 25 after exposure,
3. passive avoidance: days 35-45 after exposure,
4. hot-plate test: days 50 and 51 after exposure,
5. active avoidance: day 54 after exposure.

The data were analyzed by ANOVA and comparisons among treatments were made using Sheffe's test, or Tukey's test for 2-way ANOVA.

There was no significant effect of 1,2,4-trimethylbenzene exposure on body weight gain during the 4-week exposure. Passive-avoidance learning was significantly ($p<0.001$) retarded in groups exposed to 491 or 1227 mg/m^3 of 1,2,4-trimethylbenzene and tested 35-45 days after the end of the exposure period. Retardation of passive-avoidance learning was more pronounced in the 491 mg/m^3 exposure group than in the 1227 mg/m^3 group. In the hot-plate test following foot shock, evaluation of rats 50 days following termination of exposures to 491 or 1227 mg/m^3 of 1,2,4-trimethylbenzene revealed significantly ($p<0.01$) increased paw-lick latency times, in comparison to unexposed controls. There was no significant change in the active avoidance test, although there was a trend toward decreased avoidance responses with increasing 1,2,4-trimethylbenzene exposure concentration. Short-term working memory did not appear to be adversely affected by 1,2,4-trimethylbenzene exposure. In the open field test there was no significant effect on spontaneous movement or on rearing behavior; however, there was a significant ($p<0.05$) increase in grooming behavior of animals exposed to 1,2,4-trimethylbenzene at 491 mg/m^3. Although grooming behavior also was increased above controls in the 123 and 1227 mg/m^3 groups, the difference was not statistically significant. The results of these experiments suggest that 4-week exposures at concentrations that produced no overt clinical signs of toxicity can produce long-term effects on the functional state of the rat central nervous system. Based on findings of significantly retarded passive avoidance learning and increased paw-lick latency in rats of the 491 and 1227 mg/m^3 exposure groups, the 123 mg/m^3 group represented a NOAEL and the 491 mg/m^3 group represented a LOAEL (6 hours/day, 5 days/week) for persistent neurotoxic effects.

Gralewicz and Wiaderna (2001) employed the same general protocol used by Gralewicz et al. (1997a) in a comparative study of the behavioral effects of repeated inhalation exposure to individual trimethylbenzene isomers or *m*-xylene. The study included a group of 11 adult male Wistar rats exposed to 100 ppm (491 mg/m^3) of 1,2,4-trimethylbenzene (purity not stated) and a control (air only) group of 10 male rats. Exposures were for 6 hours/day, 5 days/week for 4

weeks. The sequence of behavioral testing varied slightly from that employed by Gralewicz et al. (1997a) and included:

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.

No significant exposure-related effects were seen regarding body weights or short-term working memory (as determined in the radial arm maze test) for any of the trimethylbenzene isomers or *m*-xylene. Acquisition, but not retention, of the two-way active avoidance response was significantly impaired in all solvent-exposed groups. Results of other behavioral tests demonstrated exposure-related effects for each of the solvents. In the case of 1,2,4-trimethylbenzene, significantly increased spontaneous locomotor activity in the open field, impaired passive avoidance learning and significantly longer paw-lick latencies in the hot-plate test 24 hours after foot shock were observed. These results support the findings of the earlier study (Gralewicz et al., 1997a) in which the 491 mg/m^3 (6 hours/day, 5 days/week) exposure level represented a LOAEL for neurotoxic effects in 1,2,4-trimethylbenzene-exposed rats.

Gralewicz et al. (1997b) investigated the effect of a 4-week (6 hours/day, 5 days /week) inhalation exposure to 1,2,4-trimethylbenzene (purity not stated) at concentrations of 0, 25, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) on the occurrence of spike-wave discharges (SWD) in the neurocortex of male Wistar rats (9-10/group). Bursts of SWD increase in number and/or duration with advancing age and it was hypothesized that exposure to neurotoxic solvents may accelerate the aging process in the brain. Electrodes were implanted into the fronto-parietal cortex and into the dorsal hippocampus. One-hour EEG recordings were performed immediately before initiation of exposure, at the end of the exposure period, 1 month later and 3 months later. The occurrence of SWD bursts is limited to the state of awake immobility. The number and total duration of SWD bursts were determined from each EEG. The data were analyzed by ANOVA and multiple comparisons among treatments was performed with Tukey's test. The study results included information regarding mean body weights, but the study report did not provide details of body weight data collection.

The study authors (Gralewicz et al., 1997b) indicated that 1,2,4-trimethylbenzene exposure resulted in no statistically significant body weight effects. In the control and lowest (123 mg/m^3) exposure groups, the total duration of SWD showed an increasing trend with time, in comparison to pre-exposure SWD and reached statistical significance ($p<0.05$) at 3 months after exposure. In contrast, the total duration of SWD tended to decline with time in the mid- and high-exposure groups after exposure. The decrease in SWD occurrence, however, was statistically significant only for the measurements performed 1 month after the end of exposure in the mid-exposure (491 mg/m^3) group. A similar trend was seen when the number of SWD bursts per hour was determined. The frequency of SWD bursts increased with age in the control and lowest exposure groups and tended to decline with time in the mid- and high-exposure groups. The data, however, were highly variable and were statistically significantly different from pre-exposure SWD only for the highest exposure level at 3 months after exposure. Thus,

there were no clear 1,2,4-trimethylbenzene induced concentration-related effects on SWD, although the results are suggestive that long-term effects on brain activity may have occurred.

Korsak et al. (2000) exposed groups of male and female rats (10/sex/group; 20/sex/group at the highest exposure concentration) of outbred Imp:WIST to 1,2,4-trimethylbenzene (97% pure) vapors at target concentrations of 0, 123, 492 or 1230 mg/m^3 for 6 hours/day, 5 days/week for 3 months. Animals were observed twice daily for clinical signs of toxicity. Body weights were recorded prior to initiation of exposures and weekly thereafter and food consumption was measured weekly. 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. Organ weights were determined for lungs, liver, spleen, kidneys, adrenals, heart and gonads. 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.

Clinical findings were unremarkable (Korsak et al., 2000). No significant exposure-related effects were seen regarding food consumption or body weights. The few differences in some relative or absolute organ weights did not appear to be of toxicological relevance. Significant concentration-related trends ($p<0.01$) for decreased numbers of red blood cells and increased numbers of white blood cells were noted for male (but not female) rats. In the male rats of the highest 1,2,4-trimethylbenzene exposure group (1230 mg/m^3), both red and white blood cell counts were significantly different ($p<0.01$) from those of control males. A significant trend ($p<0.01$) for concentration-related decreased reticulocyte counts was observed in female rats and the difference was significant ($p<0.05$) in the 1230 mg/m^3 group. Hematological testing also revealed a significant trend ($p<0.01$) for decreased clotting time in female (but not male) rats; the decrease reached the level of statistical significance ($p<0.05$) in the 492 and 1230 mg/m^3 groups. Clinical chemistry results were unremarkable, with the exception of significantly increased serum sorbitol dehydrogenase in all 1,2,4-trimethylbenzene-exposed groups of male rats (not concentration related). Histopathological examinations revealed exposure-related significantly increased severity of pulmonary lesions, which included increased proliferation of peribronchial lymphatic tissue in males of the mid- (but not highest) exposure level, increased alveolar macrophages in males of the highest exposure level and increases in interstitial lymphocytic infiltrations in males of the mid- (but not highest) exposure level and females of the highest exposure level. No significant exposure-related changes were seen in the other examined organs and tissues. The mid exposure level of 492 mg/m^3 can be considered a LOAEL for hematological and respiratory effects in this study. The low exposure level of 123 mg/m^3 is a NOAEL.

Korsak et al. (1997) exposed male Wistar rats of IMP:DAK outbreed stock (10/group) to 1,2,4-trimethylbenzene (≥97% pure) at concentrations of 0, 25, 100 or 250 ppm (0, 123, 491 or 1227 mg/m^3) for 90 days (6 hours/day, 5 days/week). Lung lavage fluid was collected 24 hours after termination of the subchronic exposure and centrifuged at 400 g for 10 minutes. Differential counts of bronchoalveolar lavage (BAL) cell smears were determined by light microscopy after staining and the trypan blue test was used to determine cell viability. Total

protein concentration, mucoprotein concentration, lactate dehydrogenase and acid phosphatase activity were determined in the BAL supernatant.

All rats exposed to 1,2,4-trimethylbenzene for 90 days survived the experiment and there were no significant differences in final body weight. Statistically significant increases were observed in total cell and macrophage numbers in BAL of all treated groups after 90 days in comparison to controls. Significant increases were also observed in total protein, lactate dehydrogenase (LDH) and acid phosphatase (AP) in BAL fluid of all treated groups. However, the observed increases in these parameters were either at or near their highest observed response at the lowest exposure concentration, and there was no indication of further concentration-related increases. For the observed effects, the lowest exposure level used (123 mg/m^3) would be a LOAEL; however, the toxicological significance of these effects is not clear.

In a study by IBT (1981), groups of 5 male and 5 female COBS rats were exposed to 49 or 480 mg/m^3 MCS-1809 6 hours/day, 5 days/week for 4 weeks (IBT, 1981). MCS-1809 was identified as a compound containing 75% 1,2,4-trimethylbenzene and 25% C9 aromatics (Monsanto, 1992). The test atmosphere was generated by passing the MCS-1809 through a nebulizer; no information on the particle size distribution was reported. Based on the vapor pressure of 1,2,4-trimethylbenzene, it is likely that the animals were predominantly exposed to 1,2,4-trimethylbenzene vapors rather than a mist. The following parameters were used to assess toxicity: 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 480 mg/m^3 groups (tissues from the 49 mg/m^3 group were examined if significant findings were found in the 480 mg/m^3 group).

Exposure to MCS-1809 did not result in deaths. Clinical signs of toxicity in the 480 mg/m^3 group included ataxia and hypoactivity that persisted between exposures, ptosis, red ocular discharge, and ruffed fur. Less pronounced hypoactivity and ruffed fur were observed in the 49 mg/m^3 group. In the 480 mg/m^3 group, significant decreases in body weight gain (35% lower in the males; no significant alteration in females), increases in absolute (females only) and relative liver weights and decreases in absolute and relative spleen weights (females only) were observed. A significant increase in absolute liver weight was also observed in the 49 mg/m^3 female rats. Histological alterations were limited to focal or diffuse testicular atrophy in 3/5 male rats exposed to 480 mg/m^3 in the absence of statistically significant changes in testis weight; no testicular effects were observed in the 49 mg/m^3 (testes examined in four rats from this group) or control groups. This study identified a NOAEL of 49 mg/m^3 and LOAEL of 480 mg/m^3 (6 hours/day, 5 days/week) for clinical signs of toxicity (persistent ataxia and hypoactivity, ptosis, ocular discharge), decreased body weight gain, and histopathological evidence of testicular atrophy. The increased absolute liver weight observed in the 49 mg/m^3 female rats was not considered adverse because no histological alterations were observed at the 49 or 480 mg/m^3 concentrations.

Bättig et al. (1958) exposed 8 male rats (strain not reported) to air concentrations of 1700 ppm of Fleet-X DV 99 solvent for 4 months (8 hours/day, 5 days/week). Other rats (sex, strain,

and number not specified) were exposed to 500 ppm for 70 days (8 hours/day, 5 days/week). As described earlier, Fleet-X DV 99 is a solvent containing 97.5% aromatic hydrocarbons (>50% 1,2,4-trimethylbenzene and >30% 1,3,5-trimethylbenzene) and 2.5% of paraffinic and naphthenic hydrocarbons. The 500- and 1700-ppm concentrations would be approximately 2523 and 8155 mg/m^3, respectively, if the aromatic hydrocarbon fraction of the vapors were comprised exclusively of trimethylbenzenes. Within 2 weeks of exposure, 4 of the 8 rats exposed to 1700 ppm died and were replaced, while none of the animals in the 500-ppm group died. Histopathologic examinations, performed only on 1700-ppm animals that died, revealed cloudy swelling and fatty infiltration in the kidneys, peripheral fatty infiltration in the liver, an increase in secondary nodules in the spleen and marked congestion of the pulmonary capillaries with alveolar wall thickening. Alterations in differential white blood cell counts (increase in the percentage of segmented neutrophilic granulocytes and a decrease in the percentage of lymphocytes) were reported at 500 ppm. Increases in drinking water consumption (43-45% higher than in the control group) were observed in the 1700-ppm group. The authors reported that during the exposure period, the 1700-ppm animals were initially "highly excited and aggressive" followed by a period of narcosis and ataxia. Because histopathology was only performed on the animals that died, no histopathology data are available on the 500-ppm rats. Due to the limited scope of the study, a NOAEL or LOAEL cannot be identified. The high concentration of 1700 ppm (8155 mg/m^3) is a FEL for mortality.

Bernshtein (1972) exposed rats (number, sex and strain not specified) to 1000 mg/m^3 (200 ppm) of a mixture of trimethylbenzenes for 6 months (4 hours/day, 6 days/week). An inhibition of phagocytic activity of the leukocytes was reported. This study was summarized by Sandmeyer (1981) and further experimental details were not provided.

Korsak et al. (1997) examined the effect of acute exposures (6 min) to the trimethylbenzene isomers, 1,2,3-trimethylbenzene (90-95% pure), 1,3,5-trimethylbenzene (99% pure) and 1,2,4-trimethylbenzene (97% pure) on the respiratory rate of Balb/C male mice (8-10/group) at concentrations ranging from 253 to 1591 ppm (1926-9453 mg/m^3). The concentration depressing mouse respiratory rate by 50% (RD_{50}) was calculated by least squares regression and the Kruskall-Wallis test was applied for evaluation of protein and enzyme levels in the BAL fluid. All three trimethylbenzene isomers showed irritating effects on the respiratory tract and caused concentration-dependent decreases in respiratory rate. The concentration depressing the respiratory rate in mice to 50% was 519 ppm (2547 mg/m^3) for 1,2,4-trimethylbenzene.

The developmental toxicity of 1,2,4-trimethylbenzene was assessed by Saillenfait et al. (2005). Groups of mated (sperm-positive) Sprague-Dawley rats (24/group) were exposed (whole body) to 1,2,4-trimethylbenzene (97% pure) at vapor concentrations of 0, 100, 300, 600 or 900 ppm (0, 491, 1475, 2950 or 4425 mg/m^3) for 6 hours/day on gestation days 6 through 20. Maternal food consumption was recorded for the intervals of gestation days 6-13 and 13-21. Maternal body weights were recorded weekly during gestation. At necropsy on gestation day 21, 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. Half of the live fetuses from each litter were prepared for visceral examination, the others were subjected to skeletal examination.

All dams survived to scheduled necropsy (Saillenfait et al., 2005). No clinical signs of 1,2,4-trimethylbenzene-induced toxicity were observed. Maternal food consumption was significantly ($p<0.01$) depressed in the 600- and 900-ppm groups (approximately 12-14% and 15-19%, respectively, relative to controls). The 900-ppm dams exhibited significantly reduced mean body weight gain (22-52% lower than controls) throughout the exposure period. Significantly ($p<0.01$) reduced body weight gain (30% lower than controls) was observed in the 600-ppm group, but only during the first week of 1,2,4-trimethylbenzene exposure. 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). There were no other significant indications of maternal or fetal toxicity. This study identified a NOAEL of 300 ppm (1475 mg/m^3) and a LOAEL of 600 ppm (2950 mg/m^3, 6 hours/day on gestation days 6 through 20) for maternal and fetal body weight effects. However, the observed fetal toxicity was likely secondary to maternal toxicity because the decreased fetal body weight was noted only at exposure levels resulting in significantly depressed maternal body weight gain.

Other Studies

Limited genotoxicity data suggest that 1,2,4-trimethylbenzene is not mutagenic. 1,2,4-trimethylbenzene produced negative results in the Ames test with *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 not cytogenic in the mouse micronucleus test, but elicited a positive response in sister chromatid exchange (SCE) tests with bone marrow cells of Imp:Balb/c mice treated *in vivo* (Janik-Spiechowicz et al., 1998).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR 1,2,4-TRIMETHYLBENZENE

Limited information is available regarding the oral toxicity of 1,2,4-trimethylbenzene in humans and animals. The nephrotoxicity study by Borriston Laboratories (1984) is too limited in scope to be used to identify a NOAEL or LOAEL for 1,2,4-trimethylbenzene, although the 2000 mg/kg dose (1429 mg/kg-day) is clearly a FEL for increased mortality. The study of Maltoni et al. (1997) is also unsuitable for derivation of an RfD, as only one dose level was employed, decreased survival occurred at this dose level and reporting of the results was inadequate. Thus, the database for 1,2,4-trimethylbenzene is inadequate to derive a provisional RfD.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR 1,2,4-TRIMETHYLBENZENE

Several studies have examined the inhalation toxicity of mixtures predominantly containing one or more trimethylbenzene isomers, or pure 1,2,4-trimethylbenzene. Significant

increases in the incidence of CNS toxicity (vertigo, dizziness, headaches) and non-significant increases in the incidences of respiratory effects (bronchitis) and hematological effects (hyperchromic anemia and blood clotting alterations) have been observed in workers exposed to 10-60 ppm (49-295 mg/m^3) of a solvent containing >80% trimethylbenzene isomers (Bättig et al., 1958). Many of these effects reported in humans have been observed in experimental animals repeatedly exposed to 1,2,4-trimethylbenzene or other trimethylbenzene isomers or trimethylbenzene mixtures. For example, hematological effects have been reported in experimental animals exposed to a trimethylbenzene mixture (Bernshtein, 1972) or 1,2,4-trimethylbenzene (Korsak et al., 2000). Signs of adverse CNS effects have been observed in animals exposed to mixtures containing 1,2,4-trimethylbenzene (Bättig et al., 1958; IBT, 1981) or 1,2,4-trimethylbenzene alone (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a, 1997b; Korsak and Rydzyński, 1996). Results of other animal studies provide evidence of 1,2,4-trimethylbenzene-induced respiratory effects (Korsak et al., 1997, 2000). Other effects observed in animal studies include testicular atrophy in rats exposed to 480 mg/m^3 (98 ppm) of a mixture containing 75% 1,2,4-trimethylbenzene for 4 weeks (6 hours/day, 5 days/week) (IBT, 1981) and lung, liver, kidney and spleen effects in rats exposed to 1700 ppm (8155 mg/m^3) of a solvent containing >80% trimethylbenzene isomers for 4 months (8 hours/day, 5 days/week) (Bättig et al.,1958).

The lowest estimated level of occupational exposure to the solvent Fleet-X DV 99 (>80% 1,2,4- and 1,3,5-trimethylbenzene) in the study of Bättig et al. (1958) was 10 ppm (49 mg/m^3). Assuming that the solvent exclusively contained trimethylbenzene isomers, the 49 mg/m^3 exposure concentration can be considered to represent a LOAEL. Although the Bättig et al. (1958) report provides the lowest inhalation LOAEL (49 mg/m^3) of any study, it may be an inappropriate study for consideration as the principal study for a number of reasons. Importantly, Bättig et al. (1958) identified spectrophotographically the presence of various aromatic hydrocarbons, to include naphthenic and paraffenic compounds, in addition to 1,2,4-trimethylbenzene in the solvent mixture. While 1,2,4-trimethylbenzene comprised up to 50% of the Fleet-X DV 99 mixture, it is virtually impossible to confidently attribute human toxicities solely to 1,2,4-trimethylbenzene (i.e. the study LOAEL is for the mixture not the individual compound). Additional concerns that warrant exclusion of the Bättig et al. (1958) human study from consideration include inappropriate selection of a human control population [e.g. nutritional status (Vit. C deficient)], and the fact that average Fleet-X DV 99 solvent exposure duration, for the 27 exposed workers examined, was not reported.

An advantage of some of the animal models of 1,2,4-trimethylbenzene inhalation exposure over the Bättig et al. (1958) human study is that controlled atmospheres involved the compound of interest at relatively high purities (e.g. 97% 1,2,4-trimetylbenzene in the Korsak et al., 2000 study). However, available repeated exposure inhalation studies in animals are limited to subchronic exposure duration (4 weeks to 3 months) in which the lowest identified LOAEL for 1,2,4-trimethylbenzene was 491 mg/m^3 (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1997a; Korsak and Rydzyński, 1996); furthermore, many of the effects observed in these rodent studies are of unclear toxicological significance and/or have concentration-responses that are difficult to interpret.

Provisional RfCs may be derived based on adverse pulmonary or hematological effects reported in male or female rats, respectively, exposed to 1,2,4-trimethylbenzene (97% pure) for 3 months (Korsak et al., 2000). The selection of the Korsak et al. (2000) study as the basis for deriving RfCs is supported by previous observations in rats (Korsak et al., 1997) and humans (Bättig et al., 1958) exposed to pure 1,2,4-trimethylbenzene or a mixture of trimethylbenzenes, respectively, for ≥ 90 days. Indeed, pulmonary lesions and hematological abnormalities in rats exposed to pure 1,2,4-trimethylbenzene for 3 months (Korsak et al., 2000) are consistent with observations in humans following presumably longer duration exposure to a mixture containing 1,2,4-trimethylbenzene (Bättig et al., 1958).

Subchronic p-RfC

The subchronic p-RfC for 1,2,4-trimethylbenzene is derived from the NOAEL of 25 ppm (123 mg/m^3) identified in the Korsak et al. (2000) rat subchronic inhalation study. Two different toxic effects (pulmonary or hematological) were identified in male or female rats, respectively, in this study at the same LOAEL/NOAEL. As such, two separate subchronic p-RfC derivations are presented below to identify the most sensitive endpoint. Under an assumption of category 3 for decreased clotting time in female Imp:WIST rats, an adjusted experimental NOAEL can be derived using the NOAEL of 123 mg/m^3 and the exposure duration data from Korsak et al. (2000) as follows:

$$NOAEL_{[ADJ]}\ (mg/m^3) = \text{rat NOAEL}\ (mg/m^3) \times 6hr/24hr \times 5\ days/7\ days$$
$$= 123\ mg/m^3 \times 0.25 \times 0.71$$
$$= 21.8\ mg/m^3$$

According to equation (4-48) for extrarespiratory effects [Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994)], a human equivalent concentration ($NOAEL_{[HEC]}$) can be calculated as follows:

$$NOAEL_{[HEC]}\ (mg/m^3) = NOAEL_{[ADJ]}\ (mg/m^3) \times (H_{b/g})_A/(H_{b/g})_H$$

*blood:gas (b/g) partition coefficients for 1,2,4-trimethylbenzene could not be located, therefore a default value of 1 is used for the term $(H_{b/g})_A/(H_{b/g})_H$. The human $NOAEL_{[HEC]}$ is equivalent to the duration adjusted rat NOAEL of 21.8 mg/m^3. A **subchronic p-RfC of 7E-2 mg/m^3** based on a hematological effect is derived by dividing the $NOAEL_{[HEC]}$ of 21.8 mg/m^3 by a composite UF of 300, as follows:

UF (animal to human) = 3
UF (interindividual variability) = 10
UF (database deficiencies) = 10

$$\text{Subchronic p-RfC} = NOAEL_{[HEC]} / UF$$
$$= 21.8\ mg/m^3 / 300$$
$$= 0.07\ mg/m^3 \text{ or } 7E\text{-}2\ mg/m^3$$

Under an assumption of category 1 for pulmonary toxicity in male rats, the same duration adjusted rat NOAEL of 21.8 mg/m^3 is obtained as shown above. Histopathological observations in lung tissue of male Imp:WIST rats exposed to 1,2,4-trimethylbenzene for 3 months indicated inflammatory lesions primarily in the bronchiolar region. Therefore, according to equation 4-22 for Category 1 tracheobronchial effects [Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994)], a $NOAEL_{[HEC]}$ can be calculated as follows:

$$NOAEL_{[HEC]}\ (mg/m^{3)} = NOAEL_{[ADJ]}\ (mg/m^3) \times RGDR_{TB}{}^{\dagger}$$
$$= 21.8\ mg/m^3 \times 1.6$$
$$= 34.9\ mg/m^3$$

† Derivation of the $RGDR_{TB}$ can be found in Appendix 1

A **subchronic p-RfC of 1E-1 mg/m^3** based on pulmonary effects is derived by dividing the $NOAEL_{[HEC]}$ of 34.9 mg/m^3 by the same composite UF of 300 identified above:

$$\text{Subchronic p-RfC} = NOAEL_{[HEC]} / UF$$
$$= 34.9\ mg/m^3 / 300$$
$$= 0.1\ mg/m^3 \text{ or } 1E\text{-}1\ mg/m^3$$

The composite UF includes a factor of 3 for extrapolation from animal to human, 10 for interindividual variability, and 10 for database deficiencies. The reduced uncertainty of 3 for animal to human extrapolation is due in part to both the conversion of the rat NOAEL to a human equivalent concentration as well as the consistency of hematological and pulmonary toxicity between rats and humans exposed to 1,2,4-trimethylbenzene. The database deficiencies include lack of developmental toxicity studies in a second species, multigeneration reproductive toxicity studies, and a lack of confidence in the large majority of available animal studies reporting effects of undetermined toxicological significance with concentration-responses that are difficult to interpret. The derivations shown above clearly indicate that decreased clotting time in female rats due to subchronic inhalation exposure to 1,2,4-trimethlybenzene is the more sensitive or health protective endpoint under consideration.

Chronic p-RfC

The **chronic p-RfC of 7E-3 mg/m^3** based on decreased clotting time in female rats (Korsak et al., 2000) is derived by dividing the $NOAEL_{[HEC]}$ of 21.8 mg/m^3 by a composite UF of 3000, as follows:

$$\text{Chronic p-RfC} = NOAEL_{[HEC]} / UF$$
$$= 21.8\ mg/m^3 / 3000$$
$$= 0.007 \text{ or } 7E\text{-}3\ mg/m^3$$

As for the chronic RfC, the composite UF includes a factor of 10 for extrapolation from subchronic to chronic exposure, 3 for extrapolation from animal to human, 10 for interindividual variability, and 10 for database deficiencies.

Confidence in the principal study (Korsak et al., 2000) is low. While it is remarkable that hematological and pulmonary effects are apparently conserved from rats (Korsak et al., 2000) to humans (Bättig et al., 1958), the concentration-response for either compartment in rats (particularly male rats) is difficult to interpret. Specifically, the low inhalation concentration (123 mg/m^3) in female rats from the Korsak et al. (2000) study was clearly a NOAEL for decreased clotting time (hematological compartment); this NOAEL was also identified for pulmonary effects (e.g. proliferation of peribronchial lymphatic tissue, interstitial lymphocytic infiltration of parenchyma, bronchitis and bronchopneumonia) in male rats. Interestingly, female rats seemed slightly more resistant to these pulmonary effects. However, the overall commutative score, following statistical trend analysis, of all pulmonary lesions suggested that the lungs of male and female Imp:WIST rats are significantly affected by inhalation exposure to 1,2,4-trimethylbenzene at the mid dose of 492 mg/m^3 (Korsak et al., 2000). However, paradoxically, in male rats of the high dose group the pulmonary effects decreased compared to animals in the mid dose group. This counter-intuitive concentration-response relationship might suggest a concentration-dependent transition in mode of action for pulmonary toxicity (note the increase in absolute lung weight of male Imp:WIST rats at the mid concentration of 492 mg/m^3, which is the concentration at which inflammatory foci were identified in lung tissue, and yet in the high concentration group lung weight decreased back to control levels (Korsak et al., 2000); more work would be required to verify.).

According to the derivations provided above it appears that the hematological endpoint (i.e. decreased clotting time in female rats) may be a more appropriate endpoint to consider for inhalation exposure to 1,2,4-trimethylbenzene. Further work in this area is certainly warranted. The human occupational report from Bättig et al. (1958) identified a lower inhalation effect level (e.g. LOAEL = 49 mg/m^3) compared to any of the available animal data. However, the utility of this study in derivation of RfCs is limited by poor reporting of results, undetermined exposure levels, the lack of statistical analysis of results, the lack of information on the exposed and control groups (e.g., age, education level, length of employment), small group sizes and possibly a poorly matched control group (as evidenced by increased incidence of vitamin C deficiency in controls). Also, the controls worked in adjacent rooms and the possibility that they also may have been exposed to trimethylbenzene cannot be excluded. Confidence in the database is low because the database is lacking developmental toxicity data in a second species and reproductive toxicity studies. Reflecting low confidence in the principal study and database, confidence in the provisional subchronic and chronic RfC values is low.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 1,2,4-TRIMETHYLBENZENE

Weight-of-evidence Classification

No information was located regarding the carcinogenicity of 1,2,4-trimethylbenzene or mixtures of trimethylbenzene isomers in humans. The database of information regarding the carcinogenicity of trimethylbenzene in animals is limited to a single carcinogenicity study in which male and female Sprague-Dawley rats (50/sex/group) were administered 1,2,4-trimethylbenzene via oral gavage at doses of 0 or 800 mg/kg for 104 weeks (Maltoni et al.,

1997). Although quantitative survival data were not included in the study report, the authors noted "intermediate" and "slight" reduction in the survival of 1,2,4-trimethylbenzene treated male and female rats, respectively. Under the conditions of the study, oral exposure to 1,2,4-trimethylbenzene did not cause a statistically significant increase in the incidence of animals bearing either malignant tumors or benign and malignant tumors (combined) or in the incidence of neuroesthesioepitheliomas. The study of Maltoni et al. (1997) included a single animal species (rat) and a single 1,2,4-trimethylbenzene dose level (800 mg/kg). Based on limitations in study design and reporting of results and the lack of additional carcinogenicity data in animals, the database of information for 1,2,4-trimethylbenzene is inadequate to establish the potential carcinogenicity of 1,2,4-trimethylbenzene. Limited genotoxicity data demonstrated that 1,2,4-trimethylbenzene was not mutagenic in several strains of *Salmonella typhimurium*, and did not elicit cytogenicity in the mouse micronucleus test, but did elicit a positive response in sister chromatid exchange (SCE) tests with bone marrow cells of Imp:Balb/c mice treated *in vivo* (Janik-Spiechowicz et al., 1998). These data provide inadequate evidence for genotoxic activity.

Collectively, the available carcinogenicity and genotoxicity data do not adequately assess the carcinogenic potential of 1,2,4-trimethylbenzene in humans or animals. Under the current U.S. EPA (2005) cancer guidelines, the human and animal data are inadequate for a determination of the human carcinogenic potential of 1,2,4-trimethylbenzene.

Quantitative Estimates of Carcinogenic Risk

There are no appropriate human or animal data from which to derive an oral slope factor or inhalation unit risk for 1,2,4-trimethylbenzene.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. Documentation of the threshold limit values for chemical substances. 7th Edition. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 2005. TLVs® and BEIs®: Threshold Limit Values for Chemical Substances and Physical Agents, Biological Exposure Indices. Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2006. Toxicological Profile Information Sheet. Available at http://www.atsdr.cdc.gov/toxpro2.html

Bättig, K., E. Grandjean, L. Rossi and J. Rickenbacher. 1958. [Toxicological investigations of trimethylbenzene.] Archiv fuer Gewerbepathol. Gewerbehyg. 16:555-566. [German, with English translation]

Bernshtein, L.M. 1972. No title provided. Vopr. Gig. Tr. Profzabol. Mater. Nauch. Konf. Vol. 53. (Cited in Sandmeyer, 1981)

Borriston Laboratories, Inc. 1984. Four-week oral nephrotoxicity screening study in male F344 rats. EPA Document No. FYI-AX-0784-0280.

CalEPA (California Environmental Protection Agency). 2006. Air - Chronic RELs. California Office of Environmental Health Hazard Assessment. Available at http://www.oehha.ca.gov/air/chronic_rels/AllChrels.html

Gralewicz, S. and D. Wiaderna. 2001. Behavioral effects following subacute inhalation exposure to *m*-xylene or trimethylbenzene in the rat a comparative study. Neurotoxicology. 22:79-89.

Gralewicz, S., D. Wiaderna and T. Tomas. 1997a. Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4-trimethylbenzene) in the rat. Neurotox. Teratol. 19:327-333.

Gralewicz, S., D. Wiaderna and T. Tomas. 1997b. Retardation of the age-related increase in spontaneous cortical spike-wave discharges (SWD) in rats after a 28-day inhalation exposure to an industrial solvent, pseudocumene (1,2,4-trimethylbenzene). Int. J. Occup. Med. Environ. Health. 10:213-222.

IARC (International Agency for Research on Cancer). 2006. IARC search page. Available at http://www.iarc.fr/index.html

IBT (Industrial Bio-Test Laboratories, Inc.). 1981. Four-week subacute aerosol inhalation toxicity study with MCS-1809 in albino rats. Prepared for Monsanto Company, St. Louis MO.

Janik-Spiechowicz, E., K. Wyszńska and E. Dziubaltowska. 1998. Genotoxicity evaluation of trimethylbenzenes. Mutat. Res. 412:299-305.

Korsak, Z. and K. Rydzyński. 1996. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. Int. J. Occup. Med. Environ. Health. 9:341-349.

Korsak, Z., K. Rydzyński and J. Jajte. 1997. Respiratory irritative effects of trimethylbenzenes: An experimental animal study. Int. J. Occup. Med. Environ. Health. 10:303-311.

Korsak, Z., J. Stetkiewicz, W Majcherek, I. Stetkiewicz, J. Jajte and K. Rydzyński. 2000. Sub-chronic inhalation toxicity of 1,2,4-trimethylbenzene (pseudocumene) in rats. Int. J. Occup. Med. Environ. Health. 13(2):155-164.

Maltoni, C., A. Ciliberti, C. Pinto, M. Soffritti, F. Belpoggi and L. Menarini. 1997. Results of long-term experimental carcinogenicity studies of the effects of gasoline, correlated fuels, and major gasoline aromatics on rats. Ann. New York Acad. Sci. 837:15-52.

Monsanto. 1992. Cover letter submitted to EPA's Office of Toxic Substances with the IBT (1981) study.

NIOSH (National Institute for Occupational Safety and Health). 2006. Online NIOSH Pocket Guide to Chemical Hazards. Available at http://www.cdc.gov/niosh/npg

NTP (National Toxicology Program). 2006. Management Status Report. Available at http://ntp-server.niehs.nih.gov/

OSHA (Occupational Safety and Health Administration). 2006. OSHA Standard 1910.1000 Table Z-1. Part Z, Toxic and Hazardous Substances. Available at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992

Saillenfait, A., F. Gallissot, J. Sabate and G. Morel. 2005. Developmental toxicity of two trimethylbenzene isomers, mesitylene and pseudocumene, in rats following inhalation exposure. Food Chem. Toxicol. 43:1055-1063.

Sandmeyer, E.E. 1981. Aliphatic hydrocarbons. In: Patty's Industrial Hygiene and Toxicology. Vol. 3. G.D. Clayton and R.E. Clayton, Eds. John Wiley and Sons, Inc., New York. pp. 3300-3302.

U.S. EPA. 1987a. Health Effects Assessment for Trimethylbenzenes. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1987b. Drinking Water Health Advisory for 1,2,4-Trimethylbenzene. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Drinking Water, Washington, DC.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994a. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1994b. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Prepared by the Office of Health and Environmental Assessment, Research Triangle Park, NC. EPA/600/8-90/066F.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. Annual Update. FY-1997. Office of Research and Development, Office of Emergency and Remedial Response, Washington, DC. July 1997. EPA/540/R-97/036. NTIS PB97-921199.

U.S. EPA. 2004. 2004 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. EPA/822/R-02/038. Available at http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf

U.S. EPA. 2005. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, Washington, DC. EPA/630/P-03/001B. Available at www.epa.gov/cancerguidelines

U.S. EPA. 2007. Integrated Risk Information System (IRIS). Online. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. http://www.epa.gov/iris/

WHO (World Health Organization). 2006. Online Catalogs for the Environmental Criteria Series. Available at http://www.inchem.org/pages/ehc.html

APPENDIX 1

According to equation 4-22 for Category 1 tracheobronchial effects [Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994)], a $NOAEL_{[HEC]}$ is calculated using the duration adjusted animal NOAEL and a dosimetric adjustment factor (DAF). In this case the DAF is the RGDR for the tracheobronchial region of the lung ($RGDR_{TB}$). The $RGDR_{TB}$ is calculated as follows:

$$RGDR_{TB} = (RGD_{TB})_A/(RGD_{TB})_H = \frac{(V_E/SA_{TB})_A}{(V_E/SA_{TB})_H} \quad \frac{(e^{-SA_{ET}/V_E})_A}{(e^{-SA_{ET}/V_E})_H}$$

where,

Rat

V_E = 160.07 ml/min or 0.16 L/min (derived using equation 4-4, default body wt. for Wistar rats, and the default intercept and coefficient values provided in Table 4-6)

SA_{TB} = 22.5 cm^2 (Table 4-4)

SA_{ET} = 15.0 cm^2 (Table 4-4)

Human

V_E = 13.8 L/min (default value based on human body weight of 70 kg)

SA_{TB} = 3,200 cm^2 (Table 4-4)

SA_{ET} = 200.0 cm^2 (Table 4-4)

$$= \frac{(0.16 \text{ L/min} / 22.5 \text{ cm}^2)_A}{(13.8 \text{ L/min} / 3{,}200 \text{ cm}^2)_H} \quad \frac{(e^{-15.0 \text{ cm2}/ 0.16 \text{ L/min}})_A}{(e^{-200.0 \text{ cm2}/ 13.8 \text{ L/min}})_H} \ ^*$$

* the exponential portion of the equation is much smaller than 1; thus this half of the equation is negligible.

$$= 0.007 / 0.0043$$

$$RGDR_{TB} = 1.6$$