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The sub-chronic oral toxicity of 1,3,5-trimethylbenzene in Sprague–Dawley rats

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ABSTRACT

The systemic toxicity of a trimethylbenzene isomer and constituent of C9 aromatic solvents (1,3,5-trimethylbenzene, 135-TMB) was studied in Sprague–Dawley rats following a 90-day oral gavage exposure to 0, 50, 200 and 600 mg/kg/day. No statistically significant effects on body weight, body weight gain or food consumption were observed at study termination. Treatment-related changes in clinical chemistry parameters at the end of the 90-day dosing period were limited to small, but statistically significant, increases in phosphorus levels in high dose males and females. Liver enlargement in high dose male/female rats was considered an adaptive response as this was reversible and was not associated with histopathological lesions or increased liver enzyme markers indicative of liver damage. Kidney weight changes were limited to a small, but statistically significant, increase in relative weights in high dose males. This was not associated with histopathological lesions and thus not considered toxicologically relevant. Overall, the No-Observed-Adverse-Effect-Level (NOAEL) was the highest concentration tested (600 mg/kg/day). The results of the present study are relevant for assessing the risk of trimethylbenzenes through the oral route of exposure and provide a basis for the development of provisional screening values for trimethylbenzene isomers while avoiding the uncertainty associated with route-to-route extrapolation.

1. Introduction

1,3,5-Trimethylbenzene (135-TMB), also known as mesitylene, is an isomeric C9 benzene derivative consisting of three methyl groups placed symmetrically on the benzene ring. Along with 124- and 123-TMB isomers, 135-TMB is a naturally occurring component of crude petroleum and coal tar and, thus, may be a component of more narrowly refined products of crude oil distillation containing C9 aromatics. Some of these products include aviation fuels, paint thinners, specialty solvents, printing inks, cleaners, dyes and resins. The primary manufacturing process for trimethylbenzenes is referred to as catalytic reforming and involves the dehydrogenation of cycloparaffins present in petroleum refinery naphthas (with poor octane ratings) in the presence of a catalyst. The subsequent high octane "catalytic reformate" is further distilled to remove C6–C8 components and used mostly as a component of gasoline to improve octane ratings (Firth, 2008).

The kinetics of 135-TMB absorption following oral and inhalation exposure, distribution and metabolism have been described in humans and laboratory animals (Jarnberg et al., 1996; Jones et al., 2006; Kostrzewski et al., 1997). Relative respiratory uptake was rapid in human volunteers exposed to 135-TMB during light worker activity and large volumes of distribution coupled with four distinct elimination half-lives (1–2 min, 21–27 min, 4–5 h and 120 h), suggest preferential distribution to deeply perfused tissues (Eide and Zahlsen, 1996; Huo et al., 1989; Jarnberg et al.,

Abbreviations: 123-TMB, 1,2,3-trimethylbenzene; 124-TMB, 1,2,4-trimethylbenzene; 135-TMB, 1,3,5-trimethylbenzene; 5′-NT, 5′-nucleotidase; ACGIH, American Conference of Governmental Industrial Hygienists; ALT, alanine aminotransferase; AP, alkaline phosphatase; ASVCP, American Society for Veterinary Clinical Pathology; BW_A, animal body weight; BW_H, human body weight; DMBA, dimethyl benzoic acid; DMHA, dimethyl hippuric acid; DNEL, derived no effect level; ECETOC, European Center for Ecotoxicology and Toxicology of Chemicals; ESTP, European Society of Toxicologic Pathology; GGT, γ-glutamyltransferase; LOAEL, Low-Observable-Adverse-Effect-Level; NOAEL, No-Observable-Adverse-Effect-Level; $NOAEL_{ADJ}$, adjusted No-Observable-Adverse-Effect-Level; $NOAEL_{HED}$, human equivalent No-Observable-Adverse-Effect-Level; NTP, National Toxicology Program; PBPK modeling, physiologically based pharmacokinetic modeling; POD, point of departure; RfD, reference dose; SCOEL, Scientific Committee for Occupational Exposure; TBA, total bile acid; TLV, threshold limit values; UF_A, interspecies uncertainty factor; UF_H, intraspecies uncertainty factor; UF_S, subchronic to chronic uncertainty factor.

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1996; Kostrzewski et al., 1997; Meulenberg and Vijverberg, 2000). Metabolism of 135-TMB and other TMB isomers mainly occurs in the liver and involves side-chain oxidation and hydroxylation of one of three methyl groups to form alcohols and dimethyl benzoic acids (DMBA), which can then be conjugated by sulfates, glucuronidation or glycine (to form the dimethyl hippuric acid (DMHA)) and excreted in the urine (Huo et al., 1989; Kostrewski and Wiaderna-Brycht, 1995; Kostrzewski et al., 1997; Laham and Potvin, 1989; Mikulski and Wiglusz, 1975; Swiercz et al., 2003; Wiglusz, 1979). Although very little 135-TMB is eliminated unchanged, it has been detected in expired air and urine (Conkle et al., 1975; Janasik et al., 2008; Krotoszynski et al., 1977).

In humans, primary effects of a substance containing 80% trimethylbenzenes and 20% other solvents (10–60 ppm) were limited to respiratory irritation and transient acute central nervous system (CNS) effects which resolved upon cessation of exposure (Battig et al., 1956). None of these symptoms were reported in human volunteers exposed to 25 ppm 135- or 124-TMB (Jarnberg et al., 1996; Jarnberg et al., 1998; Jarnberg et al., 1997; Jones et al., 2006) or 150 mg/m^3 (~30 ppm) 135-TMB for 8-h (Kostrewski and Wiaderna-Brycht, 1995; Kostrzewski et al., 1997). Based on the human observational data from Battig et al. (1956), additional toxicological data, and volunteer studies, early recommendations for occupational exposure limits for these solvents were in the range of approximately 35–50 ppm (Carpenter et al., 1975; Gerarde, 1960; Nau et al., 1966). The American Conference of Governmental Industrial Hygienists (ACGIH) proposed 25 ppm as 8-h threshold limit values (TLV) for all three isomers of trimethylbenzene (ACGIH, 2013). In Europe, the Scientific Committee for Occupational Exposure Limits (SCOEL) recommended an 8 h indicative occupational exposure limit of 20 ppm (100 mg/m^3) based on results of animal studies (SCOEL, 2013).

The toxicology information on C9 aromatics is primarily from studies of complex aromatic solvents and catalytic reformate, which are comprised of trimethylbenzene and ethyltoluene isomers. These studies included assessments of repeated inhalation exposure (Clark et al., 1989), as well as studies of the potential for these substances to cause developmental and reproductive effects (Lehotzky et al., 1985; Ungvary et al., 1983). A further series of studies of complex C9 aromatic substances provided more information on developmental toxicity (McKee et al., 1990), more complete information on reproductive toxicity (McKee et al., 1990), and also addressed the potential for mutagenic (Schreiner et al., 1989) and neurological effects (Douglas et al., 1993). Studies of individual trimethylbenzene isomers indicated a similar toxicological profile. There were no apparent target organ effects in repeated inhalation toxicity studies of 123-TMB (Korsak et al., 2000b), 124-TMB (Korsak et al., 2000a) or 4-ethyltoluene (Swiercz et al., 2000). There was no evidence of developmental toxicity in separate studies of two of the TMB isomers (Saillenfait et al., 2005).

An overall conclusion from these studies on complex substances and individual isomers was that these substances did not produce specific organ or nervous system effects and developmental delays were observed only at maternally toxic levels. Subsequently, an oral repeat dose toxicity study on 135-TMB was conducted to provide data that might be useful in assessing the potential risks to humans from exposure to trimethylbenzene isomers in drinking water. The objective of the present paper is to provide a detailed evaluation of the potential systemic toxicity of 135-TMB in the rat following 90-day once-daily oral gavage administration, as a preferred basis for the development of substance-specific oral screening values for regulatory purposes. Although an exposure study via drinking water would more closely mimic a relevant route of human exposure, oral gavage exposure (one of four options provided by the EPA TSCA testing guideline 40 CFR 798.2650) was considered the most feasible option due to the poor water solubility of the test material.

2. Materials and methods

2.1. Materials

The test substance was a pure sample of 1,3,5-trimethylbenzene (CAS # 108-67-8; Lot # 4388-01; 99% purity) (Koch Chemical Company, Corpus Christi, TX). The corn oil vehicle (Lot # 66,580) that was used in study was obtained from Best foods (Englewood Cliffs, NJ). A suitable aliquot of test substance was weighed out and diluted to volume with corn oil for each dosing solution. Stock solutions of test substance in corn oil were prepared weekly during the study and stored at room temperature. An equal volume of corn oil dispensed at the time of dosing solution preparation was stored similarly for use as the vehicle control.

2.2. Test substance analysis

The test substance was analyzed for identity by IR spectrum (Perkin Elmer Model 783), purity using gas chromatography (Varian 3700), composition and stability. Measured purity at the end of the dosing period was 99.3% 135-TMB and 0.69% 124-TMB. Solutions of the test substance in corn oil were analyzed for concentration, homogeneity and stability. Mean analyzed concentrations were within 97–100% of target concentrations. Analyzed concentration values for 0, 10 and 120 mg/ml (corresponding to 0, 50 and 600 mg/kg body weight) at weeks 1, 7 and 13 are provided in Table 1.

2.3. Animals

Sixty male and sixty female Sprague–Dawley CD (Crl:CD® BR) rats, approximately 6 weeks of age were purchased from Charles River Laboratories (Portage, MI) for use in this study. Randomized samples of 10 rats of each sex were weighed (133–158 g) and held in quarantine for 2 weeks prior to dosing initiation to assess health status. Food and water were provided *ad libitum* except for approximately 16–20 h (no food) prior to scheduled blood collection or necropsy. Rats were singly housed in stainless steel wire mesh cages and held in an air conditioned room controlled for temperature, humidity and lighting during the treatment and recovery phases of the study.

2.4. Experimental design

Dose selection was guided by a prior pilot study in which Sprague–Dawley rats were administered 0, 60, 150 and 600 mg/kg/day of test material, 7 days/week for 14 consecutive days (FHR, 1995). Treatment-related statistically significant effects were

Table 1
Analysis of dosing solutions of 1,3,5-trimethylbenzene in corn oil.

Target concentration (mg/ml)	Analyzed concentration (mg/ml)		
	Week 1[a]	Week 7[b]	Week 13[b]
0	BDL	–	–
10 (50 mg/kg)	9.78	9.60	9.92
40 (200 mg/kg)	39.04	–	–
120 (600 mg/kg)	120.4	128.2	114.6

BDL – Below detection limit.
[a] Values represent means of duplicate analysis for 0 mg/ml and 6 replicates for 10, 40, and 120 mg/ml.
[b] Values represent means of duplicate analyses.