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Species and gender differences in the carcinogenic activity of trimethylolpropane triacrylate in rats and mice

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ABSTRACT

Trimethylolpropane triacrylate (TMPTA) is a multifunctional monomer with industrial applications. To determine the carcinogenic potential, male and female F344/N rats and B6C3F1/N mice were administered TMPTA (0, 0.3, 1.0, or 3.0 mg/kg) in acetone dermally for 2 years. There were no differences in the body weights and survival in the treated animals compared to controls. Nonneoplastic skin lesions at the site of application included epidermal hyperplasia and hyperkeratosis in both rats and mice. There were no incidences of tumors at the site of application in rats and mice. Rare malignant liver neoplasms were observed in female mice that included hepatoblastoma in the 0.3 and 3.0 mg/kg groups, and hepatocholangiocarcinoma in the 1.0 and 3.0 mg/kg groups. The incidences of uterine stromal polyp and stromal polyp or stromal sarcoma (combined) in female mice occurred with positive trends and the incidences were significantly increased in the 3.0 mg/kg group. A marginal increase in the incidences of malignant mesothelioma in male rats may have been related to TMPTA treatment. In conclusion, our studies show that TMPTA is a dermal irritant in both rats and mice of either sex. Increased incidences of tumor formation were observed in female mice and male rats.

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1. Introduction

Trimethylolpropane triacrylate (TMPTA) is a multifunctional monomer with a wide range of industrial applications as a cross-linking agent, reactive diluent, and chemical intermediate. It is used in the production of ultraviolet-curable inks, acrylic glues, adhesives, and anaerobic sealants (Bjorkner, 1984; Voog, 1992). Based on the 2006 Inventory Update Reporting, a national production volume of TMPTA ranged from 10 to 50 million pounds (United States Environmental Protection Agency, 2006). Workers involved in the manufacturing, processing, product handling, and application of TMPTA are at risk of exposure. Furthermore, a potential exists for widespread exposure of consumers through the use of TMPTA in products such as latex paints, furniture and floor polishes (Dearfield et al., 1989; Voog, 1992). The most relevant route of exposure in humans is the dermal route, and TMPTA has been shown to be moderately absorbed through the skin in rats and mice (National Toxicology Program, 2005). In male rats, the percent dose absorbed was 55.7%, 32.7%, or 18.7% at 72 h following dermal application of 1.7, 15.2, or 130 mg/kg, respectively. In male B6C3F1 mice, 75% of the dose was absorbed 72 h after a single application of 1.2 mg/kg. TMPTA is an irritant based on positive results with the irritancy test, but is not a contact sensitizer based on negative results with the local lymph node assay conducted by NTP (National Toxicology Program, 2005).

A carcinogenicity study in male mice was reported in the literature (Andrews and Clary, 1986). In that study, no neoplasms occurred in 50 male C3H/HeJ mice dermally administered 100 mg/kg of a solution of 5% TMPTA in mineral oil to the interscapular region twice per week for up to 80 weeks. Because the study was conducted only in male mice with a single dose exposure, it was considered inadequate for assessment of the carcinogenic potential of TMPTA. To further investigate this compound, the NTP conducted a series of short- and long-term toxicity studies.

The NTP 2- and 13-week dermal studies showed that TMPTA affected the site of application (SOA) in F344 rats and B6C3F1 mice (Doi et al., 2005; National Toxicology Program, 2005). The microscopic examination of the skin (SOA) showed epidermal hyperplasia, hyperkeratosis and chronic inflammation. No systemic toxicity was observed except for some changes in organ weights, which were considered, unrelated to the treatment. In the Tg. AC

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dermal 26-week studies, TMPTA produced tumors at the site of application in a dose-related fashion (Doi et al., 2005). To further investigate the carcinogenicity of TMPTA, 2-year dermal studies in F344/N rats and B6C3F1/N mice were conducted.

2. Materials and methods

2.1. Chemicals

Technical grade TMPTA was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot. The chemical was identified as TMPTA by infrared, and proton and carbon 13-nuclear magnetic resonance spectroscopy. The purity was determined using gas chromatography with flame ionization detection and high-performance liquid chromatography (HPLC) with ultraviolet detection. Karl Fischer titration indicated an average water content of 0.10%. HPLC/mass spectrometry analysis indicated that all impurities appeared to be consistent with TMPTA adducts and that neither hydroquinone nor methyl hydroquinone was detected above 0.1% of the total peak area. The overall purity was determined to be greater than 78%.

2.2. Animal

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY). Rats were quarantined for 13 days and mice were quarantined for 12 days. Rats were approximately 6 weeks old and mice approximately 5–6 weeks old at the start of the studies. Rats and mice were housed individually. Feed and water were available *ad libitum*. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and in accordance with all relevant animal care and use policies and applicable federal, state, and local regulations and guidelines.

2.3. Experimental design

Groups of 65 male and 65 female rats and mice received dermal applications of 0, 0.3, 1.0, or 3.0 mg/kg TMPTA 5 days per week for up to 104–105 (rats) or 105–106 (mice) weeks. All doses were administered in acetone at volumes of 0.5 mL/kg for rats and 2.0 mL/kg for mice. The single daily doses were applied to a clipped area in the interscapular region of the back using a positive displacement micropipetter. All animals were observed twice daily. Body weights were recorded initially, approximately weekly for the first 13 weeks, at 4-week intervals thereafter, and at study termination. Clinical findings were recorded weekly from day 3 for male rats, day 4 for female rats and male mice and day 5 for female mice and at 4-week intervals beginning week 5 for all animals. At 2 weeks, 13 weeks, and 12 months, skin from the site of application was collected from interim evaluation animals, fixed in formalin, and examined microscopically. At the end of the study, complete necropsies and microscopic examinations were performed on all core study animals, including those found dead or euthanized as moribund during the study. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined.

2.4. Statistical analysis

For tumor and non-neoplastic lesion incidences, continuity-corrected, variance-adjusted Poly-3 tests were used to test for dose-related trends and pairwise differences between each dose group and the control group (Bailer and Portier, 1988; Bieler and Williams, 1993; Portier and Bailer, 1989). The Poly-3 test is a modified Cochran–Armitage test that adjusts for survival by modifying the tumor/lesion proportions within each group to more closely represent the number of animals-years at risk. Animals surviving the entire two years or having the tumor or lesion of interest are assigned a risk weight of 1, while animals that die tumor/lesion-free before the end of the study are assigned a risk weight equal to the proportion of study time survived raised to the third power. *p*-Values from these tests are one-sided.

3. Results

3.1. Rats

There was no effect on survival or body weight throughout the study (Table 1). No chemical-related clinical findings were observed. In male rats, there was a positive trend in the incidences of malignant mesothelioma of the testicular tunica vaginalis. The incidence in 3.0 mg/kg males was significantly greater than the vehicle control incidence and exceeded the historical control ranges for dermal studies and for all routes of administration by only one tumor (Table 1). Microscopically, malignant mesotheliomas were papillary and consisted of one or more layers of neoplastic mesothelial cells covering pedunculated fibrovascular stalks (Fig. 1). In all cases, they were present in the tunics around the testes with dissemination into the peritoneal cavity.

In addition, there were increased incidences of nonneoplastic skin lesions at the site of application including epidermal hyperplasia and hyperkeratosis (Table 1). The incidences of these lesions in male rats administered 1.0 or 3.0 mg/kg were significantly increased. Microscopically, epidermal hyperplasia was defined as increased cell layers in the epidermis. The severity grade was considered to be minimal when the epidermis was thickened to three to four cell layers and mild when there was a thickening of the epidermis to five to six cell layers. Hyperkeratosis was defined as thickening of the stratum corneum with concurrent expansion of the stratum granulosum. The severity grade was considered to be minimal if the layers in the stratum corneum were slightly thickened by a thin amount of loosely packed keratin and mild when thickened by a dense, compact band of keratin.

Compared to the vehicle controls, male rats administered 0.3 mg/kg had increases in the incidences of basal cell adenoma (0/50, 3/50, 0/50, 2/50), basal cell carcinoma (0/50, 2/50, 1/50,

Table 1
Survival, body weights and incidences of neoplastic and nonneoplastic lesions in F344/N rats in the 2-year study.

Dose (mg/kg)	Survival[a]	Final mean body weights (% control)	Neoplastic lesion All organ	Nonneoplastic Lesion Skin			
			Malignant mesothelioma	Epidermal Hyperplasia		Hyperkeratosis	
Males							
0	23/50		0[b,c]	1	(1.0)[d]	2	(1.0)
0.3	18/50	101	2	0		4	(1.0)
1.0	28/50	99	2	12**	(1.0)	33**	(1.0)
3.0	23/50	97	5*	28**	(1.1)	49**	(1.0)
Females							
0	27/50		0	0		0	
0.3	31/50	99	0	4	(1.3)	11**	(1.0)
1.0	24/50	98	0	11**	(1.0)	42**	(1.0)
3.0	32/50	99	0	25**	(1.0)	50**	(1.0)

* $p < 0.05$ Significantly different from the control group by the Poly-3 test.
** $p < 0.01$ Significantly different from the control group by the Poly-3 test.
[a] Number of animals surviving to study termination/number initially in group.
[b] Number of animals with lesion.
[c] Historical incidences for 2-year dermal study vehicle controls (all vehicles): 8/250, range 0–8%; all routes: 40/1249, range 0–8%.
[d] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.