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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.

Keywords

Trimethylolpropane triacrylate; Skin; Liver; Uterine; Carcinogenicity

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

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Conflict of Interest

The authors declare no conflicts of interest.

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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 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, 0/50), and basal cell adenoma or basal cell carcinoma (combined) (0/50, 5/50, 1/50, 2/50) in the skin. The combined incidence of basal cell adenoma and carcinoma was statistically significant in the 0.3 mg/kg group and exceeded the historical control range for all routes of study (0–8%), but only by a single neoplasm. The increased incidence of basal cell adenoma or basal cell carcinoma (combined) was statistically significant in the 0.3 mg/kg group, the lowest dose group. There was no dose relationship for these lesions, the incidences of basal cell carcinoma alone were not statistically significant and did not exceed the historical control range for all routes of study (0–6%), and none of these lesions was at the site of application. Therefore, the increased incidences of these lesions compared to concurrent controls were not considered treatment-related.

In female rats, there was no treatment-related increase in incidences of neoplastic lesions. At the site of application, the incidences of epidermal hyperplasia were significantly increased at 1.0 and 3.0 mg/kg, and the incidences of hyperkeratosis were significantly increased in all dosed groups (Table 1).

Interim evaluations of the proliferative skin effects of dermal exposure to TMPTA were conducted at weeks 2 and 13 and month 12 (Table 2). Histopathologic evaluation of the skin from the site of application identified treatment-related increases in the incidences of epidermal hyperplasia at all three time points in both sexes. There were also treatment-

related increases in the incidences of sebaceous gland hyperplasia at 2 and 13 weeks in the male rats, and at 13 weeks in the female rats. At 12 months, there were treatment-related increases in the incidences of hyperkeratosis in both sexes. The dosed rats had up to five layers of epidermal cells (control rats had one to two layers). Sebaceous gland hyperplasia was characterized by a slight increase in the size of the sebaceous glands relative to the vehicle controls. Hyperkeratosis was characterized by an increase in the amount of keratin on the epidermal surface. At all three time points these changes were considered to be minor, but the increases in the severities of these lesions were clearly dose related.

3.2. Mice

Dermal application of TMPTA for 2 years did not affect survival or body weight throughout the study (Table 3). No chemical-related clinical findings were observed. In males, no neoplastic lesion was identified as treatment related. In females, although not statistically significant, there were increased incidences of hepatoblastoma in the 0.3 and 3.0 mg/kg groups and hepatocholangiocarcinoma in the 1.0 and 3.0 mg/kg groups of females (Table 3). The historical control range for hepatoblastoma in female B6C3F1 mice is low (0–2%), while hepatocholangiocarcinoma has not been seen in historical controls. Based on the rarity of these neoplasms in female mice and their absence in the concurrent vehicle controls, hepatoblastoma and hepatocholangiocarcinoma, when taken together, were considered to be treatment-related lesions. Female mice exposed to TMPTA showed positive trends in the incidences of hepatocellular carcinoma. However, increased incidences in treated groups were not significant and not dose related; therefore, this neoplasm was not considered as treatment related. Microscopically, hepatoblastomas were noted as irregular masses with blood-filled cystic spaces, necrosis and hemorrhage, and they generally had scant stroma and were composed of small, deeply basophilic cells with scant cytoplasm and elongated nuclei (hepatoblasts) (Fig. 2). Hepatocholangiocarcinomas were recorded as a pattern similar to hepatocellular carcinomas with small areas of ducts often containing both neoplastic hepatocytes and neoplastic biliary epithelial cells often inconspicuous in the primary tumor and more obvious in metastases, and containing large necrotic or cystic areas within tumors (Fig. 3). Hepatocellular adenomas were usually solitary or multiple masses composed of well-differentiated hepatocytes with variable size and tinctorial characteristics. Hepatocellular carcinomas were noted to have trabecular patterns, with trabeculae at least three cells thick. The incidences of eosinophilic focus (0 mg/kg, 15/50; 0.3 mg/kg, 23/50; 1.0 mg/kg, 21/50; 3.0 mg/kg, 21/50) in 0.3 mg/kg females and Kupffer cell pigmentation (4/50, 5/50, 10/50, 4/50) in 1.0 mg/kg females were significantly greater than those in the vehicle controls. The relationship of these nonneoplastic lesions to TMPTA administration is uncertain.

Incidences of uterine stromal polyp and stromal polyp or stromal sarcoma (combined) in female mice showed positive trends as well as significant increases in the 3.0 mg/kg group; the incidences in the 3.0 mg/kg females also exceeded the ranges in historical controls from dermal studies and from all routes of administration (Table 4). In addition, there was one female in the control group with a uterine sarcoma of uncertain origin. Since the origin of the uterine sarcoma was uncertain, the diagnosis of uterine sarcoma was not included with the other uterine tumors. Microscopically, stromal polyps were pedunculated masses that

protruded into the lumen, were often lined by cuboidal to columnar epithelium, and were composed of abundant amounts of loose stroma containing stellate or spindle cells, numerous blood vessels, and ectatic endometrial glands (Fig. 4). Stromal sarcomas were distinguished from stromal polyps by marked cellular pleomorphism.

Compared to the vehicle control incidences, the incidences of epidermal hyperplasia, melanocyte hyperplasia, and chronic inflammation were significantly increased in core study males and females administered 3.0 mg/kg; the incidences of epidermal hyperplasia in 1.0 mg/kg females and chronic inflammation in 1.0 mg/kg males were also significantly increased (Table 5). Microscopically, epidermal hyperplasia consisted of a slight increase in the number of cell layers of the epidermis. Melanocyte hyperplasia was characterized by rare-to-multiple aggregates of cells containing dark brown granules of pigment, which were typically found in the superficial dermis. Chronic inflammation was characterized predominantly by mononuclear cells, with occasional neutrophils in the dermis. Acute inflammation, which was much less common than chronic inflammation, consisted mainly of neutrophils with fewer macrophages and lymphocytes in the dermis and epidermis adjacent to an eroded or ulcerated surface epithelium. Degenerate neutrophils, cell debris and necrotic epidermal cells, and proteinaceous fluid frequently formed a crust overlying the eroded or ulcerated epidermis.

Interim evaluations of the proliferative skin effects of dermal exposure to TMPTA were conducted at weeks 2 and 13 and month 12 (data not shown). Histopathologic evaluation of the skin from the site of application identified slight treatment-related increases in the incidences of hyperplasia and inflammation or chronic active inflammation at all three time points in both sexes. Additionally, there were treatment-related increases in the incidences of sebaceous gland hyperplasia at the 13-week time point in both sexes. The dosed mice had up to five layers of epidermal cells (vehicle control mice had one to two layers). Sebaceous gland hyperplasia was characterized by a slight increase in the size of the sebaceous glands relative to controls. The inflammation consisted of infiltrates of predominantly lymphocytes, with occasional macrophages, and the chronic active inflammation was characterized by infiltrates of predominantly macrophages with fewer neutrophils. The inflammatory cells were scattered throughout the dermis, multifocally in minimal inflammation, and more diffusely in mild and moderate inflammation. At all three time points, these changes were considered to be minor, but the increases in the incidences of these lesions were clearly dose related.

In male mice administered 3.0 mg/kg, there were significantly increased incidences of hyperplasia in the adrenal medulla (1/49, 4/49, 3/46, 10/50). The incidence in 3.0 mg/kg males was significantly greater than that in the vehicle control, and exceeded the range in all routes for historical controls (0–8%). Microscopically, the lesion was focal, characterized by a circumscribed increase in the number and tinctorial basophilia of the chromaffin cells.

4. Discussion

TMPTA is a multifunctional monomer with a wide range of industrial applications. From the available data in the literature, the critical effects of TMPTA are skin and eye irritation

(Kanerva et al., 1998; Mancuso and Berdondini, 2008; Mortensen, 1992; National Toxicology Program, 2005). Repeated dermal exposure to humans or animals leads to contact dermatitis and corrosion of the skin (Bjorkner et al., 1980; Clemmensen, 1984; Dahlquist et al., 1983; Maurice and Rycroft, 1986; Nethercott, 1978; Nethercott et al., 1983; Parker and Turk, 1983). It is a moderate to strong sensitizer in experimental animals and humans. In dermal carcinogenicity studies on 10 related acrylates and methacrylates, results were negative for TMPTA associated carcinogenic activity in male mice (Andrews and Clary, 1986). However, the study was conducted only in male mice with a single dose exposure. Therefore, it was considered inadequate for assessment of the carcinogenic potential of TMPTA.

The dose concentrations selected for 2-year studies reported here were based on 3-month dermal toxicity studies performed at doses ranging from 0.75 to 12 mg TMPTA/kg body weight in acetone (Doi et al., 2005; National Toxicology Program, 2005). Non-neoplastic skin lesions observed at the site of application in those studies were epidermal hyperplasia, hyperkeratosis, necrosis, and chronic inflammation at or above 3.0 mg/kg in both rats and mice. Based on this information, the doses selected for 2-year dermal studies were 0, 0.3, 1.0, and 3.0 mg/kg.

In the 2-year studies reported here, there were no differences in body weights or survival of dosed animals compared to the vehicle controls. There were no clinical findings of toxicity related to TMPTA administration. However, TMPTA increased the incidences of epidermal hyperplasia, characteristic of tumor promotion (Rundhaug and Fischer, 2010), at the site of application in a dose-dependent manner in rats and mice at all the time points examined. Dose-dependent, significant increases in the incidences of hyperkeratosis at the site of application were also observed in male and female rats. Both hyperplasia and hyperkeratosis are characteristics of chronic irritant contact dermatitis (Berardesca and Distant, 1994). Our results are consistent with the dermal irritant effect of TMPTA reported in the literature (Kanerva et al., 1998; Mortensen, 1992; National Toxicology Program, 2005). Skin irritation is an inflammatory response (Corsini and Galli, 1998) and inflammation can affect all stages of carcinogenesis (Kundu and Surh, 2008). For example, reactive oxygen and nitrogen species formed by activated inflammatory cells can affect the initiation stage. During the tumor promotion and progression stage, cell proliferation, apoptosis, and angiogenesis can be affected by inflammation. Whereas there was no treatment-related inflammation in rats from 2 or 13 weeks or 12 months, it was observed in a dose-dependent manner in male and female mice at all the time points. However, severity of the chronic inflammation was minimal. The data are consistent with the study in the Tg.AC hemizygous mouse model where dose-related increases in the incidences of epidermal hyperplasia and minimal chronic inflammation were observed at the site of application; however, there were dose-related increases in the incidences of squamous cell papilloma at the site of application (National Toxicology Program, 2005).

Although skin was identified as a target organ in the subchronic toxicity and transgenic mouse studies (National Toxicology Program, 2005), TMPTA did not increase any treatment-related neoplasm in the skin of male or female rats or mice at the site of application in the current studies. Our data are consistent with the previous study (Andrews

and Clary, 1986), which found no evidence of dermal carcinogenic activity in male mice. However, in the current studies, there were increased incidences of tumors at sites other than skin.

A marginal increase in the incidences of malignant mesothelioma of the testicular tunica vaginalis was observed in male rats. Malignant mesothelioma is a neoplasm arising from the surface serosal lining cells of the pleural, peritoneal, and/or pericardial cavities and/or from the testicular tunica vaginalis (Attanoos and Gibbs, 1997; Maronpot et al., 2009). It is a common spontaneous lesion of the peritoneal cavity in male F344/N rats, often arising from the tunica vaginalis of the testis (Hall, 1990). In this study, all incidences were present in the tunics around the testes with dissemination into the peritoneal cavity. There was a positive trend in the incidences of mesothelioma; the incidence in 3.0 mg/kg males was significantly greater than in the concurrent vehicle controls receiving acetone. The incidences of mesothelioma exceeded the historical control ranges for dermal studies (all vehicles) and for all routes of exposure by only one tumor. Because of the variability of these tumors in the historical controls (range 0–8%) and the fact that the incidence in the high dose group was only one tumor outside of the historical control range, the marginally increased incidences may have been related to TMPTA administration.

In the female mice, carcinogenic activity related to TMPTA administration was observed at two sites, liver and uterus. Incidences of hepatoblastoma (HB) and hepatocholangiocarcinoma (HCCC) of the liver, two uncommon malignancies, were increased in the dosed groups of mice. HBs are considered a primitive, poorly differentiated malignant variant of hepatocellular neoplasms. They are noted as irregular masses with blood-filled cystic spaces, necrosis and hemorrhage, and they generally have scant stroma composed of small, deeply basophilic cells with scant cytoplasm and elongated nuclei. HB is a rare neoplasm in mice and spontaneous incidence in female B6C3F1 mice has been reported to be less than 1% in females (Harada et al., 1996; Turusov et al., 2002) and, up to 3% or more in males, which is comparable to the NTP historical control data. In the NTP historical control data, the incidence of HB in female mice was 2/250 with range of 0–2% in dermal studies and 4/1195 with range of 0–2% in all routes. In contrast, the incidence of HB in male mice was 14/250 with range of 2–10% in dermal studies and 61/1149 with range of 0–34% in all routes all vehicle studies in the past 5 years. HBs can be seen arising from preexisting neoplasms or “de novo” from hepatic parenchyma. When a HB is seen to be clearly arising from a hepatocellular neoplasm, only the HB is recorded for incidence data. Increases in the incidences of HBs in the 0.3 and 3.0 mg/kg females (8% and 6%, respectively) above historical control values from NTP studies during the past 5 years (<1%) were considered due to TMPTA administration. In 20 of 24 studies in the current historical control database, no HBs were observed in the control groups. In the other 4 studies, only 1 HB was observed in the control groups. Incidences in the current study were 0, 4, 0, and 3 (vehicle control, low, middle, and high dose groups, respectively). The incidences in the dosed groups were considered due to treatment since they exceeded the concurrent and historical control ranges.

HCCCs are rare, aggressive hepatic neoplasms of the mouse that frequently metastasize. Up to 84% of HCCCs metastasize compared to about 25% of hepatocellular carcinomas and

HBs (Adams et al., 2011). They have areas of hepatocellular carcinoma that are accompanied by variable-sized areas of well- to poorly-differentiated neoplastic bile ducts and biliary epithelium. The neoplastic cell differentiation is often more obvious in metastases. Spontaneous occurrence of this mixed tumor is very rare in B6C3F1/N mice in NTP studies. In the current study, HCCC occurred in 1.0 and 3.0 mg/kg females (2% and 4%, respectively). Although the incidences were not significantly increased over concurrent controls, HCCC has not been seen in the current historical controls [historical incidence for 2-year dermal studies (all vehicles): 0/250; (all routes): 0/1,195] in female mice. In male mice, the incidence of HCCC is 2/250 with range of 0–4% in dermal studies and 13/1149 with range of 0–8% in all routes in the historical control data. From 1982 to 2008, 164 cases (95 in males and 69 in females) occurred in only 74 of more than 500 completed NTP studies. Control female mice had many fewer incidences than males (<0.5% and <1%, respectively; (Bach et al., 2010) and NTP database). Of the 74 studies identified to have at least one occurrence of HCCC, none appeared related to treatment. Since occurrence of HCCC is rare, we interpret the incidences in the dosed groups of the current study to be due to treatment since they exceeded the concurrent and historical control ranges. Taken together, the occurrence of two rare variants of hepatocellular neoplasms (i.e. HBs and HCCCs) provided evidence of a chemical-related manifestation of hepatocellular neoplasms with a highly malignant phenotype in the liver of female mice.

Incidences of uterine stromal polyp or stromal sarcoma (combined) in female mice occurred with a positive trend as well as a significant increase in the 3.0 mg/kg group. Uterine stromal polyps have been seen in control B6C3F1 mice (Tamano et al., 1988) particularly in older mice, with a mean incidence of $2.0\% \pm 2.2\%$ and a range of 0–8% among all studies in the NTP historical control database. The incidences in this study were dose related and the incidence in the 3.0 mg/kg group exceeded the historical control range for all dermal studies regardless of vehicle and for all routes. Therefore, it was concluded that the increased incidences were related to TMPTA administration. Previous studies with B6C3F1 mice showed that TMPTA increased the relative length of estrous stages in female mice (National Toxicology Program, 2005). Although the contribution of hormones is not clearly shown in rodents, development of stromal polyps in rodents is usually associated with aging (Davis, 2012). In humans, the peak incidence of uterine sarcomas is between 50 and 65 years of age (Seddon and Davda, 2011). The expression of both estrogen and progesterone receptors are documented in human endometrial stromal sarcomas (Seddon and Davda, 2011). In fact, in clinical settings, aromatase inhibitors, gonadotropin-releasing hormone analogues, and progestins are effectively used for treatment of endometrial stromal sarcoma in women (Rauh-Hain and del Carmen, 2013). Similar mechanistic-based studies in rodents demonstrating clear endocrine perturbations are lacking.

A number of polyfunctional acrylates including TMPTA were tested for carcinogenic activity and reported by Andrews and Clary (1986). In that study, male mice were topically administered 100 mg/kg TMPTA in mineral oil twice weekly for 80 weeks. The conclusion from that study was that skin was the major target of toxicity as shown by increased incidences of skin lesions in the treated animals; however, no skin or systemic tumors were observed. The current study showed increases of tumors at sites other than skin, suggesting systemic toxicity in TMPTA treated animals. In fact, NTP absorption and metabolism

studies showed that [^{14}C]-TMPTA is well absorbed from skin in rats and mice, parent chemical/metabolites are distributed throughout the body, and excretion is mainly via urine (National Toxicology Program, 2005).

TMPTA tested in the Tg.AC hemizygous mouse model demonstrated increased squamous cell papilloma formation at the site of dermal application in a dose-dependent manner (National Toxicology Program, 2005), suggesting that TMPTA was likely to be carcinogenic in a 2-year bioassay; this was shown in the current study where TMPTA showed carcinogenic activity in female mice thereby further supporting the value of the Tg.AC hemizygous mouse model in predicting carcinogenic activity. Interestingly, in the 2-year study, TMPTA did not increase tumor formation at the site of application in the skin, and this discrepancy could be due to increased sensitivity of the Tg.AC hemizygous mouse skin to tumor promoters (Leder et al., 1990). The Tg.AC hemizygous mouse contains an oncogene, v-Ha-ras transgene (Leder et al., 1990), so this model is genetically initiated and sensitive to the dermal tumor promoters. For example, abrasion (wounding) or application of a known tumor promoter was enough to induce papillomas in the skin in the Tg.AC hemizygous mouse model (Cannon et al., 1997; Leder et al., 1990). The Tg.AC hemizygous mouse model is expected to respond to both genotoxic and nongenotoxic carcinogens (Spalding et al., 2000; Tennant et al., 2001). The results from a limited number of genotoxicity assays with TMPTA are mixed. Evidence of TMPTA-induced chromosomal damage *in vitro* was seen in mouse lymphoma L5178Y tk^{+/−} cells (Cameron et al., 1991; Dearfield et al., 1989; Moore, 1989). However, no evidence of genotoxicity was seen with the bioassay lot of TMPTA in the Ames assay (bacterial mutagenicity test), and no evidence of chromosomal damage was seen *in vivo* in male or female B6C3F1 mice in a 3-month dermal study or Tg.AC hemizygous mice in a 6-month dermal study (National Toxicology Program, 2005). Thus, it is not possible, based on the current evidence, to assign a genotoxic or nongenotoxic mode of action to TMPTA.

In conclusion, our studies show that TMPTA is a dermal irritant at the site of application in rats and mice, males and females. Increased tumor incidences were observed in male rats and female mice away from the site of application. These results indicate concern for chronic human exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Malignant mesothelioma in the mesentery of a male F344/N rat administered 3.0 mg/kg TMPTA in the 2-year dermal study. The malignant mesothelioma is papillary and consists of one or more layers of neoplastic mesothelial cells. H&E.

Fig. 2.

Hepatoblastoma in the liver of a male B6C3F1/N mouse administered 3.0 mg/kg TMPTA in the 2-year dermal study. An expansile mass within the hepatic parenchyma is composed of small, deeply basophilic neoplastic cells and contains blood-filled cystic spaces. H&E.

Fig. 3.

Hepatocholangiocarcinoma in the liver of a male B6C3F1/N mouse administered 1.0 mg/kg TMPTA in the 2-year dermal study. The neoplasm contains both neoplastic hepatocytes and neoplastic bile duct epithelial cells with necrotic and cystic areas. H&E.

Fig. 4.

Stromal polyp in the uterus of a female B6C3F1/N mouse administered 3.0 mg/kg TMPTA in the 2-year dermal study. The polyp is a pedunculated mass that protrudes into the lumen and contains marked cellular pleomorphism. H&E.

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		Nonneoplastic Lesion	
			All organ	Skin		
Males						
			Malignant mesothelioma	Epidermal Hyperplasia	Hyperkeratosis	
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.

Table 2
Incidences of nonneoplastic skin lesions at the site of application in the F344/N rats treated for designated time periods.

Dose	Epidermal hyperplasia			Sebaceous gland hyperplasia			Inflammation		Hyperkeratosis	
	2W	13W	12M	2W	13W	12M	2W	12M	12M	12M
<i>Male</i>										
0	0 ^a	0	0	0	0	0	0	0	0	0
0.3	0	0	0	0	1	(1.0)	0	0	0	0
1.0	4	(1.0) ^b	3	(0.6)	4	(1.3)	0	0	1	(0.2)
3.0	5	(1.2)	5	(2.2)	3	(1.0)	4	(1.0)	5	(2.0)
<i>Female</i>										
0	0	0	0	0	0	0	0	0	0	0
0.3	0	2	(1.0)	1	(0.2)	0	0	0	0	0
1.0	3	(1.3)	1	(1.0)	0	0	0	1	(1.0)	0
3.0	4	(1.0)	4	(1.0)	2	(0.4)	0	4	(1.0)	0
									1	(0.2)
									5	(1.2)

W = weeks.
M = Months.
N = 5.

^a Number of animals with lesion.

^b Average severity grade of lesions in affected animals; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Table 3
Survival, body weights and incidences of neoplasms of the liver in B6C3F1/N mice in the 2-year study.

Dose (mg/kg)	Survival ^a	Final mean body weights (% control)	Neoplastic lesion			
			Liver			
			Hepatoblastoma	Hepatocolangiocarcinoma	Adenoma	Carcinoma
<i>Males</i>						
0	30/50		5 ^{b,c}	2 ^d	34	22 ^e
0.3	35/50	100	5	0	35	14
1.0	29/50	99	4	3	32	19
3.0	38/50	100	5	0	34	25
<i>Females</i>						
0	39/50		0 ^f	0 ^g	34	12 ^{*h}
0.3	31/50	104	4	0	34	13
1.0	30/50	101	0	1	31	10
3.0	30/50	101	3	2	36	19

* $p < 0.05$ Significant dose-related trend 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): 14/250, range 2–10%; all routes: 61/1149, range 0–34%.
^d Historical incidences for 2-year dermal study vehicle controls (all vehicles): 2/250, range 0–4%; all routes: 13/1149, range 0–8%.
^e Historical incidences for 2-year dermal study vehicle controls (all vehicles): 101/250, range 28–56%; all routes: 399/1149, range 16–56%.
^f Historical incidences for 2-year dermal study vehicle controls (all vehicles): 2/250, range 0–2%; all routes: 4/1195, range 0–2%.
^g Historical incidences for 2-year dermal study vehicle controls (all vehicles): 0/250; all routes: 0/1195.
^h Historical incidences for 2-year dermal study vehicle controls (all vehicles): 63/250, range 6–46%; all routes: 144/1195, range 0–46%.

Table 4

Incidences of neoplasms of the uterus in female B6C3F1/N mice in the 2-year study.

Dose (mg/kg)	Neoplastic lesion		
	Uterus		
	Stromal polyp	Stromal sarcoma	Stromal polyp or stromal sarcoma
0	0 ^{**} , ^{a,b}	0 ^c	0 ^{**} , ^d
0.3	1	0	1
1.0	2	0	2
3.0	5 [*]	1	6 [*]

N = 50.

* $p < 0.05$ Significantly different from the control group by the Poly-3 test.

** $p < 0.01$ Significant dose-related trend by the Poly-3 test.

^a Number of animals with lesion.

^b Historical incidences for 2-year dermal study vehicle controls (all vehicles): 5/250, range 0–6%; all routes: 24/1198, range 0–8%.

^c Historical incidences for 2-year dermal study vehicle controls (all vehicles): 0/250; all routes: 2/1198, range 0–2%.

^d Historical incidences for 2-year dermal study vehicle controls (all vehicles): 5/250, range 0–6%; all routes: 26/1198, range 0–8%.

Table 5
Incidences of nonneoplastic lesions of the skin at the site of application in B6C3F₁/N mice in the 2-year study.

Dose (mg/kg)	Nonneoplastic lesion				
	Skin				
	Epidermal hyperplasia	Melanocyte hyperplasia	Chronic inflammation	Ulcer	Acute inflammation
<i>Males</i>					
0	10 ^a	0	13	0	1
0.3	7	0	17	2 (3.0)	2
1.0	15	0	26 ^{**}	0	0
3.0	44 ^{**}	19 ^{**}	43 ^{**}	2 (3.0)	3
<i>Females</i>					
0	7	1	37	0	1
0.3	7	1	36	0	1
1.0	15 [*]	3	43	3 (3.3)	2
3.0	34 ^{**}	33 ^{**}	48 ^{**}	3 (3.3)	4

N = 50.

* $p < 0.05$ 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 with lesion.

^b Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.